Notice number: 5.001

COMMISSION OF INQUIRY INTO DNA PROJECT 13

Section 5(1)(d) of the *Commissions of Inquiry Act 1950*

STATEMENT OF THOMAS NURTHEN

I, **Thomas Edmund Kersey Nurthen**, care of Forensic Biology Division, Forensic Science Queensland, Reporting Scientist, do solemnly and sincerely declare that:

1. On 19 October 2023, I was requested to provide a statement responding to Notice 5.001 "Requirement to Give Information in a Written Statement".

2. The Notice 5.001 requiring me to provide a written statement relates to matters largely in 2007 and 2008, some 15-16 years ago. Since that time, I have worked on a significant quantity of procedures, protocols, validations and other matters at Forensic Biology Division, Forensic Science Queensland.

Identification

Question 1(a) - State your full name

3. My name is Thomas Edmund Kersey Nurthen.

Question 1(b) - State your qualifications, skills or experience relevant to forensic science and DNA

4. I obtained a Bachelor of Science from the University of Queensland in 1998.

5. I obtained a Bachelor of Science with Honours from the University of Queensland in 2001.

Question 1(c) - State the period(s) of time you have been or were employed by or otherwise engaged with Queensland Health, Queensland Health Forensic and Scientific Services (QHFSS) and/or Forensic Science Queensland, and in what roles and when

6. I am currently employed by Forensic Biology Division, Forensic Science Queensland as a Reporting Scientist. I have been employed as a Reporting Scientist by Queensland Health, Queensland Health Forensic and Scientific Services and now Forensic Science Queensland since 2012.

7. From October 2008 to 2012, I was employed as a Senior Scientist Quality & Projects, DNA Analysis, Forensic and Scientific Services.

8. From June 2006 to October 2008, I was employed as a Senior Scientist, Automation and Laboratory Information Management Systems (**LIMS**) implementation project, DNA Analysis, Forensic and Scientific Services. It was a temporary project position.

9. From September 2004 to June 2006, I was employed as a Scientist, Forensic Biology, Queensland Health Scientific Services.

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Thomas Nurthen Witness

10. Prior to my employment with Queensland Health Scientific Services, I was employed in the following positions:

    (a) Technician, Clinical Chemistry Department, West Middlesex University Hospital NHS Trust, London from April 2003 to April 2004;

    (b) Scientist, Endocrinology Department, Sullivan Nicolaides Pathology from June 2002 to February 2003;

    (c) Technical Officer (temporary), Endocrinology, Queensland Health, Royal Brisbane Hospital from March 2002 to April 2002;

    (d) Operational Officer (part-time), Queensland Health, Central Specimen Reception, Prince Charles Hospital, Chermside from March 2001 to June 2001;

    (e) Scientist, Endocrinology Department, Sullivan Nicolaides Pathology from April 1999 to January 2001; and

    (f) Operational Officer, Chemical Pathology, Queensland Health, Royal Brisbane Hospital, from April 1998 to April 1999.

11. At the time relevant to this Commission of Inquiry, and until October 2008, I held the temporary project position of Senior Scientist on the Automation and LIMS implementation project. The implementation project was tasked with:

    (a) The validation of automated robotic systems to perform DNA extractions (DNA extractions are the breaking open of cells and cleaning of the DNA from unwanted inhibitors);

    (b) Automating the set-up of the DNA quantitation process (which is measurement of the amount of DNA in a sample) and the DNA amplification process (which are the different locations with which a person's DNA is 'copied' or 'amplified'); and

    (c) Enhancing AUSLAB to improve efficiency. The LIMS is the software that records all case information, moves samples onto worklists and send the results of the samples back to the Queensland Police Service and other stakeholders. AUSLAB is the LIMS that was used in the Forensic DNA Laboratory.

12. In this role, I led a team of five scientists and coordinated and managed the overall project. I reported directly to the Managing Scientist (this was firstly Vanessa Ientile and then later Cathie Allen).

13. In addition to leading a team, my duties and responsibilities included:

    (a) Reporting directly to the Managing Scientist with respect to how the project was progressing (including against project milestones) and escalating any issues as necessary;

    (b) Participating in Management Team meetings and decisions about the project and laboratory agenda items;

    (c) Developing functional specifications for the enhancements of AUSLAB (i.e. creating new functionality within AUSLAB that allowed, for example, the

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Thomas Nurthen Witness

integration of robotic platforms, new workflows to improve efficiency of case management of samples and improved sample auditing);

(d) Liaising with external laboratories in relation to the development and validation of the robotic systems; and

(e) Overseeing the planning of the validation experiments (which were a necessary party of continuous quality improvement and NATA accreditation requirements) and coordinating of the validation report publication (the validation reports were prepared by my direct reports and provided to me for review, editing, writing up the experiments, approval and publication).

Manual and Automated DNA Extraction Methods

Question 2 – In relation to the report being the "Project 13. Report on the Verification of an Automated DNA IQ Protocol using the Multiprobe II PLUS HT EX with Gripper Integration Platform", Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C., Ientile, V. Automation/LIMS Implementation Project, DNA Analysis FSS (August 2008) (2008 Report)1 and the abstract and introduction therein which state:

1. Abstract

A manual method for extracting DNA from forensic samples using the DNA IQ™ system (Promega Corp., Madison, WI, USA) was validated for routine use in DNA Analysis (FSS). We have verified an automated DNA IQ™ protocol in 96-well format for use on the MultiPROBE® II PLUS HT EX Forensic Workstation platforms (PerkinElmer, Downers Grove, IL, USA). Data indicate that results from the automated procedure are comparable to those from the manual procedure. Contamination checks were performed using samples prepared in checkerboard and zebra-stripe format, and results were as expected. We recommend the use of the MultiPROBE® II PLUS HT EX platforms to perform automated DNA extraction using the DNA IQ™ system.

2. Introduction

The MultiPROBE® II PLUS HT EX FORENSIC WORKSTATION platforms (PerkinElmer, Downers Grove, IL, USA) are equipped to perform automated DNA extractions, as they include a DPC shaker and individual heat controllers to enable on-board lysis and incubation steps. Currently in DNA Analysis, the MultiPROBE® platforms allow walk-away operation of PCR setup protocols for DNA quantitation and amplification.

The DNA IQ™ protocol has been verified or validated by various laboratories for use on the MultiPROBE® II PLUS platform. The laboratories that perform an automated DNA IQ™ protocol include PathWest (Western Australia), Forensic Science South Australia (South Australia) and Centre of Forensic Sciences in Toronto (Ontario). The MultiPROBE® II PLUS instrument comes pre-loaded with an automated DNA IQ™ protocol. Unlike the other laboratories, however, we did not validate the included protocol, but instead validated a manual DNA IQ™ protocol which was based on the CFS automated protocol (PerkinElmer, 2004), followed by verification of an automated protocol based on the validated manual method.

The verified automated DNA IQ™ protocol is identical to the validated manual protocol used in-house: there are no differences in reagents or volumes. The adopted DNA IQ™ protocol differs slightly, however, from the manufacturer's protocol, as it includes a lysis step using Extraction Buffer (10mM Tris, 1mM EDTA, 100mM NaCl, 20% w/v SDS) in the presence of Proteinase K, before incubating in the DNA IQ™ Lysis Buffer. Furthermore, the lysis incubation conditions were lowered from 70°C to 37°C in order to accommodate extraction of DNA from heat labile materials such as nylon and polyester. In addition, the automated protocol utilises the SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA) for simultaneous processing of samples in a 96-well format.

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Thomas Nurthen Witness

Manual Method

Question 2(a) describe, with precision, the "manual method" for extracting DNA from forensic samples using the DNA IQ™ system referred to in the first line of the Abstract to the 2008 Report (Manual Method), including whether the Manual Method:

(i) was devised within the QFSS Forensic DNA Analysis laboratory (Laboratory); or

(ii) was otherwise a modification of an existing manual method (and if so which method)

14. The Manual Method for extracting DNA from forensic samples using the DNA IQ™ system was validated by Project 11.

15. Project 11 concerned the validation of a manual method for DNA extraction of blood and cell stains on forensic samples using the DNA IQ™ system. The evaluation methods used, and the results and discussion, are reported in Project 11 *Report on the Validation of a manual method for Extracting DNA using the DNA IQ System* dated August 2008 (**Project 11 Report**).

16. **Attached and marked Exhibit TN-01** is a copy of the Project 11 Report.

17. To the best of my recollection, there were three different DNA IQ™ extraction protocols used in the Laboratory during early 2008:

(a) On deck lysis (this protocol is largely performed on the MultiPROBE® II);

(b) Manual DNA IQ™ extraction (this protocol is a fully manual task without the use of the MultiPROBE® II); and

(c) Off deck lysis (this protocol is performed on the MultiPROBE® II instrument with some additional tasks performed off the instrument).

18. The 'on deck lysis' protocol was first introduced in around 2007 when the MultiPROBE® II was first introduced into the Laboratory for the purposes of DNA extraction. The MultiPROBE® II was a liquid handling instrument that ran the DNA extraction protocols, including the DNA IQ system. A DNA extraction protocol is the method which is programmed into the instrument and instructs the instrument about how to process a sample.

19. **Attached and marked Exhibit TN-02** is the internal Standard Operating Procedure (**SOP**) 24897V1 valid from 24 October 2007, which sets out the 'on deck lysis' protocol.

20. **Attached and marked Exhibit TN-03** is the internal SOP 24897V2 valid from 11 January 2008, which sets out the 'on deck lysis' protocol and the Manual DNA IQ™. This SOP was an updated version of **TN-02** and it incorporated, amongst other things, the Manual Method.

21. The Manual DNA IQ™ extraction method was adapted from the Centre of Forensic Sciences (**CFS**) in Toronto, Ontario, Canada and PerkinElmer Protocol.

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22. **Attached and marked exhibit TN-04** is a copy of the *Modification of Automated DNQ IQ™ System for Mixed Casework Sample DNA Isolation* (PPN 8842157) **(CFS Protocol)**. The CFS Protocol was for the automated method using the MultiPROBE® II machine. On review of the document, I understand the PerkinElmer Protocol was created by CFS and the manufacturer of the MultiPROBE® II machine distributed this protocol.

23. The Laboratory adapted the CFS Protocol because it was devised by CFS for DNA IQ extraction using the MultiPROBE® II machine. The Manual Method was a fully manual task without the use of a MultiPROBE® II machine. The CFS Protocol required adaption by the Laboratory to make it suitable for a fully manual extraction method.

24. Adaption of protocols was, and remains to this day, usual and common practice by laboratories due to:

    (a) the ever evolving and developing nature of science and scientific techniques;

    (b) carrying out of scientific experiments for the validation of protocols and the advancement of scientific technique; and

    (c) manufacturers of equipment continual advancements in product range and services, requiring the constant adaptation of protocols to cater for new products and equipment.

25. The adapted Manual DNA IQ™ extraction method was as follows:

    (a) Set one ThermoMixer at 37°C and another at 65°C.

    (b) Ensure that appropriately sized samples are contained in a sterile 1.5mL tube. For every sample, prepare three set of labelled tubes: spin baskets (for every tube except the extraction control), 2 mL SSI tubes and Nunc™ tubes.

    (c) Prepare Extraction Buffer and add 300 µL to each tube. Close the lid and vortex before incubating the tubes at 37°C on the ThermoMixer at 1000 rpm for 45 minutes.

    (d) Remove the tubes from the ThermoMixer and transfer the substrate to a DNA IQ™ Spin Basket seated in a labelled 1.5 mL Microtube using autoclaved twirling sticks. Then transfer the liquid to a labelled 2 mL SSI sterile screw cap tube.

    (e) Centrifuge the spin basket on a benchtop centrifuge at room temperature for 2 minutes at its maximum speed. Once completed, remove the spin basket and collect the remaining solution and pool with the original extract in the 2 mL SSI sterile screw cap tube, then vortex.

    (f) Add 550 µL of Lysis Buffer to each tube.

    (g) Dispense 50 µL of DNA IQ™ Resin – Lysis Buffer solution (7 µL Resin in 43 µL Lysis Buffer) to each tube. Invert the resin tube regularly to keep the beads suspended while dispensing to obtain uniform results.

    (h) Vortex each tube for 3 seconds at high speed then place in a multitube shaker set at 1200 rpm to incubate at room temperature for 5 minutes.

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Thomas Nurthen Witness

(i) Vortex each tube for 2 seconds at high speed before placing the tubes in the magnetic stand. Separation will occur instantly.

Note: If resin does not form a distinct pellet on the side of the tube, or if the pellet has accidentally mixed with the solution while in the stand, vortex the tube and quickly place back in the stand.

(j) Carefully remove and discard all of the solution without disturbing the resin pellet on the side of the tube. If some resin is drawn up in tip, gently expel resin back into tube to allow re-separation.

(k) Remove the tube from the magnetic stand; add 125 µL of prepared Lysis Buffer and vortex for 2 seconds at high speed.

(l) Return tube to the magnetic stand, allow for separation and then remove and discard the Lysis Buffer.

(m) Remove tube from the magnetic stand; add 100 µL of prepared 1X Wash Buffer and vortex for 2 seconds at high speed.

(n) Return tube to the magnetic stand, allow for separation and then remove and discard all Wash Buffer.

(o) Repeat Steps 13 to 14 two more times for a total of three washes. Be sure that all of the solution has been removed after the last wash.

(p) In a biohazard cabinet, place the lids of the tubes upside down on a Kimwipe, in their respective order, and the tubes into a plastic rack, and air-dry the resin for 5-15 minutes at room temperature. Do not dry for more than 20 minutes, as this may inhibit removal of DNA. Once dry, screw on the lids.

(q) To each samples then add 50 µL of Elution Buffer very gently on the top of the magnetic pellet. Do not mix.

(r) Close the lid and then incubate the tubes in the ThermoMixer at 65°C for 3 minutes with no shaking and another 3 minutes shaking at 1100 rpm.

(s) Remove the tubes and vortex for 2 seconds at high speed. Immediately place the tube in the magnetic stand. Tubes must remain hot until placed in the magnetic stand or yield will decrease.

(t) Carefully transfer the supernatant containing the DNA to the respective labelled Nunc™ tubes.

(u) Remove tubes from the magnetic stand and add carefully another 50 µL of Elution Buffer above the magnetic pellet.

(v) Repeat step 30 to 32 [*sic*]. The final volume after this elution should be approximately of 95 µL of DNA solution.

(w) DNA can be stored at 4°C for short-term storage or at -20 or -70°C for long-term storage.

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Thomas Nurthen Witness

Question 2(b) describe, with precision, the method by which the Manual Method's "routine use" in DNA Analysis (FSS) was validated

26. Project 11 validated the Manual Method's extraction of DNA and routine use in DNA Analysis.

27. As the team leader on the Automation and LIMS project it is likely that I contributed to the writing of the Project 11 Report, and its various iterations, however I cannot now identify by looking at the report which sections I may have written or contributed to.

28. The method by which the Manual Method's "routine use" in DNA Analysis (FSS) was validated is outlined in the following paragraphs of the Project 11 Report at **TN-01**:

    (a) Paragraph 4 documents the equipment and materials used to validate the Manual Method's "routine use" in DNA Analysis;

    (b) Paragraph 5 documents the validation methods for:

        (i) sensitivity, reproducibility (linearity) and yield (paragraph 5.3);

        (ii) inhibition challenge (paragraph 5.4);

        (iii) substrates (paragraph 5.5);

        (iv) mixture students (paragraph 5.6);

        (v) substrate size (paragraph 5.7); and

        (vi) extraction using the DNA IQ™ System (Promega Corp.) (paragraph 5.8).

    (c) Paragraph 6 documents the results of the validation and discussion.

29. The adapted manual DNA IQ™ protocol is described in paragraph 5.8 of the Project 11 Report and is set out at paragraph 25 above.

Question 2(c) state whether, and if so how, the Manual Method differed from or otherwise modified the DNA IQ™ protocol that was "verified or validated by various laboratories for use on the Multiprobe® II PLUS platform" (as stated in the second line of the second paragraph of the Introduction to the 2008 Report)

30. In 2007, the laboratories that performed an automated DNA IQ protocol included PathWest (Western Australia), Forensic Science South Australia (South Australia) and CFS in Toronto, Ontario.

31. The CFS Protocol (**TN-04**), and the Manual Method were broadly the same and involved roughly the same steps. The main differences were:

    (a) the elution step in the CFS Protocol specified a single elution step whereas the Manual Method used a double elution step. My recollection is that our experience was that DNA was still bound to the beads after a single elution step and a double elution step allowed recovery of additional DNA; and

    (b) the CFS Protocol specified different consumables (i.e. plasticware) and hardware (i.e. heaters and centrifuges).

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Thomas Nurthen Witness

32. **Attached and marked TN-05** is a copy of the PathWest protocol, *PerkinElmer MultiPROBE 11 Robotics Validation for Crime Scene Samples* (**PathWest Protocol**).

33. The Pathwest Protocol and the Manual Method were generally the same and involved roughly the same steps. The main differences were:

    (a) the PathWest Protocol used an incubation temperature of 65°C whereas the Manual Method used an incubation temperature of 37°C;

    (b) the PathWest Protocol used a single elution step whereas the Manual Method used a double elution step for the reasons explained at paragraph 31(a)above; and

    (c) the PathWest Protocol used different consumables (i.e. they used different chemicals from different manufacturers of chemicals) and hardware.

34. I do not have a copy of the Forensic Science South Australia DNA IQ protocol and therefore cannot comment on whether, and if so how, the Manual Method differed from that protocol or was implemented.

Question 2(d) state when the Manual Method was so devised

35. I do not recall the specific date the Manual Method was devised but it would have been in 2007.

Question 2(e) identify those within the Laboratory responsible for devising the Manual Method

36. The Automation team comprised of myself, Dr Vojtech Hlinka, Iman Muharam, Breanna Gallagher, Generosa Lundie, and Cecilia Iannuzzi. I reported to Vanessa Ientile

37. I do not recall who within the Automation team was responsible for adapting the Manual Method to include a double elution step. It is very likely that I was involved in that decision within the Automation Team.

Question 2(f) state the reason(s) why the Laboratory chose to devise and to implement the Manual Method

38. The Manual Method was necessary first and foremost as a reference point to assess the efficiency of the automated DNA IQ™ protocol.

39. The Manual Method also meant, in the event the machines malfunctioned or went offline, the Laboratory could continue processing samples and extracting DNA manually.

CFS Automated Protocol

Question 2(g) describe, with precision, the "CFS automated protocol (PerkinElmer, 2004)" (CFS Automated Protocol) referred to in the seventh line of the second paragraph of the Introduction to the 2008 Report

40. The CFS Protocol is **attached and marked exhibit TN-04.** The protocol is set out at figure 1A and figure 1B of the CFS Protocol.

Manual DNA IQ™ Protocol

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Thomas Nurthen Witness

Question 2(h) describe, with precision, the "manual DNA IQ™ protocol" (Manual DNA IQ™ Protocol)" referred to in the seventh line of the second paragraph of the Introduction to the 2008 Report, including whether it:

(i) was developed or otherwise supplied by the manufacturer of the MultiPROBE® II PLUS HT EX FORENSIC WORKSTATION platform;

(ii) was devised within the Laboratory; or

(iii) was otherwise a modification of an existing Manual DNA IQ™ protocol (and if so which method)

41. I understand the "Manual DNA IQ™ Protocol" is the Manual Method.

42. The Manual Method/Manual DNA IQ™ Protocol was a modification of the CFS Protocol by the Laboratory.

Question 2(i) describe, with precision, the method by which the Manual DNA IQ™ Protocol was validated

43. I refer to my response to question 2(b) at paragraphs 26-29 above.

Question 2(j) state whether, and if so how, the Manual DNA IQ™ Protocol differed from or otherwise modified the DNA IQ™ protocol that was "verified or validated by various laboratories for use on the Multiprobe® II PLUS platform" (as stated in the second line of the second paragraph of the Introduction to the 2008 Report)

44. I refer to my response to question 2(c) at paragraphs 30-34 above.

Question 2(k) state when the Manual DNA IQ™ Protocol was so devised

45. I refer to my response to question 2(d) at paragraph 35 above.

Question 2(l) identify those within the Laboratory responsible for devising the Manual DNA IQ™ Protocol

46. I refer to my response to question 2(e) at paragraphs 36-37 above.

Question 2(m) state the reason(s) why the Laboratory chose to devise Manual DNA IQ™ Protocol

47. I refer to my response to question 2(f) at paragraphs 38-39 above.

Automated DNA IQ™ Protocol

Question 2(n) state whether the "automated DNA IQ™ protocol" referred to in the first line of the third paragraph of the Introduction to the 2008 Report (Automated DNA IQ™ Protocol) is the same as the automated protocol the subject of the 2008 Report. If it is not, then state the reasons why and describe any differences

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Thomas Nurthen Witness

48. To the best of my recollection, the "automated DNA IQ™ protocol" referred to in the first line of the third paragraph of the Introduction to the 2008 Report (Automated DNA IQ™ Protocol) is the same as the automated protocol the subject of the 2008 Report.

Question 2(o) state whether, and if so how, the Automated DNA IQ™ Protocol differed from or otherwise modified:

(i) the Manual Method;

(ii) the DNA IQ™ protocol that was "verified or validated by various laboratories for use on the Multiprobe® II PLUS platform" (as stated in the second line of the second paragraph of the Introduction to the 2008 Report);

(iii) the CFS Automated Protocol; and

(iv) the Manual DNA IQ™ Protocol

49. **Attached and marked TN-02, TN-03 and TN-06-TN-13** are SOPs for the Automated DNA IQ Protocol:

(a) SOP QIS#24897V1 valid from 24 October 2007 (**TN-02**);

(b) SOP QIS#24897V2 valid from 11 January 2008 (**TN-03**);

(c) SOP QIS#24897V3 valid from 27 March 2008 (**TN-06**);

(d) SOP QIS#24897V4 valid from 21 May 2008 (**TN-07**);

(e) SOP QIS#24897V5 valid from 29 June 2009 (**TN-08**);

(f) SOP QIS#24897V6 valid from 13 August 2009 (**TN-09**);

(g) SOP QIS#24897V7 valid from 9 November 2010 (**TN-10**);

(h) SOP QIS#24897V8 valid from 27 June 2012 (**TN-11**);

(i) SOP QIS#24897V9 valid from 3 January 2014 (**TN-12**);

(j) SOP QIS#24897V10 valid from 12 June 2015 (**TN-13**); and

(k) SOP QIS#24897V11 valid from 30 January 2017 (**TN-14**).

50. The differences between each of the Automated DNA IQ Protocol are documented in the above QIS documents. As can be observed from the various iterations of the SOPs as above at paragraph 49, there were multiple versions of the Automated DNA IQ Protocol.

51. To answer this question I have reviewed the following documents:

(a) Project 11 Report (**TN-01**);

(b) SOP QIS#24897V1 (**TN-02**);

(c) SOP QIS#24897V2 (**TN-03**);

[redacted] [redacted]

Thomas Nurthen Witness

(d) CFS Protocol (**TN-04**);

(e) PathWest Protocol (**TN-05**); and

(f) 2008 Report (**TN-18**).

52. I have limited my review to version 1.1 of the Automated Protocol (**TN-02**) because it is the version referred to at paragraph 5.6 of the 2008 Report (**TN-18**).

53. As stated above at paragraph 20, SOP QIS# 24897V2 (**TN-03**) incorporated, amongst other things, the Manual Method.

54. The table below sets out at a high level, the differences between the Manual Method (**TN-03**) and the Automated Protocol version 1.1 which is set out at SOP QIS#24897V1 (**TN-02**).

55. Additionally, there would have been changes made to the procedure, performance files and calibrations within the MultiPROBE® II PLUS platform. At the time of signing this statement, I have not been able to access and review the different versions of the WinPrep program. As I understand it, we do not have the WinPrep program installed on the Laboratory's systems.

Difference	**Manual Method/Manual DNA IQ Protocol (TN-03)**	**Automated DNA IQ™ Protocol (Version 1.1) (TN-02)**
Extraction buffer	300 µL	500 µL
Elution	Double 50 µL elution	Double 60 µL elution
Plasticware	1.5 mL tubes, DNA IQ™ Spin Baskets	Deep well plates SlicPrep™ 96 Device
Hardware	ThermoMixer Comfort heater shaker MagneSphere magnetic separation stand Vortex mixer	Heater tiles DPC Deck shaker PKI magnet

56. The table below sets out at a high level, the differences between the PathWest Protocol (**TN-05**) and the Automated DNA IQ Protocol (**TN-02**).

Difference	**Automated DNA IQ™ Protocol (Version 1.1) (TN-02)**	**PathWest Protocol (TN-05)**
[redacted]	[redacted]	

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Thomas Nurthen

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Witness

Extraction buffer	500 µL	300 µL
Incubation temperature	37 degrees Celsius	65 degrees Celsius
Elution	Double 60 µL elution	Single 35 µL elution
Plasticware	SlicPrep™ 96 Device Deepwell plates	Deepwell plate Whatman plate

57. The Automated DNA IQ Protocol (**TN-02**) and the CFS Protocol (**TN-04**) differed in the following respects

Difference	**Automated DNA IQ™ Protocol (Version 1.1) (TN-02)**	**CFS Protocol (TN-04)**
Extraction buffer	500 µL	300 µL
Elution	Double 60 µL elution	Single 35 µL elution
Plasticware	SlicPrep™ 96 Device Deepwell plates	Innovative Microplate 1.4 mL V-bottom polypropylene Deepwell plate Whatman 2 mL square deep well plate Greiner 96-Well V-Bottom PP Microplates ABI PRISM® 96-well Optical Reaction Plate w/ Barcode, Code 128 ABI PRISM® Optical Adhesive Covers
Hardware	Centrifuge PKI magnet	MagnaBot 96 Magnetic Separation Device MagnaBot Spacer, ¼ inch Foam (Cat #Z3301)

58. As above at paragraph 41, I understand the "Manual DNA IQ™ Protocol" is the Manual Method. For that reason, I have not done a comparison as requested in Question 2(o)(iv).

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Thomas Nurthen Witness

Question 2(p) state when the Automated DNA IQ™ Protocol was so devised

59. The Automated DNA IQ protocol was devised at the same time as the Manual Method in 2007.

Question 2(q) identify those within the Laboratory responsible for devising the Automated DNA IQ™ Protocol

60. No one person within the Automated Team was responsible for devising the Automated DNA IQ Protocol. It was a collaborative process. The Automated DNA IQ Protocol was the result of various discussions, team meetings, testing, experiments and actions by all team members over the life of the automation project to devise an automated method which was fit for purpose within the Laboratory.

Question 2(r) state the reason(s) why the Laboratory chose to devise the Automated DNA IQ™ Protocol rather than use the manufacturer method

61. It was necessary for the Laboratory to devise the Automated DNA IQ Protocol. This was because:

    (a) The decision by the Laboratory to use the SlicPrep™ 96 Device meant the manufacturer's method was not fit for purpose. Based on discussions in 2005 with the manufacturer (Promega), the SlicPrep™ 96 Device was identified by the manufacturer as a potential solution to tip clogging by substrates. Tip clogging occurs when the tip gets substrate stuck on or in the tip and cannot aspirate or dispense properly. This can affect volumes and is a contamination risk. **Attached and marked TN-15** is a file note of discussions between the Laboratory and the manufacturer regarding the use of the SlicPrep™ 96 Device. At that time, tip clogging by substrates was identified by the manufacturer as a real issue. **Attached and marked TN-16** is an article dated September 2005. Attached and marked **TN-17** is an article dated February 2006.

    (b) The Laboratory chose to use a double elution step as our experience was that DNA was still bound to the beads after a single elution step and a double elution step allowed recovery of additional DNA; and

    (c) The Laboratory used an increased extraction buffer volume. The Laboratory tested multiple volumes (300 µL, 350 µL, 400 µL, 450 µL and 500 µL) and found 500 µL was required to cover the substrate adequately.

Question 3 – State when the Laboratory received the Multiprobe II Device

62. The Laboratory received the MultiPROBE® II Device some time in December 2005.

Question 4 – For each of the Manual DNA IQ™ Protocol and the Automated DNA IQ™ Protocol, describe, with precision and completeness, what, if anything was done to the device to modify it, including whether any of the manufacturer's factory settings were changed, and if so which ones and how (including but not limited to temperature settings, reagents and volumes)

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Thomas Nurthen Witness

63. The Laboratory modified the removable heating tile used on the MultiPROBE® II Device.

64. The heating tile was physically modified (i.e. cut down) to allow the SlicPrep™ 96 Device to fit. The SlicPrep™ 96 Device is a 96 well spin basket designed to rapidly remove or separate substrates and lysates. The advantage of the SlicPrep™ 96 Device was it removed the substrates and lysates before completing the extraction and eliminated tip clogging. The SlicPrep™ 96 Device was such a new product that there was no off the shelf heating tile on the market.

65. The Laboratory did not otherwise modify the MultiPROBE® II.

66. In answering this question I am not taking into account the calibration of various parts of the MultiPROBE® II including arms and tip probes. Calibration involved teaching the robot how to use different plasticware. I do not consider calibrations to be a modification of the manufacturer's factory settings.

67. It is unclear to me from the question posed how manufacturer factory settings could be changed/modified for a manual protocol. No changes or modifications were made in my view.

Question 5 - State when the modifications were made

68. The modification to the heating tile for the SlicPrep™ 96 Device was made in or around October 2007.

Question 6 - Identify those within the Laboratory responsible for the modifications

69. I was responsible for the modification to the first heating tile for the SlicPrep™ 96 Device. I cut down the heating tile to accommodate the SlicPrep™ 96 Device. The SlicPrep™ 96 Device second heating tile was cut down by the campus workshop.

70. After the heating tiles were cut down, they were checked in the Laboratory for even distribution of heat and verified, as reported in paragraph 5.5 in the 2008 Report (further discussed below).

Question 7 - State the reasons why the modifications were made

71. The heating tile was physically modified (i.e. cut down) so that it could accept the SlicPrep™ 96 Device.

Question 8 - Describe your role in the preparation of the 2008 Report

72. **Attached and marked Exhibit TN-18** is a copy of what I understand is being referred to in this Commission of Inquiry as the 2008 Report.

73. As the team leader on the Automation and LIMS project, I would have contributed to the writing of the Project 13 Report and its various iterations, however I cannot now identify by looking at the report which sections I may have written or contributed to.

[redacted] ..... [redacted] .....

Thomas Nurthen Witness

74. The 2008 Report is a draft. I have formed this view because the document contains incomplete sentences, for example paragraph 5.6.4, the paragraph under the heading 'Checkerboard1' on page 9 of 18, the second paragraph on page 10 of 18. It identifies the staff donors by their initials. Any identifying information should be removed from a final report. There are also incomplete sections in the report which are highlighted in red.

75. I have searched for a final version of the 2008 Report and cannot find one.

76. I have searched for and located 10 iterations of the 2008 Report, which are attached marked **TN-19** to **TN-27** as follows:

    (a) 11 October 2007 V0.1 (**TN-19**);

    (b) 29 July 2008 V0.2 (**TN-20**);

    (c) 12 August 2008 V0.3 (**TN-21**);

    (d) 20 August 2008 V.04 (**TN-22**);

    (e) 26 August 2008 9.26am V.05 (**TN-23**);

    (f) 26 August 2008 3.05pm V0.6 (**TN-24**);

    (g) 4 September 2008 V0.7 (**TN-25**);

    (h) 5 September 2008 V0.8 (**TN-26**);

    (i) 9 September 2008 V.09 (**TN-27**); and

    (j) 11 December 2008 V0.10 (**TN-17**).

77. I believe that a different validation document was used as a template for the 2008 Report. I believe this because:

    (a) On review of Version 0.1 of the 2008 Report, the authors listed and the report date are identical to *'Project 1: Report on the Verification of automated Quantifier™ Human DNA Quantification Setup using the MultiPROBE II Plus HT EX with Gripper™ Integration Platform'* (**Report 1**). A copy of Report 1 is **attached and marked TN-28**;

    (b) The sentences which appear in the Abstract *'Data indicates that result from the automated procedure are comparable to those form the manual procedure. Contamination checks were performed using samples prepared in checkerboard and zebra-stripe format, and results were as expected'* also appear in Report 1;

    (c) The whole of the Introduction in Report 1 is the same as the Introduction in Version 0.1 of the 2008 Report, with the exception that Report 1 refers to DNA Analysis and the 2008 Report refers to Forensic Biology; and

    (d) Figure 8 in version 0.1 of the 2008 Report is the same as Figure 8 in Report 1.

Question 9 - Describe the directions you received in relation to the preparation of the 2008 Report, and identify the person or persons from whom you received those directions.

[redacted] ..... [redacted].

Thomas Nurthen Witness

78. I did not receive any express directions for the creation and drafting of the 2008 Report. It was implicit that a report would be prepared for Project 13 in accordance with the Laboratory's validation SOP requirements.

79. The experimental work was completed but was not written into a final validation report.

Question 10 - State the substance of the communications (including discussions) that occurred between you, any other authors of the 2008 Report and/or any supervisor or person in a position of management concerning the purpose(s) or intended purpose(s) of the 2008 Report, including by identifying with whom those communications took place and when.

80. The purpose of the 2008 Report was to document the results of the experiments that had been undertaken for Project 13.

81. It was always the intention to finalise the 2008 Report. I cannot now recall exactly why the 2008 Report was not finalised, other than the contamination issues were the highest priority from July 2008, and by October 2008 I had moved roles into the Quality & Projects team.

Question 11 - Identify the persons to whom was the 2008 Report was distributed.

82. To the best of my knowledge, the 2008 Report was not distributed. It was not distributed by me either to management or externally.

83. However, none of the drives were 'locked down' and, as a consequence, the 2008 Report could have been accessed and/or edited by any number of staff at the Laboratory.

Question 12 - In relation to the matters stated in the 2008 Report, state

Question 12(a) - how the conclusion on page 1 of the 2008 Report that "Data indicate that results from the automated procedure are comparable to those from the manual procedure" was reached, including:

(i) any discussions or communications between any of the named authors of the 2008 Report and any supervisor or person in a position of management in relation to that conclusion or the referenced data; and

(ii) how that conclusion can be reconciled with the data and figures outlined in part 6.4 of the 2008 Report.

84. The conclusion on page 1 of the draft 2008 Report (**TN-17**) is left over from Report 1 (**TN-28**). It was not reflective of Project 13.

85. There were no discussions or conversations between any of the named authors of the 2008 Report (**TN-17**) and any supervisor or person in a position of management in relation to the conclusion or the referenced data in the Abstract because they were left over from Report 1 (**TN-28**).

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Thomas Nurthen Witness

86. The conclusion in the Abstract cannot be reconciled with the data and figures outlined in part 6.4 of the 2008 Report (**TN-17**) because the conclusion in the Abstract is left over from Report 1 (**TN-28**).

Question 12(b) - how the recommendations summarised on page 18 of the 2008 Report were decided, including by identifying:

(i) your role in the decision;

(ii) with whom and when you communicated (including by way of discussion) with any other person in connection with the recommendations and the decision to make them

87. I do not recall who drafted the recommendations in the 2008 Report. I cannot recall any discussion in connection with the recommendations and I cannot comment on how they were decided.

88. The draft recommendations first appear in Version 3 of the draft, last saved on 12 August 2008, after Project 13 went live on 29 October 2007 (**TN-21**).

89. The draft recommendations do not accord with the view I expressed to Vanessa Ientile prior to Project 13 going live. My view was that we were not ready to go live because the yields from the Automated DNA IQ Protocol were too low at that time. My concern was that the yields would not be as sensitive to extract lower amounts of DNA. Based on my review of my records, I expressed my view to Vanessa Ientile at our weekly project update meetings, including on 9 October 2007 and 16 October 2007.

90. **Attached and marked TN-29** is Vanessa Ientile's handwritten meeting notes from our weekly project update meeting on 9 October 2007.

91. **Attached and marked TN-30** is Vanessa Ientile's handwritten meeting notes from our weekly project update meeting on 16 October 2007.

Question 13 – In relation to your written statement, provided to the Sofronoff Inquiry and dated 17 October 2022, why did that statement not contain any mention or explanation of the 2008 Report.

92. **Attached and marked TN-31** is the notice 2022/00178 to give information in a written statement that was issued to me in the Sofronoff Inquiry. The notice was dated 14 September 2022. The notice required me to give information in a written statement by COB 20 September 2022. I provided a written statement on 17 September 2022. My statement with exhibits was 418 pages long.

93. The questions I was asked to respond to are listed in notice 2022/00178 (**TN-30**). I answered each question truthfully to the best of my knowledge and based on my review of emails and files in my possession.

94. Question 5 to 18 related to DNA IQ. A number of questions referred to "DNA IQ problems". Specifically:

[redacted] ..... [redacted] .....

Thomas Nurthen Witness

(a) Question 6 asked me to "Explain what problems with DNA IQ were experienced in approximately 2008. Explain to the best of your knowledge, how these problems were first detected."

(b) Question 7 asked me to "Identify each OQI and adverse event that relates to DNA IQ problems at around this time, or has since been linked to DNA IQ problems from around this time."

(c) Question 8 asked me "What actions did the management committee and/or staff at the DNA laboratory take in response to the discovery of the problem? Provide a clear timeline which covers the problems identified, the decisions taken in response and by whom, and how those decisions were implemented."

(d) Question 9 asked me "Was the cause of the issues or problems relating to DNA IQ identified? If yes, what was it?"

(e) Question 10 asked me "What immediate action was taken after the cause of the issues or problems was identified?"

(f) Question 11 asked me "Outline your role in responding to issues with DNA IQ, and any audits completed in relation to any OQI concerning DNA IQ, including audit 9175. Provide an explanation of the findings of audit 9175 and actions taken in response to the audit. When were the follow-up actions finalised?"

(g) Question 12 asked me to "Identify each staff member involved in detecting and responding to the problems with DNA IQ, and the nature of each person's involvement."

(h) Question 13 asked me to "Identify whether any issue or problem with respect to DNA IQ was audited by an external agency? If yes, when did that occur and in respect of what particular issue or issues. Who decided that should occur, provide:

(i) Instructions;

(ii) List of material; and

(iii) The report, including any draft report."

(i) Question 14 asked me "How were the results of the audit communicated to the DNA laboratory?"

(j) Question 15 asked me "What permanent changes, or amendments to SOPs, were made as a result of identifying the problems related to DNA IQ?"

(k) Question 16 asked me to "Explain what communications were made to external agencies, including the Queensland Police Service, the Office of the Director of Public Prosecutions, and the Queensland Courts, about the problems with DNA IQ and when communications were made. Attach copies of any emails or letters sent to the external agencies."

[redacted] ..... [redacted] .....

Thomas Nurthen Witness

(l) Question 17 asked me "Did the problems with DNA IQ lead to the retraction or amendment of results in these cases? If yes, how many cases were affected? By what means were the amendment and retraction of the results communicated?"

(m) Question 18 asked me "Has the DNA laboratory since returned to using DNA IQ processes, systems and/or products? Have there been any further problems with DNA IQ systems or products? Explain all future problems in detail, including what has been done in response to them. Attach any OQIs, Adverse Entry Log's or record of the problem being identified and investigated."

95. The problems with DNA IQ that were experienced in 2008 concerned sample cross contamination. I gave detailed written answers to each of the questions posed to me in respect of the problem of sample cross contamination. That was the problem that was recorded in an OQI#19330 on 21 April 2008 and in a subsequent contamination events as recorded in OQI#19349 on 23 April 2008, OQI#19477 on 12 May 2008, OQI#19767 on 14 June 2008 and OQI#19768 on 30 July 2008.

96. In response to Question 7, I provided a table which listed the OQIs which were raised in relation to DNA IQ problems in 2008. Seventeen OQIs are listed, all of them concerning a single contamination event between casework samples or multiple contamination events between caseload samples.

97. It did not occur to me in giving my written statement to the Sofrnoff Inquiry to mention or explain the 2008 Report. This is because:

(a) The Laboratory's focus in 2008 was determining the cause of, and taking action in response to, contamination events. I provided a detailed written statement to the Sofrnoff Inquiry about those concerns.

(b) The concerns I had and raised with Vanessa Ientile about yield levels prior to Project 13 going live were resolved. Efficiency was considered as part of validation process to reintroduce the use of the MultiPROBE® II PLUS machines in 2009. **Attached and marked TN-32** is the Re-implementing the Automated DNA IQ™ extraction protocol on the MultiPROBE® II PLUS HT EX Forensic Workstation platforms, and associated processes Interim Report – Extraction Platform B, dated April 2009. The testing results (Figure 8) indicate that the modified automated DNA IQ™ procedure is very sensitive and able to isolate low copy number DNA samples at a very high recovery rate that is close to 100%. Therefore I did not consider yields to be a DNA IQ 'problem' from reimplementation onwards.

(c) The 2008 Report was a draft.

I have not conferred or had any discussion with other witnesses in preparing my statement.

All the facts and circumstances declared in my statement, are within my own knowledge and belief, except for the facts and circumstances declared from information only, and where applicable, my means of knowledge and sources of information are contained in this statement.

I make this solemn declaration conscientiously believing the same to be true and by virtue of the provisions of the *Oaths Act 1867*.

[redacted] ..... [redacted] .....

Thomas Nurthen Witness

TAKEN AND DECLARED before me Brisbane on 25 October 2023

[redacted] ..... [redacted] .....

Thomas Nurthen Witness

EXHIBITS INDEX

Exhibits Index – Thomas Nurthen Statement

Question	Exhibit	Document Title
2(a)	TN-01	Project 11 *Report on the Validation of a manual method for Extracting DNA using the DNA IQ System* dated August 2008
2(a)	TN-02	Standard Operating Procedure (**SOP**) QIS#24897V1 valid from date 24 October 2007
2(a)	TN-03	SOP QIS#24897V2 valid from date 11 January 2008
2(a)	TN-04	Modification of Automated DNQ IQ™ System for Mixed Casework Sample DNA Isolation (PPN 8842157) (**the CFS protocol**)
2(c)	TN-05	PathWest protocol, *PerkinElmer MultiPROBE 11 Robotics Validation for Crime Scene Samples* (**PathWest protocol**)
2(p)	TN-06	SOP QIS#24897V3 valid from date 27 March 2008
2(p)	TN-07	SOP QIS#24897V4 valid from date 21 May 2008
2(p)	TN-08	SOP QIS#24897V5 valid from date 29 June 2009
2(p)	TN-09	SOP QIS#24897V6 valid from date 13 August 2009
2(p)	TN-10	SOP QIS#24897V7 valid from date 9 November 2010
2(p)	TN-11	SOP QIS#24897V8 valid from date 27 June 2012
2(p)	TN-12	SOP QIS#24897V9 valid from date 3 January 2014
2(p)	TN-13	SOP QIS#24897V10 valid from date 12 June 2015
2(p)	TN-14	SOP QIS#24897V11 valid from date 30 January 2017
2(r)	TN-15	File Note between the laboratory and the manufacturer regarding the use of the SlicPrep device dated 19 July 2005
2(r)	TN-16	Article dated September 2005
2(r)	TN-17	Article dated February 2006
8	TN-18	2008 Report version 1.0 – Date of last save 11 December 2008
8	TN-19	Project 13 Report, version 0.1 – Date of last save 11 October 2007
8	TN-20	Project 13 Report, version 0.2 – Date of last save 29 July 2008
8	TN-21	Project 13 Report, version 0.3 – Date of last save 12 August 2008
8	TN-22	Project 13 Report, version 0.4 – Date of last save 20 August 2008
8	TN-23	Project 13 Report, version 0.5 – Date of last save 26 August 2008 at 9.26am
8	TN-24	Project 13 Report, version 0.6 – Date of last save 26 August 2008 at 3.05pm
8	TN-25	Project 13 Report, version 0.7 – Date of last save 4 September 2008

[redacted] ..... [redacted]

Thomas Nurthen Witness

8	TN-26	Project 13 Report, version 0.8 – Date of last save 5 September 2008
8	TN-27	Project 13 Report, version 0.9 – Date of last save 9 September 2008
8	TN-28	Project 1: Report on the Verification of automated Quantifier™ Human DNA Quantification Setup using the MultiPROBE II Plus HT EX with Gripper™ Integration Platform (**Report 1**)
12(b)	TN-29	Vanessa Ientile's handwritten meeting notes from meeting dated 9 October 2007
12(b)	TN-30	Vanessa Ientile's handwritten meeting notes from our meeting dated 16 October 2007
13	TN-31	Notice 2022/00178 dated 14 September 2022
13	TN-32	Re-implementing the automated DNA IQ™ extraction protocol on the MultiPROBE® II PLUS HT EX Forensic Workstation platforms, and associated processes Report dated April 2009

[redacted] .....

Thomas Nurthen

[redacted]

Witness

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Project 11. Report on the Validation of a manual method for Extracting DNA using the DNA IQ™ System

August 2008

Automation and LIMS Implementation Project Team,

DNA Analysis

Forensic And Scientific Services

Clinical and Scientific Services

Queensland Health

safe | sustainable | appropriate

Queensland Government
Queensland Health

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Project 11. Report on the Validation of a Manual Method for Extracting DNA using the DNA IQ™ System

Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C., Ientile, V.
Automation/LIMS Implementation Project, DNA Analysis FSS (August 2008)

1. Abstract

The DNA IQ™ system was found to be the most suitable kit for extracting cell and blood samples that are analysed in DNA Analysis FSS (refer to Project 9). This DNA extraction system, based on magnetic bead technology, was found to generate results that were comparable or better than the current Chelex®-100 protocol. We have validated a manual DNA IQ™ method for extracting DNA from forensic samples, and incorporated studies on sensitivity and consistency, inhibition, substrate type, substrate size, and mixture studies. This manual DNA IQ™ method is suitable for verification on the automated MultiPROBE® II PLUS HT EX extraction platforms.

2. Introduction

A previous evaluation of various DNA extraction systems that were designed specifically for forensic samples was performed in order to select a suitable extraction technology for extracting various sample types that are processed in DNA Analysis FSS. DNA IQ™ was identified as a suitable kit for extracting forensic samples, and was found to outperform both the current Chelex®-100 protocol and also all the other kits evaluated. The results of the evaluation are reported in Project 9 (Gallagher *et al*., 2007a).

DNA purification with silica matrices, either in membrane- or bead-form, commonly uses the affinity of DNA for silica without the need for hazardous organic reagents. However, these systems tend to require extensive washing to remove the guanidium-based lysis buffer. The DNA IQ™ system uses a novel paramagnetic resin for DNA isolation (Promega Corp., 2006). The DNA IQ™ System's basic chemistry is similar to other silica-based DNA isolation technologies, except that the specific nature of the paramagnetic resin, coupled with the formulation of the lysis buffer, is unique. In the DNA IQ™ System, negatively-charged DNA molecules have a high affinity for the positively-charged paramagnetic resin under high salt conditions supplied by the lysis buffer. Once DNA is bound to the magnetic resin, and the resin is immobilised by a magnet, the sample can be washed using an alcohol/aqueous buffer mixture. The high alcohol content of the wash buffer aids to maintain the DNA-resin complex in low-salt conditions, while the aqueous component functions to wash away residual lysis buffer and any inhibitors or non-DNA contaminants such as cellular debris and protein residues. DNA is released from the resin by using a low ionic strength elution buffer, and the purified DNA can be used directly in downstream applications such as PCR.

For samples that are in excess (e.g. reference samples), DNA IQ™ resin will only isolate up to a total of approximately 100ng of DNA due to bead saturation (Huston, 2002).

3. Aim

To validate a manual method for DNA extraction of blood and cell stains on forensic samples using the DNA IQ™ system (Promega Corp., Madison, WI, USA).

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4. Equipment and Materials

- DNA IQ™ System (Promega Corp., Madison, WI, USA); 100 samples, Cat.# DC6701), which includes:
  - 0.9mL Resin
  - 40mL Lysis Buffer
  - 30mL 2X Wash Buffer
  - 15mL Elution Buffer
- TNE buffer (10mM Tris, 100mM NaCl, 1mM EDTA, pH 8.0)
- MagneSphere® Magnetic Separation Stand, 12-position (Cat.# Z5342) (Promega Corp., Madison, WI, USA)
- DNA IQ™ Spin Baskets (Cat.# V1221) (Promega Corp., Madison, WI, USA)
- Microtube 1.5mL (Cat.# V1231) (Promega Corp., Madison, WI, USA)
- 95-100% ethanol
- Isopropyl alcohol
- 1M DTT (Sigma-Aldrich, St. Louis, MO, USA)
- Proteinase K (20mg/mL) (Sigma-Aldrich, St. Louis, MO, USA)
- 20% SDS (Biorad, Hercules, CA, USA)
- 0.9% saline solution (Baxter Healthcare, Old Toongabbie, NSW, Australia)
- ThermoMixer Comfort (Eppendorf, Hamburg, Germany)
- Vortex mixer
- Bench top centrifuge
- Cytobrush® Plus Cell Collector (Cooper Surgical, Inc., Trumbull, CT, USA)
- FTA® Classic Cards (Whatman plc, Maidstone, Kent, UK)
- Rayon (155C) and cotton (164C) plain dry swabs (Copan Italia S.p.A., Brescia, Italy)
- Vacuette® K2EDTA blood collection tubes (Greiner Bio-One GmbH, Frickenhausen, Germany)
- Sticky tape (BDF tesa tape Australia Pty Ltd)
- Tannic acid $C_{76}H_{52}O_{46}$ FW1701.25 (Selby's BDH, Lab Reagent >~90%)
- Urea NH_2CONH_2 FW60.06 (BDH, Molecular Biology Grade ~99.5%)
- Indigo carmine $C_{16}H_8N_2Na_2O_8S_2$ FW466.35 PN 131164-100G (Sigma-Aldrich, St. Louis, MO, USA)
- Humic acid sodium salt PN H167520-100G (Sigma-Aldrich, St. Louis, MO, USA)
- Used car motor oil, SW20/SAE50 (Caltex)
- Various clothing materials, including:
  - Best & Less Pacific Cliff, White cotton shirt, XXL
  - Big W Classic Denim, Men's Blue denim jeans, 112
  - Private Encounters, off-white nylon cami, size 14
  - Clan Laird, blue 100% wool kilt
  - Millers Essentials, blue 100% polyester camisole, size 10
  - Unknown, teal green 100% lycra swimwear
  - Leather Belt, brown

5. Methods

5.1 Cell and blood collection

Buccal cells were collected using a modified Cytobrush® protocol (Mulot *et al*., 2005; Satia-Abouta *et al*., 2002). Four donors were chosen. Each donor was asked to brush the inside of one cheek for one minute. Then, with another Cytobrush®, the other cheek was also sampled. The cells collected on the brush where then resuspended in 2mL of 0.9% saline solution. Multiple collections were taken on different days.

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Whole blood was collected from three donors by a phlebotomist as per standard collection procedures in EDTA tubes. Blood samples were refrigerated until spotting onto substrate and cell-counting step.

Table 1 lists the donor sample ID's.

Table 1. List of donor samples used for validating a manual DNA IQ™ method.

Donor ID
Cell samples
D1
D2
D3
D4
Blood samples
D1
D2
D3

5.2 Cell counting

Buccal cell suspensions were diluted using 0.9% saline solution to create a 1/10 dilution of the original sample prior to submitting for cell counting. All counts were performed by the Cytology Department, RBWH (QIS 15393).

Blood cell counting was performed on a 1mL aliquot of the original sample also by the Cytology Department, RBWH (QIS 15393).

5.3 Sensitivity, Reproducibility (Linearity) and Yield

Sensitivity and reproducibility of the DNA IQ™ kit was assessed using dilutions of cell and blood samples.

For cell samples, dilutions were made using a sample from donor 4, diluted in 0.9% saline solution. The dilutions used were:

- Neat
- $^{1}/_{10}$
- $^{1}/_{100}$
- $^{1}/_{1000}$

For blood samples, dilutions were made using a sample from donor 2, diluted in 0.9% saline solution. The dilutions used were:

- Neat
- $^{1}/_{10}$
- $^{1}/_{100}$
- $^{1}/_{1000}$

Mock samples were created from rayon and cotton swabs using the above dilutions. The swab heads were removed from the shaft using sterilised scalpel and tweezers. Swab heads were then cut into quarters and each quarter was then added to separate sterile 1.5mL tubes. To each quarter swab, 30µL of each neat sample or dilution was added to

create a total of five replicates. Samples were dried using a ThermoMixer set at 56°C over 2 hours in a Class II biohazard cabinet.

5.4 Inhibition challenge

Quartered cotton swabs in sterile 1.5mL tubes were spotted with 30µL of neat cell suspension and were dried after each addition on a ThermoMixer as described previously. Neat blood samples were also created using the same method.

All the inhibitors except for the motor oil were obtained in powder form. Before making any liquid solution of the powdered inhibitors, research was conducted to determine the likely level of each inhibitor normally encountered in the environment (Hlinka *et al*., 2007). Each solution was made at concentrations based on the information obtained (Table 2).

Table 2. Concentrations of various inh bitors used in the inhibition study.

Inhibitor	Excess/Neat Solution	Mass	Volume H_2O	Final inhibitor concentration
Tannic acid	Excess	600mg	500µL	0.705M
	Neat	200mg	500µL	0.235M
Humic acid	Excess	1g	5mL	20% (w/v)
	Neat	0.1g	5mL	2% (w/v)
Indigo carmine	Excess	0.47g	10mL	100mM
	Neat	0.047g	10mL	10mM
Urea	Excess	0.06g	1mL	1M
	Neat	0.021g	1mL	0.33M

A total of 30µL of each solution containing specified concentrations of various inhibitors was applied to the buccal cell and blood swabs prepared above. The only exception was motor oil, where only 15µL was added to the cell and blood swabs respectively. Each inhibitor sample was replicated in quadruplicate and left to dry overnight in a Class II biohazard cabinet.

To another set of prepared cell and blood swabs, an excess of each inhibitor was applied in quadruplicate for each inhibitor and allowed to dry overnight. This process was achieved by applying another solution of inhibitor exceeding the normal level (Hlinka *et al*., 2007).

5.5 Substrates

Swabs

Four cotton and four rayon swab quarters in sterile 1.5mL tubes were loaded with 30µL of neat cell or blood sample and were extracted once the sample had dried on the swab.

Tapelifts

Two donors were sampled using the tape most commonly used within the laboratory (BDF tesa tape). Strips of tape were firmly applied to the inside of the fore arm and lifted off. This process was then repeated until the tape was no longer adhesive. The tape was wrapped around sticky-side-in, forming a cylinder shape, and placed in a sterile 1.5mL tube. These samples were created in quadruplicate. Tape was not used as a substrate in the blood validation.

Fabric

The material types tested included:

- Denim jeans;

- White 100% cotton shirt;
- Blue 100% wool kilt;
- Teal green 100% lycra swimwear;
- White 100% nylon camisole;
- Blue 100% polyester camisole; and
- Brown 100% woven leather belt.

All material types except leather were sampled and ten 2.5cm x 2.5cm pieces were cut from each material and washed in 10% bleach following an in-house washing method to remove any contaminating DNA from outside the laboratory (Gallagher *et al*., 2007b). As for the leather, one strand of the leather weave was cut from the belt and washed following the same method. Once dry, the material was then cut into 0.5cm x 0.5cm pieces using sterile techniques, placed in 1.5mL tubes and 30µL of both cell sample and blood was applied to separate pieces. Each substrate sample was created in quadruplicate and dried on a ThermoMixer set at 56°C over 2 hours in a Class II biohazard cabinet.

Gum
Two types of chewing gum were chosen: (1) Wriggley's Extra White (peppermint flavour) and (2) Wriggley's Extra Green (spearmint flavour). The donor was asked to chew the gum for 30 minutes and dispose of the gum into a clip-seal plastic bag. The gum was then air dried in a Falcon tube overnight before it was frozen for roughly an hour before cutting into 3mm x 3mm x 3mm pieces and placed into sterile 1.5mL tubes. Gum substrates were not assessed for blood samples.

Cigarette butts
Two brands of cigarettes were smoked all the way through and then the butts collected. The filter paper of the butt was cut into 0.5mm^2 pieces and placed into sterile 1.5mL tubes. Cigarette butts were not assessed for blood samples.

FTA® Classic Card punches
Eight sterile 1.5mL tubes, each containing four 3.2mm FTA® Classic Card punches, were spotted with 30µL of cells or blood before being dried on a ThermoMixer. Four replicates contained sample from one donor, the other remaining four replicate tubes had a different donor sample added.

5.6 Mixture studies

Buccal cells and whole blood were obtained from a male and female donor. Dilutions were made using 0.9% saline solution to ensure that the cell concentration was equal. Dilutions were then performed on the male sample to obtain the correct ratios.

Mock samples were created using the following ratios of female to male:

- 1:1,
- 1:2,
- 1:10,
- 1:25,
- 1:50 and
- 1:100.

A total of 30µL of the female component was spotted first on to a quarter of a cotton swab in a sterile 1.5mL tube and dried on a ThermoMixer before adding another 30µL of the male component. Samples were created in quadruplicate for all ratios, for both cell and blood samples.

5.7 Substrate size

Various sizes of material were cut from a white cotton shirt:

- 0.5cm x 0.5cm,
- 1cm x 1cm,
- 2cm x 2cm.

Each piece of material was stored in individual, sterile 1.5mL tubes and 30µL of cell sample was added to the material and allowed to dry on a ThermoMixer. The same process was followed for blood samples. Five replicates were made for each sample type.

5.8 Extraction using the DNA IQ™ System (Promega Corp.)

The manual DNA IQ™ method used was based on an automated protocol developed by the Centre of Forensic Sciences (CFS) in Toronto, Ontario (PerkinElmer, 2004). A Proteinase K – SDS Extraction Buffer was made as per the recommended protocol. The 1x Extraction Buffer for one sample consisted of:

277.5µL TNE buffer
15µL Proteinase K (20mg/mL)
7.5µL 20% SDS

The TNE buffer consisted of:

1.211g Tris (10mM Tris)
2mL 0.5M EDTA (1mM EDTA)
5.844g NaCl (100mM NaCl)

The adapted manual DNA IQ™ protocol is described below:

1. Set one ThermoMixer at 37°C and another at 65°C.
2. Ensure that appropriately sized samples are contained in a sterile 1.5mL tube. For every sample, prepare three set of labelled tubes: spin baskets (for every tube except the extraction control), 2mL SSI tubes and Nunc™ tubes.
3. Prepare Extraction Buffer and add 300µL to each tube. Close the lid and vortex before incubating the tubes at 37°C on the ThermoMixer at 1000rpm for 45 minutes.
4. Remove the tubes from the ThermoMixer and transfer the substrate to a DNA IQ™ Spin Basket seated in a labelled 1.5mL Microtube using autoclaved twirling sticks. Then transfer the liquid to a labelled 2mL SSI sterile screw cap tube.
5. Centrifuge the spin basket on a benchtop centrifuge at room temperature for 2 minutes at its maximum speed. Once completed, remove the spin basket and collect the remaining solution and pool with the original extract in the 2mL SSI sterile screw cap tube, then vortex.
6. Add 550 µL of Lysis Buffer to each tube.

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7. Dispense 50µL of DNA IQ™ Resin – Lysis Buffer solution (7µL Resin in 43µL Lysis Buffer) to each tube. Invert the resin tube regularly to keep the beads suspended while dispensing to obtain uniform results.

8. Vortex each tube for 3 seconds at high speed then place in a multitube shaker set at 1200rpm to incubate at room temperature for 5 minutes.

9. Vortex each tube for 2 seconds at high speed before placing the tubes in the magnetic stand. Separation will occur instantly.

   Note: If resin does not form a distinct pellet on the side of the tube, or if the pellet has accidentally mixed with the solution while in the stand, vortex the tube and quickly place back in the stand.

10. Carefully remove and discard all of the solution without disturbing the resin pellet on the side of the tube. If some resin is drawn up in tip, gently expel resin back into tube to allow re-separation.

11. Remove the tube from the magnetic stand; add 125µL of prepared Lysis Buffer and vortex for 2 seconds at high speed.

12. Return tube to the magnetic stand, allow for separation and then remove and discard the Lysis Buffer.

13. Remove tube from the magnetic stand; add 100µL of prepared 1X Wash Buffer and vortex for 2 seconds at high speed.

14. Return tube to the magnetic stand, allow for separation and then remove and discard all Wash Buffer.

15. Repeat Steps 13 to 14 two more times for a total of three washes. Be sure that all of the solution has been removed after the last wash.

16. In a biohazard cabinet, place the lids of the tubes upside down on a Kimwipe, in their respective order, and the tubes into a plastic rack, and air-dry the resin for 5-15 minutes at room temperature. Do not dry for more than 20 minutes, as this may inhibit removal of DNA. Once dry, screw on the lids.

17. To each samples then add 50µL of Elution Buffer very gently on the top of the magnetic pellet. Do not mix.

18. Close the lid and then incubate the tubes in the ThermoMixer at 65°C for 3 minutes with no shaking and another 3 minutes shaking at 1100 rpm.

19. Remove the tubes and vortex for 2 seconds at high speed. Immediately place the tube in the magnetic stand. Tubes must remain hot until placed in the magnetic stand or yield will decrease.

20. Carefully transfer the supernatant containing the DNA to the respective labelled Nunc™ tubes.

21. Repeat step 17 to 20, transferring the supernatant to the appropriate Nunc™ tube. The final volume after the second elution should be approximately 95µL.

    Note: DNA can be stored at 4°C for short-term storage or at -20 or -70°C for long-term storage.

5.9 DNA quantitation

All DNA extracts were quantified using the Quantifiler™ Human DNA Quantitation kit (Applied Biosystems, Foster City, CA, USA) as per QIS 19977. Reaction setup was performed on the MultiPROBE® II PLUS HT EX (PerkinElmer) pre-PCR platform.

5.10 PCR amplification

DNA extracts were amplified using the AmpFℓSTR® Profiler Plus® kit (Applied Biosystems, Foster City, CA, USA) as per QIS 19976. Reaction setup was performed on the MultiPROBE® II PLUS HT EX (PerkinElmer) pre-PCR platform.

5.11 Capillary electrophoresis and fragment analysis

PCR product was prepared for capillary electrophoresis using the manual 9+1 protocol (refer to Project 15 and QIS 19978). Capillary electrophoresis was performed on an ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) under the following conditions: 3kV injection voltage, 10 sec injection time, 15kV run voltage, 100µA run current, and 45min run time. Data Collection Software version 1.1 was used to collect raw data from the ABI Prism® 3100 Genetic Analyzer. Fragment size analysis was performed using GeneScan 3.7.1. Allele designation was performed using Genotyper 3.7, with thresholds for heterozygous and homozygous peaks at 150 and 300 RFU respectively. The allelic imbalance threshold is 70%.

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6. Results and Discussion

6.1 Donor sample cell counts

Aliquots of buccal cell samples were counted at Cytology Department (RBWH) to determine the concentration of viable cells, in order to better estimate the number of cells at any particular dilution. A white cell count was not performed on all the blood samples, and therefore an estimate on the number of nucleated cells could not be determined.

6.2 Sensitivity, consistency and yield

To ensure the reliability and integrity of results for samples containing small amounts of DNA, a sensitivity study was conducted to determine the lowest concentration of DNA that provides reliable results. A consistency study was combined into the sensitivity experiment to determine the maximum acceptable difference between the results obtained. All samples were extracted in identical conditions by the same operator at the same time to minimise variability.

The cell sample used for the experiments was from donor sample 4A, which was counted to be around 3,680 nucleated cells (x 10^6/L). The blood sample used was from donor 6A, which was counted to be around 2,540 nucleated cells (x 10^6/L). The estimated amount of DNA present in each dilution is outlined in Table 3.

Table 3. Amount of DNA in each dilution, as calculated from the cell count.

Sample type	Dilution factor	Number of cells (/µL)	gDNA (ng/µL)	Theoretical total DNA on swab (ng)
Cells	Neat	3680	23.552	706.56000
	1/10	368	2.3552	70.65600
	1/100	36.8	0.23552	7.06560
	1/1000	3.68	0.023552	0.07656
Blood	Neat	2540	16.256	487.68000
	1/10	254	1.6256	48.76800
	1/100	25.4	0.16256	4.87680
	1/1000	2.54	0.016256	0.48768

The DNA yields resulted from extracting the above cell dilutions using the DNA IQ™ System is outlined in Table 4. Blood samples produced higher yields compared to cell samples. On average, blood samples on cotton swabs generated the highest yields. Cell samples on rayon and cotton swabs generated similar yields. All blood dilutions down to 1/1000 produced quantitation results, but cell samples only produced reliable quantitation results down to 1/100 dilution, possibly due to the effects of cell clumping.

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Table 4. DNA quantitation data for diluted cell and blood samples on rayon and cotton substrates.

Sample type	Dilution factor	Theoretical Input DNA (ng)	Rayon swab yield (ng)	Alleles	Cotton swab yield (ng)	Alleles	Rayon average yield (ng)	Rayon Std Dev	Recovery Rayon (%)	Cotton average yield (ng)	Cotton Std Dev	Recovery Cotton (%)
Cells	Neat	706.56000	110.0000	18	117.0000	18	134.5400	41 30	19.04	95 2800	32.69	13.48
			130.0000	18	124.0000	18						
			160.0000	18	46.8000	18						
			83.7000	7	76.6000	18						
			189.0000	17	112.0000	18						
	1/10	70.65600	10.1000	18	12.8000	18	10.4520	1.44	14.79	10.4820	2.52	14.84
			12.7000	18	6.3100	18						
			9.5500	18	11.5000	18						
			9.0100	18	10.1000	18						
			10.9000	18	11.7000	18						
	1/100	7.06560	0.6350	0	0.0000	0	0.9254	0.64	13.10	0.1270	0.18	1 80
			0.4930	0	0.0000	0						
			1.4000	5	0.2770	0						
			1.7900	14	0.3580	0						
			0.3090	0	0.0000	0						
	1/1000	0.7656	0.0000	0	0.3630	0	0.0166	0.04	2.17	0.0726	0.16	9.48
			0.0000	0	0.0000	0						
			0.0000	0	0.0000	0						
			0.0831	0	0.0000	0						
			0.0000	0	0.0000	0						
Blood	Neat	487.68000	216.0000	18	718.0000	18	317.0000	102.36	65.00	447.0000	196.46	91.66
			447.0000	18	297.0000	18						
			215.0000	18	595.0000	18						
			383.0000	7	326.0000	18						
			324.0000	18	299.0000	18						
	1/10	48.76800	113.0000	18	126.0000	18	124.7800	28.10	255.86	97.6600	21.66	200.25
			107.0000	18	91.9000	18						
			145.0000	18	75.4000	18						
			95.9000	18	81.0000	18						
			163.0000	18	114.0000	18						
	1/100	4.87680	14.3000	18	15.9000	18	12.4800	1.62	255.91	16.7600	4.69	343.67
			12.5000	13	12.1000	18						
			13.2000	18	20.8000	18						
			9.9000	18	22.4000	18						
			12.5000	18	12.6000	18						
	1/1000	0.48768	0.7300	18	2.3700	18	0.8894	0.20	182.37	3.0200	0.85	619.26
			0.6990	18	3.1300	18						
			1.1800	18	3.6300	18						
			0.8670	18	1.9700	18						
			0.9710	18	4.0000	18						

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The average yield observed within cell and blood samples on either rayon or cotton swabs were comparable (Figure 1). Some inconsistencies were present in cell samples at the lower dilutions of 1/100 and 1/1000 due to unreliable quantitation data at these low dilutions. Blood samples were found to generate higher average yields than cell samples and gave unexpectedly higher recovery values, despite the fact that the input DNA amount was 2-fold higher for cells compared to blood samples (Table 4). This discrepancy may have arisen from inconsistencies in cell suspension uniformity during dilutions of the original cell or blood sample, resulting in inaccurate estimates for average cell concentrations.

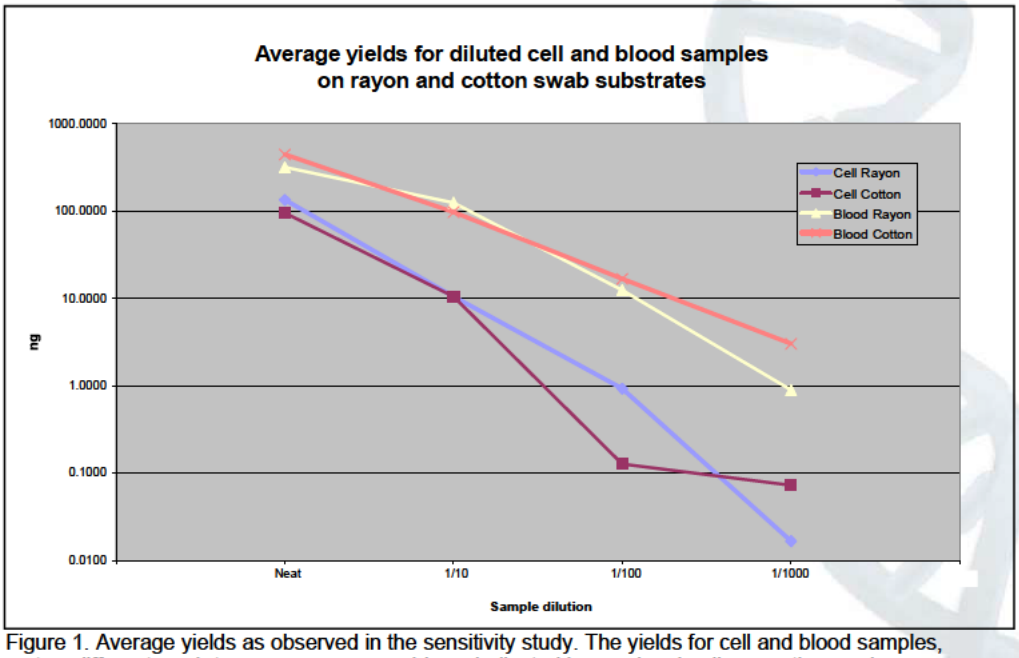

Figure 1. Average yields as observed in the sensitivity study. The yields for cell and blood samples, on two different swab types, were comparable as indicated by overlapping lines on the graph.

The dilution factor was, however, accurately reflected in the average yield for the various dilutions as displayed in Table 4 and Figure 2. An exception to this was the average yields for the neat dilutions (Figure 2). DNA IQ™ isolates a maximum of 100ng DNA as the resin is present in excess, and the system becomes more efficient with samples containing less than 50ng of DNA. Because the amount of DNA was in excess in neat samples, the observed yields varied from sample-to-sample. According to the manufacturer, the DNA IQ™ Database Protocol should be used for samples containing more than 100ng DNA to result in more consistent concentrations between the samples (Huston, 2002).

All five replicates for each neat dilution displayed the highest yields for each dilution series, as expected (Figure 2). For blood samples on rayon and cotton swabs, yields were still around 1ng for samples at the 1/1000 dilution (Figure 3).

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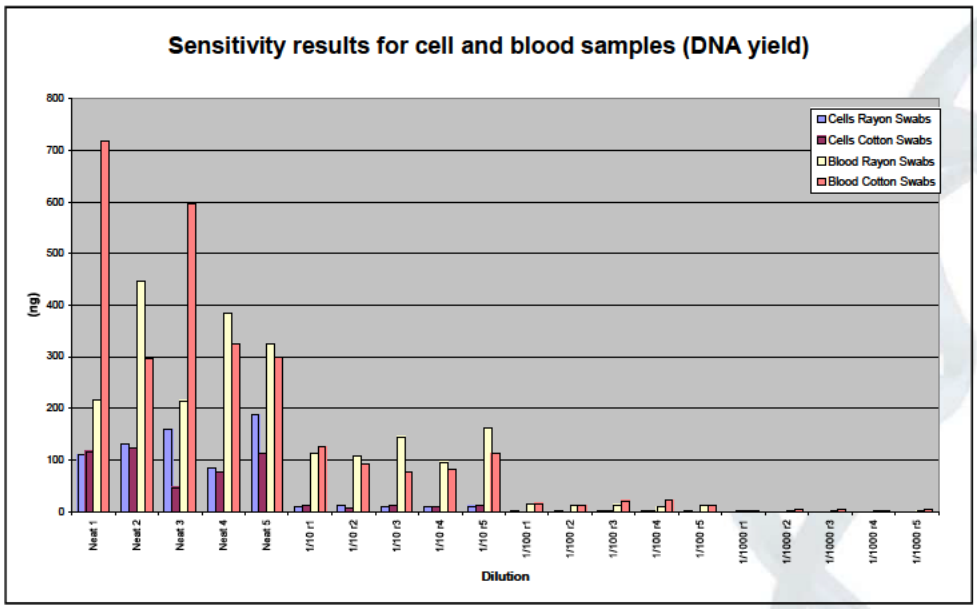

Figure 2. DNA yields (ng) observed for the sensitivity study. As expected, neat samples provided the highest yields. Yields were obtained down to 1/1000 for blood samples and 1/100 for cell samples.

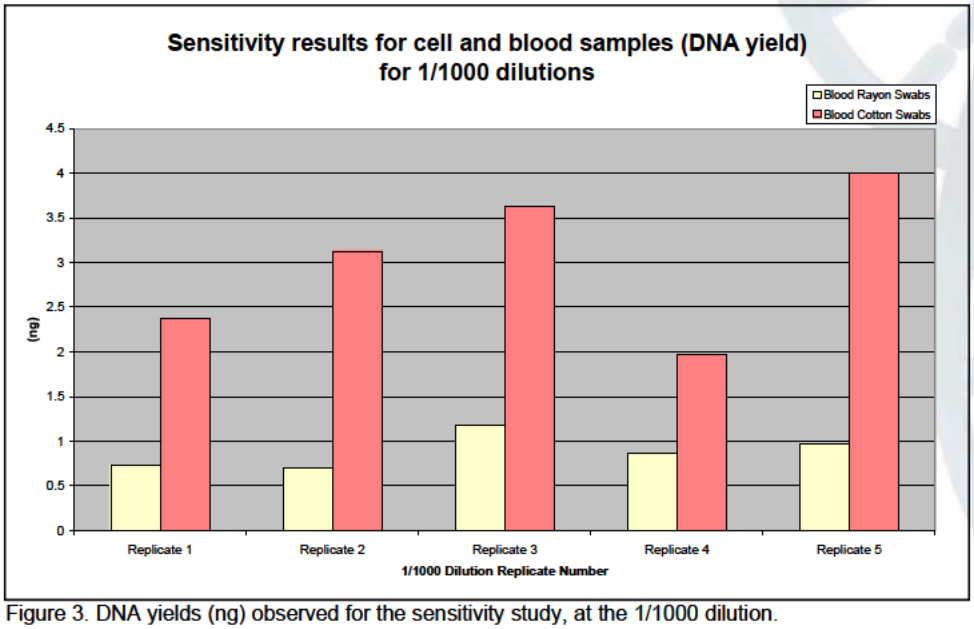

Figure 3. DNA yields (ng) observed for the sensitivity study, at the 1/1000 dilution.

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When amplified using a 9-locus STR system, all neat samples produced the expected full DNA profile (18/18 alleles), although one outlier was encountered for a cell rayon sample which produced a 7/18 partial profile (Table 4). For cell samples, full profiles could be obtained for samples that were diluted down to 1/10, with partial profiles generated from samples diluted to 1/100. For blood samples, full profiles were generally obtained from all dilutions down to 1/1000. Although two partial profiles were encountered in blood samples on rayon swabs, all blood cotton swabs produced full profiles at all dilutions.

The apparent discrepancy between the results for cell and blood samples can be attributed to inaccurate cell counts or non-uniform sample suspensions when creating the dilutions, as caused by cell clumping or cellular breakdown and precipitation.

For five replicates of each dilution, consistency was observed to vary depending on the dilution (Figure 4). Consistency, as an indication of reproducibility, was calculated as the percentage of the yield standard deviation over five replicates divided by the mean yield of all five replicates (%[SD_{yield} / $mean_{yield}$]). A value closer to 0% indicates minimal sample-to-sample variation and therefore the results are highly consistent. The mean combined reproducibility for all neat, 1/10, 1/100 and 1/1000 dilutions were 35.31%, 20.63%, 62.14% and 124.32% respectively (Figure 4), indicating that there was high reproducibility between the neat and 1/10 dilutions across the four sample types, and reduced reproducibility at the lower 1/100 and 1/1000 dilutions. Overall, the blood samples on rayon and cotton both exhibited high reproducibility across all dilutions at an average of 30.54% and 22.45% respectively (Figure 5). The cell rayon and cotton samples were more variable across all dilutions, producing lower reproducibility at an average of 84.23% and 105.19% respectively (Figure 5). The poor performance of the cell samples can be attributed to inconsistencies in quantitation data observed at the lower 1/100 and 1/1000 dilutions.

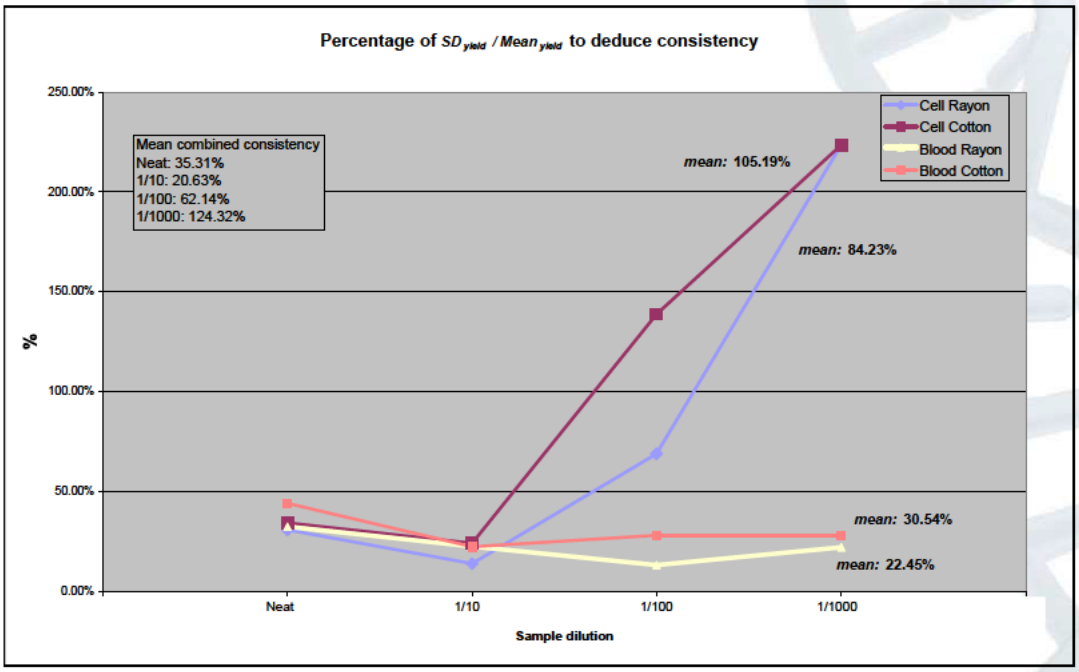

Figure 4. Reproducibility between replicates for cell and blood samples diluted down to 1/1000.

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6.3 Inhibition

Forensic samples that are commonly submitted for DNA analysis often contain inhibitors. These inhibitors may inhibit or significantly reduce the efficiency of a DNA extraction system, either by interfering with cell lysis or interfering by nucleic acid degradation or capture, therefore manifesting as extraction inhibitors (Butler, 2005). Inhibitors can also co-extract with the DNA and inhibit downstream PCR amplification processes, therefore acting as PCR inhibitors (Butler, 2005). For example, inhibitors such as hemoglobin and indigo dye likely bind in the active site of the *Taq* DNA polymerase and prevent its proper functioning during PCR amplification.

For the inhibition study, five substances were chosen for their known ability to inhibit PCR and their likelihood of appearing in routine casework samples:

- Indigo carmine: a component of the blue-dye encountered in denim jeans (Shutler, *et al*., 1999).
- Tannic acid: a chemical used in the leather tanning process.
- Urea: a component of urine (Mahony *et al*., 1998).
- Humic acid: a component found in soil and soil products (Tsai and Olson, 1992).
- Motor oil: contains various hydrocarbons and ethanolic compounds that can inhibit PCR.

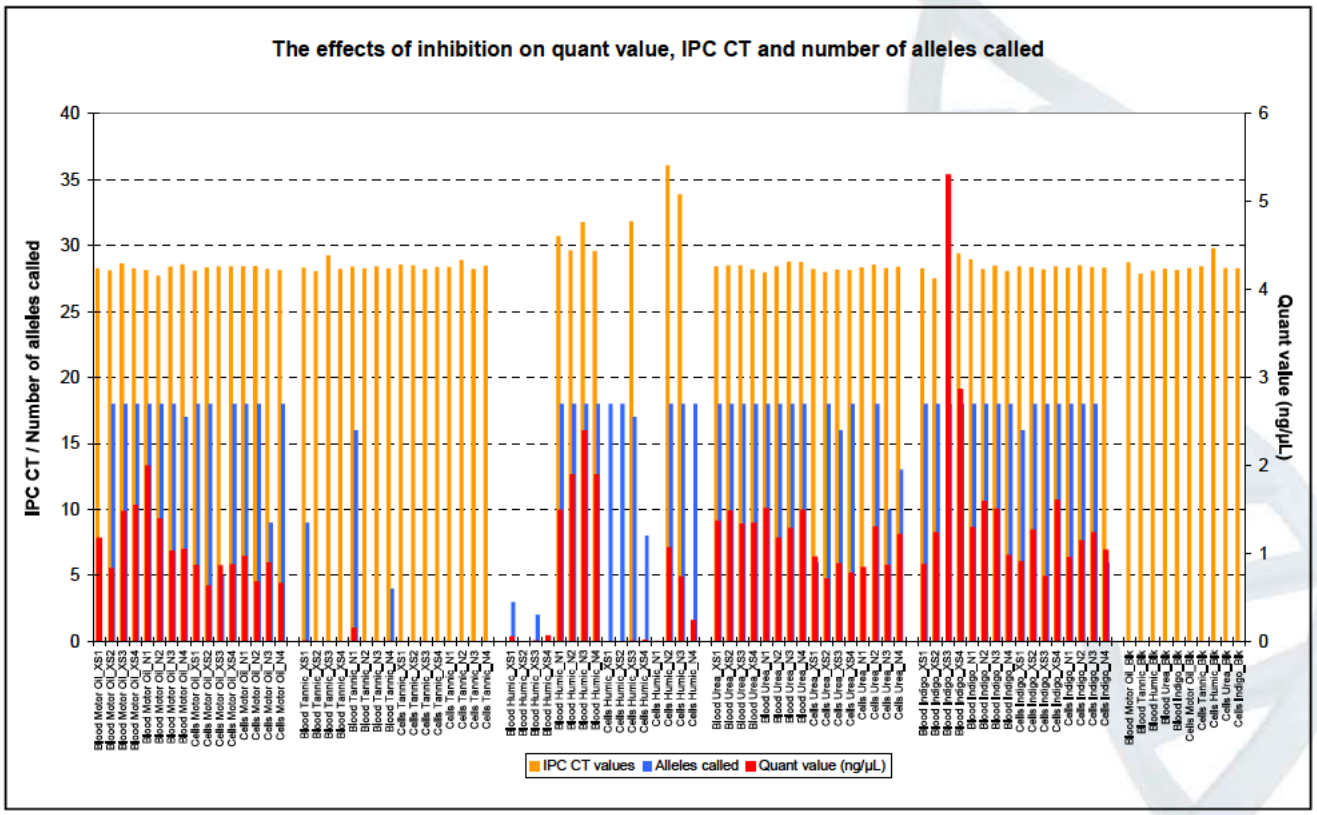

Figure 5. Effects of various inhibitors on quant value, IPC CT and number of resolved alleles.

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The observed effects of these inhibitors at neat and excess concentrations on the ability to extract, quantify and amplify various DNA samples are graphed in Figure 5. Samples were quantified using the Quantifiler™ Human DNA Quantitation Kit (Applied Biosystems) as this kit includes a built-in inhibition detector. Reaction efficiency and the presence of inhibitors can be assessed based on the performance of the internal positive control (IPC), which is known to be detected in this laboratory at around 28 cycles.

The observations that were made include:

- Samples that were spiked with motor oil, urea and indigo carmine dye did not show inhibition as determined by the IPC, and resulted in quantifiable DNA templates after extraction using DNA IQ™. The average DNA concentration observed for all samples was around 1ng/µL. The majority of samples yielded full DNA profiles, with the exception of several cell samples that were treated with urea (both at excess and neat concentrations).
- Blood and cell samples that were spiked with tannic acid did not show inhibition in Quantifiler™, as the IPC performed as expected. However, almost no amplifiable template DNA could be quantified and the majority of samples did not produce DNA profiles. This suggests that the original template DNA was degraded by application of tannic acid to the sample. It should be mentioned at this point that the tannic acid used was in the form of a yellow-brown paste substance that was applied directly to the sample swabs. The tannic acid paste, even at the neat concentration, may have been strong enough to severely fragment DNA to result in non-amplifiable templates. It was observed that three blood samples (1 with tannic acid in excess and 2 with tannic acid at neat concentration) yielded partial profiles (between 4-16 reportable alleles), and none of the cell samples produced reportable alleles. This may be caused by: (1) the concentration of viable cells in the buccal cell samples was lower than blood samples; (2) the drying of the blood stain on the substrate may have created a better barrier to protect the blood components from the degradative effects of the tannic acid.
- Blood and cell samples that were treated with humic acid in excess appeared to retain inhibition after extraction using DNA IQ™. However, at neat concentration, the effect of the humic acid inhibitor was overcome and amplifiable DNA template was purified as demonstrated by high DNA concentration yields. Residual inhibition was still present at neat concentration, as evidenced by higher CT values for the IPC (closer to 30), but full profiles were still produced. For some cell samples with humic acid in excess, the Quantifiler™ data suggested full inhibition (undetermined IPC CT and quantitation results), but two samples resulted in full DNA profiles.
- All reagent blanks were undetermined, indicating the absence of contamination in the results.

The results show that the DNA IQ™ system could be used to extract blood or cell samples that were spiked with motor oil, urea and indigo carmine at both excess and neat concentrations. Blood samples that contained humic acid in excess did not yield amplifiable template DNA, but 2 out of 4 cell samples with humic acid in excess appeared to produce full profiles. Samples that were exposed to tannic acid, at both neat and excess concentrations, resulted in non-amplifiable DNA, but the inhibitor was effectively washed out of the extract by DNA IQ™ as evidenced by the amplification of the IPC at the expected CT. Based on these results, we conclude that the DNA IQ™ system effectively removes inhibitors that are present in the original sample, resulting in a DNA extract that is of sufficient quality and is suitable for PCR amplification.

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6.4 Substrates

The substrate types examined included: swabs (cotton and rayon), tapelifts, fabric (denim, cotton, wool, lycra, nylon, polyester, leather), gum, cigarette butts, and FTA® paper. Cell and blood materials were spotted on to the substrates and extracted using DNA IQ™. The results for the two different sample types are presented in Figures 6 and 7 below.

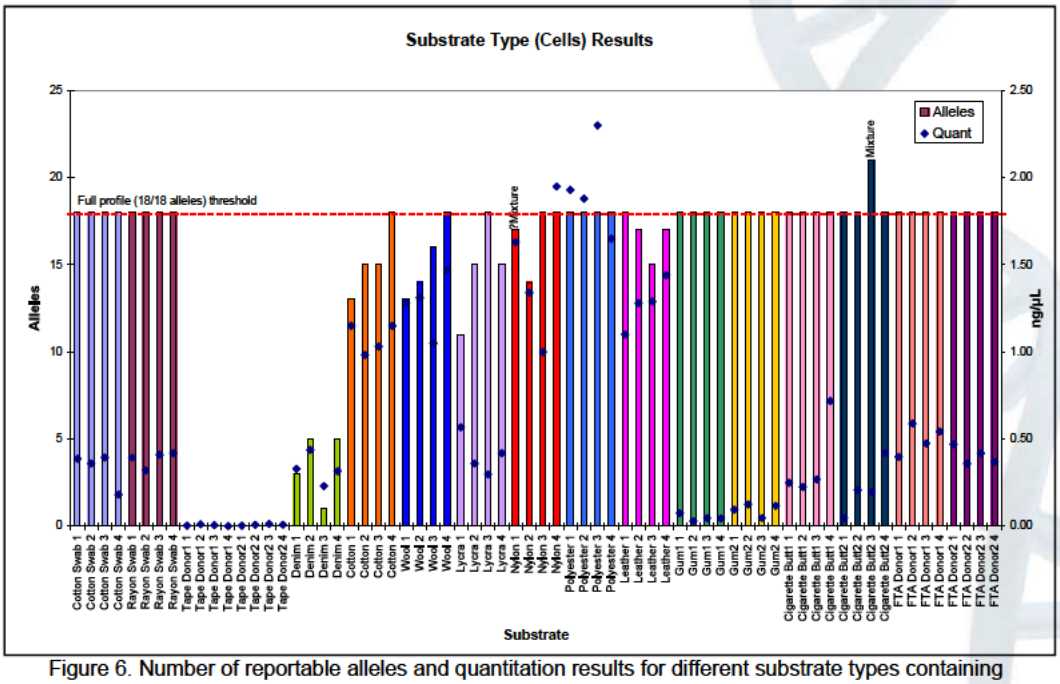

Figure 6. Number of reportable alleles and quantitation results for different substrate types containing cellular material.

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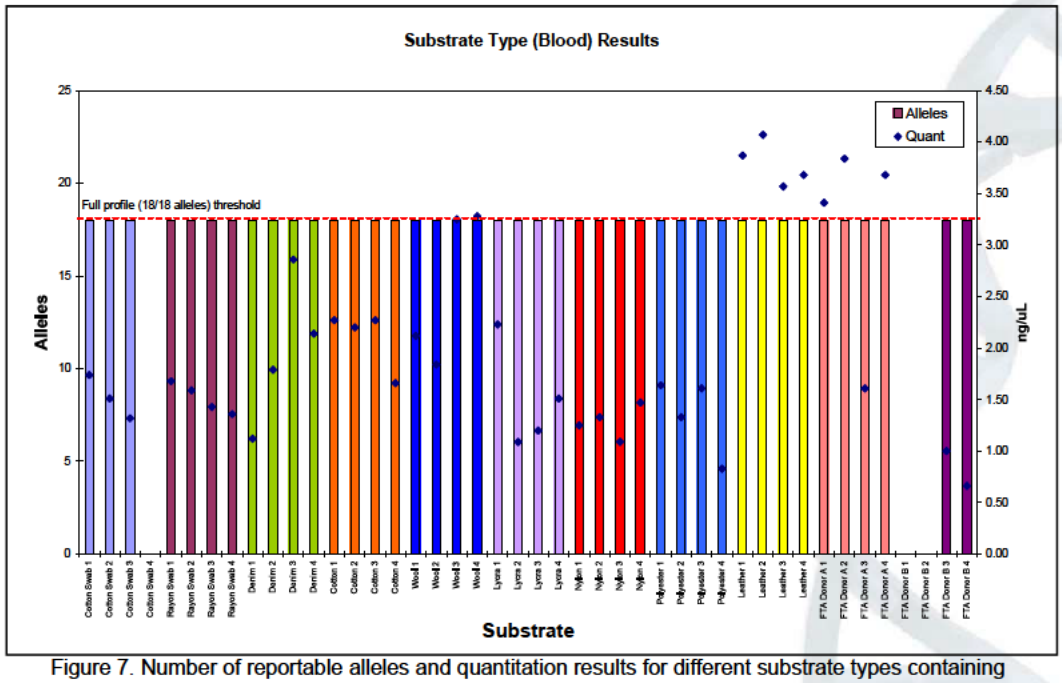

Figure 7. Number of reportable alleles and quantitation results for different substrate types containing blood material.

For cell samples:

- Full DNA profiles (18/18 alleles) were obtained from samples on cotton and rayon swabs, gum, cigarette butts and FTA® paper.
- The quantitation results for most of these samples were less than 0.5ng/µL. For gum samples, the average quantitation result was 0.072ng/µL, and therefore a PCR amplification at maximum volume (20µL) resulted in a total input DNA amount of 1.44ng which is sufficient to result in a full DNA profile.
- Tapelift samples gave an average quantitation result of 0.006ng/µL (just 0.002ng/µL higher than the observed background), and yielded no reportable alleles at all.
- The performance of clothing substrates was variable.
    - Cells on denim yielded quantitation results less than 0.5ng/µL but only partial profiles (maximum 5 reportable alleles), although Quantifiler™ results did not indicate any inhibition of the IPC. The poor performance of these samples may have been a result of sample preparation due to cell clumping.
    - Cells on cotton, wool and nylon resulted in higher quantitation values than lycra, but all substrates generated a similar number of reportable alleles (mean = 14 alleles). Only 25% of samples generated full DNA profiles.
    - **Three out of four samples on polyester produced high quantitation results (~2ng/µL) but all samples resulted in a full profile.**
    - **Cells on leather displayed an average quantitation result of 1.3ng/µL and generated more than 15 reportable alleles.**

For blood samples:

- All substrate types generated full DNA profiles.
- On average, the DNA quantitation results for all blood samples was greater than those resulted from cell samples. This is as per expected and was observed previously (see Project 9 report), because the concentration of nucleated cells in the blood samples were hypothesised to be higher than the concentration of buccal cell samples.
- Because of processing error, data was not available for the following samples: Cotton Swab 4, FTA Donor B 1 and FTA Donor B 2.

The results above are initial amplification results that do not take into account any reworking options.

We found that samples on tapelift substrates performed the worst; however this was probably due to the sampling method devised for this experiment, which did not adequately sample a sufficient number of cells.

6.5 Mixture studies

A mixture study was performed as part of the validation, however the results are not presented in this document because the mixture ratio was found to be inaccurate because cell counts were not performed on the saliva samples. Therefore, little information could be deduced from these results.

6.6 Substrate size

Blood on cotton swabs produced full DNA profiles for all sample sizes, ranging from 0.5 x 0.5cm to 2.0 x 2.0cm (Figure 8). Cells on cotton swabs did not perform as well (Figure 8), possibly due to the nature of the cells and difficulties in obtaining full DNA profiles from cell samples as observed in previous experiments.

Although the same starting amount of sample was used, it was observed that the 0.5 x 0.5cm samples generated higher quantitation results (therefore, also higher yields) compared to the 2.0 x 2.0cm samples (Figure 8). It appears that extraction efficiency decreases as the substrate surface area increases. This may be due to insufficient mixing and distribution of the lysis buffer over a larger substrate surface area, causing insufficient lysis of cellular material. This observation is in line with other reports that the DNA IQ™ system works more efficiently with smaller samples (Promega, 2006). The resulting IPC CT fell within the narrow range of 27.91 – 28.43 (mean = 28.10), indicating that both small and larger samples resulted in DNA extracts of similar quality, but the overall yield was lower for larger substrates (Figure 8 & 9).

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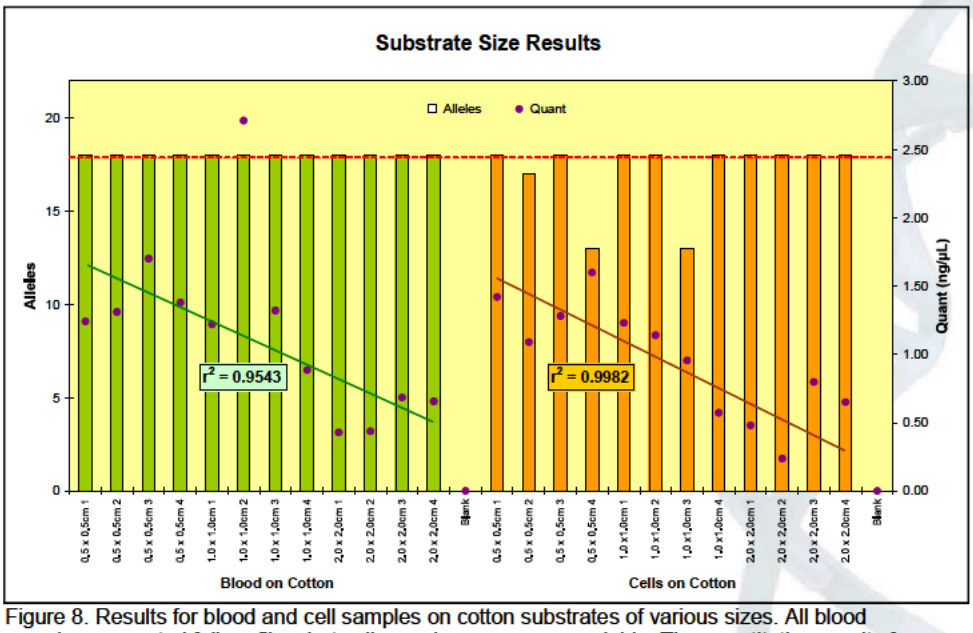

Figure 8. Results for blood and cell samples on cotton substrates of various sizes. All blood samples generated full profiles, but cell samples were more variable. The quantitation results for 0.5 x 0.5cm samples were higher than those for 2.0 x 2.0cm samples (blood $r^2 = 0.9543$*; cell $r^2 = 0.9982$; *Note: an outlier was removed from the calculation).

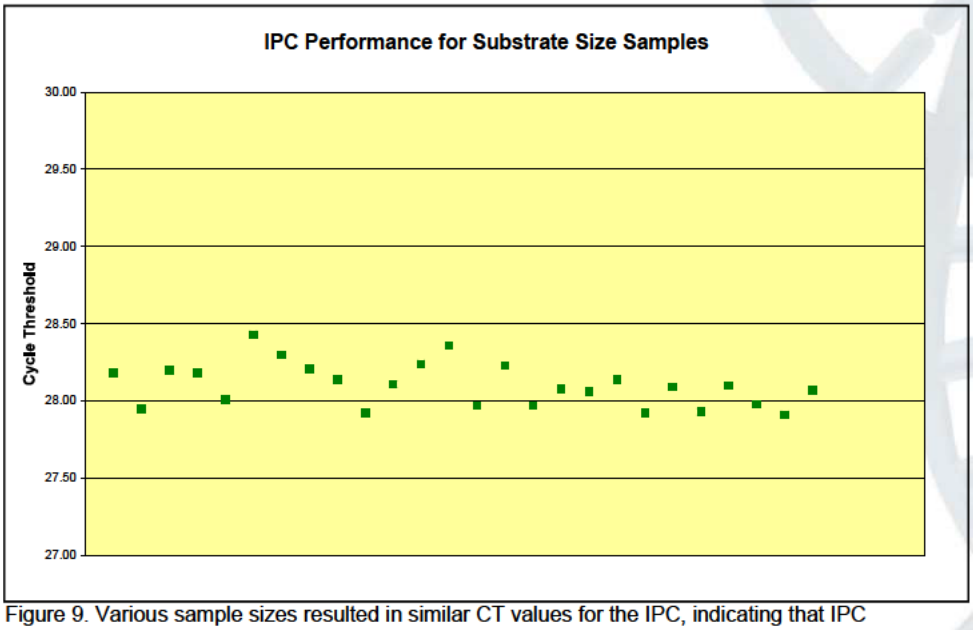

Figure 9. Various sample sizes resulted in similar CT values for the IPC, indicating that IPC performance is not affected by sample size, and that one sample size does not display a level of inhibition that is different to another sample size.

7. Summary and Recommendations

Based on the findings of this validation report, we recommend:

1. To enable processing of cell and blood samples using the validated manual DNA IQ™ protocol, except for samples on tapelift substrates.
2. To design and verify an automated protocol of the validated DNA IQ™ method for use on the MultiPROBE® II PLUS HT EX platforms, for processing blood and cell samples.

8. Acknowledgements

We wish to thank the Cytology Department at the Royal Brisbane and Women's Hospital for assistance with the cell-counting protocols.

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Automated DNA IQ™ Method of Extracting DNA from Blood and Cell Substrates

1 PURPOSE AND SCOPE

This method describes the automated DNA extraction of cell and blood samples on the PerkinElmer MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms using the Promega DNA IQ™ system.

This method applies to all Forensic Biology staff that is required to extract cell and blood samples.

Reference samples and Casework samples must be extracted separately. If Casework and Reference samples are to be extracted on the same instrument, the instrument should be decontaminated between operations.

2 DEFINITIONS

Samples	Samples awaiting DNA extraction
DNA Extracts	Samples that had DNA extraction processes performed
EB	Extraction Buffer Solution that lyses cells and breaks down proteins
LB	Promega DNA IQ™ Lysis Buffer Solution
WB	Promega DNA IQ™ Wash Buffer
DNA IQ™ Resin	Magnetic Resin Beads used to bind DNA
MP II	MultiPROBE® II Platform
DTT	1,4 Dithiothreitol
Pro K	Proteinase K
SDS	Sodium Dodecyl Sulphate
TNE	Tris, NaCl and EDTA buffer
EDTA	Ethylenediaminetetraacetate
EP-A	Extraction Platform A – back wall platform
EP-B	Extraction Platform B – side wall platform

3 PRINCIPLE

Sample Pre-lysis

The Extraction Buffer (EB) used in the pre-lysis of cellular material contains Proteinase K (Pro K), TNE (Tris, NaCl, EDTA) and SDS. TNE acts as a basic buffer with EDTA chelating ions in solution. SDS is a detergent that lyses open cell membranes. Proteinase K is added to digest protein and cellular material that interferes with the DNA binding capacity of the resin. It is also added to rapidly inactivate enzymatic activity that could potentially degrade DNA (e.g.nucleases).

Proteinase K (also known as Endoproteinase K) is a fungal enzyme from *Engyodontium album* (formerly *Tritirachium album*). It is a stable S8 family serine alkaline protease that has broad specificity and is inactivated by diisopropylfluorophosphate, phenyl-methane sulfonyl fluoride and Hg^{2+} ions. As a highly active endopeptidase, it cleaves peptide bonds adjacent/next to the carboxyl group of N-substituted (blocked alpha amino) hydrophobic, aliphatic and aromatic amino acids. Therefore, it actively hydrolyses proteins, glycoproteins, peptides and esters of amino acids. The smallest peptide that can be hydrolysed with Proteinase K is a tetrapeptide.

DNA IQ™ Kit

The DNA IQ ™ kit is designed to purify DNA from a number of different substrates and has been optimised for trace DNA samples. An in house validation was performed using a modified version of the PerkinElmer automated protocol. The protocol has been modified to incorporate a number of work practices used in Forensic Biology FSS. These are:

- The use of the Slicprep™ 96 device (Promega) for removing substrate from lysate.
- The increase of extraction buffer volume to 500µL for use with the Slicprep™ 96 device.
- The increase of Lysis Buffer volume to 957µL proportional to the increase of Extraction Buffer volume, according to the manufacturer's instructions.
- Double Elution step, with an Elution buffer volume of 60µL for a final volume of 100µL.
- The use of NUNC Bank-It tubes for storage of final extracts.

Cell lysis is performed with Promega Lysis Buffer containing Dithiothreitol (DTT). 1,4 Dithiothreitol is a reducing agent used in extractions to break disulfide bonds of proteins. The Lysis Buffer used is a proprietary buffer containing chaotropic salts required for binding of DNA to the magnetic beads. According to the MSDS, the lysis buffer contains 50-75% guanidinium thiocyanate, < 2.5% EDTA, < 2% 3-[(3-Choalamidopropryl)dimethylammonio] propanesulfonic and < 2% polyethylene glycol tert-octylphenyl ether.

The basis of the DNA IQ™ kit is a silica bead resin which contains novel paramagnetic particles. The silica bead resin usually has a DNA binding capacity of 100ng but the addition of Pro K will increase the binding capacity. Samples with small amounts of DNA are more efficient than samples with large amounts of DNA, and as a result a small sample size is critical to ensure efficient recovery of DNA.

The silica beads have a negative charge at basic and near neutral pH. The Lysis Buffer changes the pH and salt concentration of the solution and the silica becomes positively charged which is then able to bind the DNA.

Several washing steps are employed in the protocol to remove inhibitors. The first washing step is performed with Lysis Buffer. This wash ensures the DNA is bound to the resin and washes out inhibitors. The next three washing procedures are with a 1xWash Buffer. This buffer contains an alcohol/aqueous mixture which ensures the DNA is not eluted during washing by keeping the DNA dehydrated, and aqueous phase washes out the inhibitor.

Elution buffer removes the DNA from the magnetic beads. The Elution Buffer changes the salt content. Heating the complex to 65°C allows the DNA to be released from the magnetic beads. The Elution Buffer is a low ionic strength buffer that reduces the affinity of the DNA for the silica by re-hydration of the phosphate backbone.

The DNA IQ™ kit isolates DNA greater than 80bp, smaller DNA is removed selectively to prevent PCR inhibition.

MultiPROBE® II HT EX Plus with Gripper™ Integration Platform

Within Forensic Biology, blood and cell extractions are performed using 2 MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms – one primarily for Reference samples (Extraction Platform A, EP-A) and the other mainly for Casework samples (Extraction Platform B, EP-B).
Each platform uses a computer – controlled Cartesian X-Y-Z liquid handling system designed for the efficient automation of sample preparation. Liquid transfer is performed by an 8-tip System with VersaTip® and VariSpan™ options. The VersaTip® option allows the use of fixed and/or disposable tips (both clear and conductive). The VariSpan™ option permits variable probe spacing between each of the individual probes so that a wide range of labware such as micro plates, tubes, vials and reagent troughs can be accessed. Each sample probe is capable of independent motion in the Z direction due to independent Z drives.

The 8-tip System is also capable of multichannel liquid-level sensing by utilising Accusense™ technology. This technology works by each probe detecting a change in capacitance within the liquid. This capacitive mode of detection is also possible when using conductive disposable tips. Accusense™ also permits the detection of low or non-ionic polar solutions and solvents. Pipetting on the platforms is driven by 8 individually controlled syringes via the positive displacement of the system liquid (nanopure water) when transferring liquid.

The Gripper™ Integration on all the platforms (except for the Post – PCR MPII) allows for automated identification of labware via the scanning of barcodes and also allows for automated transfer of plates from one position on the deck to another. To increase deck space and capacity, all platforms include a left deck extension.

In this program a platemap is utilised to provide the necessary information for correct volumes and locations for pipetting. It also contains information regarding positions where pipetting must take place at various locations, the number and IDs of samples. Without a platemap the program will fail to work.

4 REAGENTS AND EQUIPMENT

4.1 Reagents

1. DNA IQ™ System Kit – 400 sample Kit
    - Resin
    - Lysis Buffer (LB)
    - 2x Wash Buffer (2xWB)
    - Elution Buffer (EB)
2. Tris/Sodium chloride/EDTA Buffer (TNE)
3. Proteinase K (Pro K) 20mg/mL
4. Dithiothreitol (DTT) 1M
5. 5% TriGene
6. 70% Ethanol
7. 1% Amphyl
8. 0.2% Amphyl
9. Isopropyl alcohol
10. Decon® 90 solution
11. Nanopure H_2O

Table 1. Reagent storage locations.

Reagent	Device	Storage Location
Pro K	Freezer	Room 6122
DTT	Freezer	Room 6122
20% SDS	Shelf	Room 6127 Shelf 7
Isopropyl alcohol	Shelf	Room 6127 Shelf 7
TNE pH 8 Buffer	Shelf	Room 6127 Shelf 7
DNA IQ™ Kit	Shelf	Room 6127 Shelf 5
Amphyl (1% and 0.2%)	Shelf	Room 6127 Shelf 7
Nanopure Water	Shelf	Room 6127 Shelf 7
5% TriGene	Shelf	Room 6127 Shelf 7
70% ethanol	Shelf	Room 6127 Shelf 7

Please see Table 2 for the volume of reagents for a full plate or half plate. See QIS 17165 (Receipt, Storage and Preparation of Chemicals, Reagents and Kits) for preparation of the TNE buffer. All reagents, except for the Lysis Buffer with DTT, can be made on the bench in Room 6122. DNA IQ reagents are prepared by staff performing the DNA IQ method.

Table 2. Table of reagent volumes.

		Volume for	
		96 samples	48 samples
Extraction Buffer (500 µL/sample)	TNE buffer 462.5µL	54mL	27mL
	Prot K (20 mg/mL)25.0 µL	2.9mL	1.5mL
	SDS (20 %) 12.5µL	1.5mL	0.7mL
Lysis buffer (with DTT) (1.127mL/sample)	Lysis buffer (no DTT)	130mL	66mL
	DTT (add to Lysis buffer)	1.3mL	660µL
Lysis Buffer (with DTT) Reagent Trough	From above	125mL	63mL
DNA IQ RESIN Sol (50µL/sample)	Lysis buffer (with DTT) (from above) 43µL	6mL	3mL
	DNA IQ RESIN 7µL	1mL	0.5mL
DNA IQ 1X Wash B (300µl/sample)		35mL	18mL
DNA IQ Elution B (120µl/sample)		14mL	8mL

Extraction Buffer

Prepare the buffer just before extraction in a 100mL sterile glass bottle according to the table 2. Use one aliquot of 20mg/mL Proteinase K (1.5mL) for a 48 sample run and two aliquots of Proteinase K for a 96 sample run. Remove aliquot/s of Proteinase K from the freezer, defrost, vortex and centrifuge before use. Ensure that the 20% (v/v) SDS is completely dissolved (clear) in the stock solution before making the extraction buffer. If not dissolved invert the container a few times and leave longer at room temperature.

Lysis Buffer with DTT

Lysis buffer is supplied with the kit. Lysis buffer with DTT is prepared at the start of each run. Remove the DTT from the freezer, defrost, vortex and centrifuge before use. Into a sterilised glass bottle, add 1.3mL of DTT to 130ml of Lysis buffer for 96 samples. If 48 samples are to be run, use 660µl of DTT to 66ml of Lysis buffer, again, made up in a sterile glass bottle. Make up the Lysis buffer with DTT within the Laminar Flow and ensure that full PPE is worn, including face shield. Warning: Lysis Buffer and DTT are toxic.

DNA IQ™ Resin

DNA IQ™ Resin is supplied with the kit. The resin is prepared at the start of each run in a 10mL sterile tube. Ensure the resin is properly mixed by vortexing before pipetting. Look for calculations in table 2 for the correct volumes of resin and lysis buffer (with DTT). In the 10mL tube, mix by inversion before adding to column 4 in the 12-channel reagent plate.

1X Wash buffer

2X Wash buffer is supplied with the kit. Once a new kit has been opened, add 35mL of ethanol and 35mL of isopropyl alcohol to the 2X wash buffer. Once the reagents have been added, label the lid and side of the bottle with "1X Wash buffer," initial and date.

4.2 Equipment

Table 3. Equipment used and location.

Equipment	Asset No.	Location
STORstar (B system)	10238493	6122
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (Ext A Platform)	10076438	6127
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (Ext B Platform)	10076437	6127
DPC Shaker (Ext A Platform)	N/A	6127
DPC Shaker (Ext B Platform)	N/A	6127
Automated Temperature Controller, Heat Block tiles and heat block adapters (Ext A Platform)	N/A	6127
Automated Temperature Controller, Heat Block tiles and heat block adapters (Ext B Platform)	N/A	6127
Eppendorf 5804 centrifuge	10238416	6127
Vortex	30087015	6127
Fridge	30433424	6127
Micro centrifuge	None	6127

5 SAFETY

As per the procedures in the QIS document "*Operational Practices in the DNA Dedicated Laboratories*" (QIS 17120), PPE is to be worn by all staff when performing this procedure.

The automated platforms, labware and associated equipment should be wiped with 5% TriGene™ followed by 70% ethanol before and after use.

While the MPII is running a procedure, refrain from placing any part of the body inside the cabinet. Either pause the program or use the emergency STOP button located at the front of the instrument before touching anything within the cabinet or on the deck surface.

Warning: Tris base, EDTA, SDS and Lysis Buffer are irritants. DTT is a reducing agent that destroys disulphide bonds. Chaotropic reagents such as guanidinium thiocyanate (GuSCN) are toxic. Do not breathe alcohol fumes. Handle carefully and wear appropriate PPE.

Lysis Buffer contains guanidinium thiocyanate which can be harmful if inhaled, swallowed or comes in contact with skin. Any left over Lysis Buffer with DTT is to be disposed of in a brown Winchester bottle. Never dispose down the sink. If spillage occurs, absorb with liquid-binding material (such as sand, kitty litter, etc) and dispose in a biohazard bin. Again, handle carefully and wear appropriate PPE, including face shield.

6 SAMPLING AND SAMPLE PREPARATION

Samples waiting to be extracted are stored in freezers as described in Table 4.

Table 4. Sample storage locations.

Sample type	Storage Device	Storage Location
Urgent/High/Medium Priority Samples	Freezer	6117-2
Medium Priority Samples	Walk in Freezer	6109
Low Priority Samples	N/A	

QC samples

All extraction batches require two controls to be registered. These controls, UR numbers and positions are listed below in Table 5.

Table 5. Extraction Quality Controls

QC Name	UR Number	Description
Negative Control	FBOT33	Negative Extraction control – Empty well
Positive Control	FBOT35	Positive extraction control – Known Donor dried blood swab

Registration of QC

1. Log into the **AUSLAB Main Menu**.
2. Select **1. Request Registration**.
3. Select **2. Full Reception Entry**.
4. Scan in barcode of control.
5. Enter the UR number as per Table 4 and press **[Enter]**.
6. Enter the appropriate Specimen type (e.g. Blood for blood control).
7. Request a **9PLEX** test, when prompted to enter the processing comment, enter **EXTP** (Positive extraction control) or **EXTN** (Negative extraction control).
8. Enter **LAB** in the Billing code field.
9. Press **[F4]** ***Save*** to save the Billing details.
10. Press **[F4]** ***Save*** to save the registration details.

N.B Quality controls should not have a DNA priority allocated at time of registration to ensure they are included in the top positions of a batch

Create the Extraction Batch

1. Log into the **AUSLAB Main Menu**.
2. Select **5. Workflow management**.
3. Select **1. DNA workflow table**.
4. Highlight the appropriate Extraction batch type and press **[F5]** ***Batch Allocation***.
5. Press **[F6]** ***Create batch***.
6. Press **[F8]** ***Print menu***.
7. Press **[F6]** ***Print Batch label***.
8. Press **[F7]** ***Print Plate Label***. (print 3 sets)
9. Press **[F8]** ***Print Worksheet***.
10. Press **[SF5]** ***Main menu***.
11. Press **[SF11]** ***Print***.
12. Press **[SF6]** ***Accept batch***.
13. Press **[Pause/Break]** to exit to the **Main Menu**.
14. Obtain worksheets (FBLASER3) and labels (FBLABEL13-16) from the Analytical Section printing bench (Room 6117).
15. Collect 1 NUNC and 1 STORE labware label from the roll of labels already printed in 6127.

Locating Samples

To locate samples refer to "*Analytical Sample Storage*" (QIS 24255).

Sequence Check the Sample substrates and Nunc Bank-It™ tubes

To sequence check sample substrates and storage tubes please refer to method "*Procedure for the Use of the STORstar unit for automated sequence checking*" (QIS 24256).

7 PROCEDURE

Automated setup of DNA IQ extractions in 96-deep well format is performed using the MultiPROBE® II PLUS HT EX EP-A and EP- B platforms located in Room 6127.

Refer to "*Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform*" (QIS 23939) for instructions on the use and maintenance of the MultiPROBE® II PLUS HT EX platforms.
Competent Analytical Section staff members perform all the following steps.

Figure 1. The Test Online of the program **DNA IQ Extraction_Ver1.1**.

Setting up the EP-A or EP-B MPIIs

These steps are to be carried out in the Automated extraction Room (Room 6127)

1. Turn on the instrument PC.
2. Log onto the network using the ***Robotics*** login
3. Double click the WinPrep® icon on the computer desktop (Figure 1).

Figure 2 The WinPrep® icon.

4. Log onto the WinPrep® software by entering your username and password, then press **[Enter]**.

5. Ensure that the daily/weekly start-up has been performed before running any program. If WinPrep® has been closed or been idle for a long period of time, initialise the MP II platform as described in QIS 23939.

6. Ensure the System Liquid Bottle is full before every run and perform a Flush/Wash.

7. Open the Extraction setup MP II test file in WinPrep® by selecting:
   - **File**
   - **Open**, navigate to **C:\PACKARD\MULTIPROBE\BIN\QHSS PROTOCOLS**
   - Select "**DNA IQ Extraction_Ver1.1.mpt.**"
   - Click the "**Open**" button

8. Decontaminate the required labware by wiping with 5% TriGene followed by 70% ethanol before placing the labware on to the designated grid positions as outlined in the virtual deck view within WinPrep® (Figure 2).
   - The white WALLAC Isoplates (catalogue #1450-514) that are used to support the SlicPrep™ 96 device plate must be placed into positions **E13**, **D16** and **C19**.
   - Ensure that the PKI Magnet at F16 is stable in its insert. This is critical so that beads and DNA are not lost during pipetting.

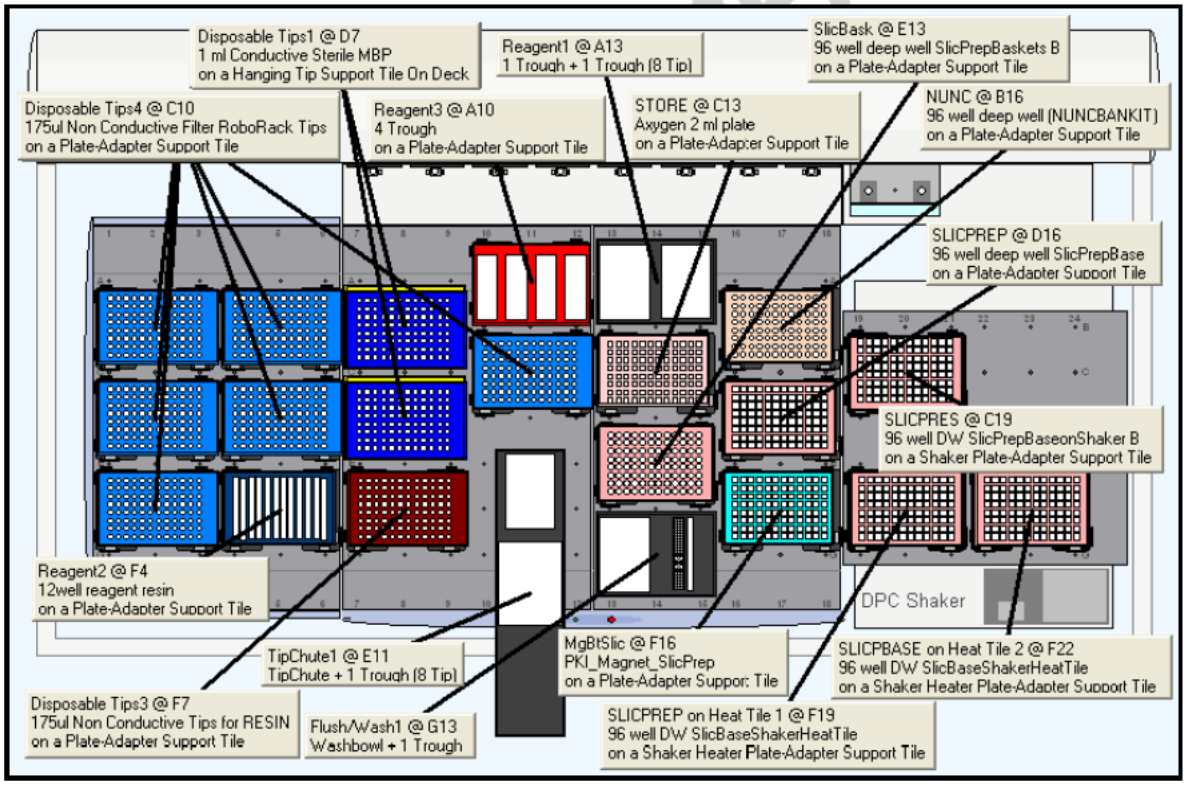


Figure 3. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

9. Ensure that the DPC Shaker and Heater Controller Box are switched on.
   For EP-A: Tile 3 should be at F19 (50^{0}C), Tile 1 at F22 (85^{0}C).
   For EP-B: Tile 1 should be at F19 (50^{0}C), Tile 2 at F22 (85^{0}C).
10. Referring to the table of reagent volumes (table 2), use the volumes of TNE, SDS and Prot K to make up the required amount of Extraction Buffer. Pour the required amounts of Extraction Buffer and Lysis Buffer (with DTT) into the labelled 150mL reagent

troughs, using the reagent volume table as a guide to the volumes. Ensure that full PPE is worn, including face shield when handling these reagents.

11. Place Lysis Buffer on the left hand side of the 2 trough holder (**A13**) and the Extraction buffer on the right hand side next to the Lysis buffer (**A13** also).

12. Using the left over Lysis Buffer, make up the Resin Solution according to table 2. Add the resin solution to the fourth channel and split the amount of elution in half between channels 11 and 12 of the 12 channel reagent plate (**F4**). Ensure that the face shield is worn while making up and dispensing the resin.

13. Record the Lot numbers of all the reagents used onto the AUSLAB worksheet.

14. To the Amphyl wash station at **A10**, add fresh 1% Amphyl to the trough on the far left hand side, 0.2% diluted Amphyl to the middle left and place an empty reagent trough in the middle right position of the station. The nanopure water needed to complete the Amphyl wash goes to position **G13** into a 160ml trough in the Flush-Wash station. Only fill to the designated level markers.

15. If not already done, label the Slicprep™ 96 device, with the AUSLAB Batch ID label with the Batch ID label on the front and the barcode on the right hand side of the plate. Place the Slicprep™ 96 device containing the substrates in the support tile position assigned in the program with the Batch label facing forward. For the Nunc plate and Axygen 2ml deep well Storage plate, label the front of the plate with the Batch ID. On the right hand side of the plates, label with corresponding Labware barcode - either the "NUNC" barcode or the "STORE" barcode, depending upon the type of plate. De-cap the Nunc tubes before placing in the support tile on the deck.

16. Transfer the batch's platemap from the Extraction folder within I:\ drive to the following file path:
    - **C:\PACKARD\EXT PLATE MAPS**

17. After ensuring that all the necessary labware has been positioned in the correct grid position as displayed within WinPrep®, click the **EXECUTE TEST** button. While the test is loading, record all run information in the Run Log book.

18. Click "**Reset Tip Boxes**" and ensure that the number of tips displayed in this window match those as available on the deck platform. Fill new tips if necessary. Click "**Close**" to accept the tip count, followed by clicking "**Next**"

19. Select the correct platemap by browsing to **C:\PACKARD\EXT PLATE MAPS**. Ensure that the plate maps selected match the batch ID affixed to the 96-well Slicprep™ 96 device in position **D16.** Once this has been done, click "**Start**", to continue.

20. After the barcodes have been read, a user prompt will appear as a reminder to:
    "**Ensure**
    1. Shaker and heat box are on.
    2. Deck has been populated correctly.
    3. The Lysis buffer is on the left side and Extraction buffer is on the right at A13."
    Click OK to continue.

21. Once the extraction buffer has been added to the plate, a user prompt will appear requesting the following:

"Cover Slicprep with the Aluminium sealing film, then place in position F19. Press OK."
Once the Slicprep™ 96 device has been covered with an aluminium seal and been placed onto the deck at the correct position, click OK on the user message.

22. After shaking, a User Prompt will appear with the following directions:
"**Remove plate, add white plastic collar and centrifuge 5mins at 3021rpm, then in the cabinet, remove the spin basket part and place it in the empty 1 ml tip container**."
Following the above steps Place the Slicprep™ 96 device into the plate centrifuge and ensure the correct balance plate is used. Once the plate has been centrifuged, carry the plate to the hood and remove the basket of the Slicprep™ 96 device, storing the basket in an empty 1mL tip box, discard the Collar. Complete the step by clicking OK.

23. Once OK has been clicked, another User message (step 15) will appear requesting:
"**Place the Slicprep in position D16. Ensure wash buffer has been added. Press OK when ready**."
Place the Wash buffer in the far right hand side of the 4 well reagent trough holder (Amphyl wash station at **A10**).

24. After the first elute where the plate has been heated to 65^{0}C and moved to the PKI Magnet, a User message (step 79) will appear requesting:
"**Push down the Slicprep on the PKI Magnet then press OK**."
Allow to the plate to cool slightly before pushing down on all four corners of the plate to lock it into place on the PKI Magnet. Once it is firmly in place, click OK to continue. After the second elute, the prompt will appear again. Repeat the steps.

25. Once the program is completed, a final User Message prompt appears asking to:
"**Remove all the plates starting with the NUNC tubes (recap).**
Place the Spin Basket into the original base.
Cover the other plate with the aluminium sealing film."
Remove and seal the Nunc Bank-It tubes first by recapping the tubes. Seal the 2mL storage plate with aluminium foil seal. Remove the Slicprep™ 96 device from the deck and replace the basket on it, make sure the basket part is fitted in the right position. Click "**OK**" to proceed. The platform will perform an Amphyl wash to decontaminate the system tubing.

26. Once the program has finished, remove the tip chute and rinse with warm tap water to help loosen and remove any residue inside the chute before cleaning with 5% TriGene and 70% ethanol. Hang the chute to dry on the rack beside the sink. If running another extraction straight after, replace the tip chute with another spare, clean chute. While wearing the face shield, remove Lysis buffer with DTT and dispose of left over reagent into a brown Winchester bottle.

Recording Reagent Details in AUSLAB

1. To record reagent lot numbers, log into the **AUSLAB Main Menu.**
2. Select **5.Workflow Management**.
3. Select **2. DNA Batch Details**.
4. Scan in the Extraction Batch ID.
5. Press **[F6]** ***Reagents***.
6. Press **[SF8]** ***Audit***.
7. Press **[F5]** ***Insert Audit Entry***, enter the lot number details and press **[Enter]**.

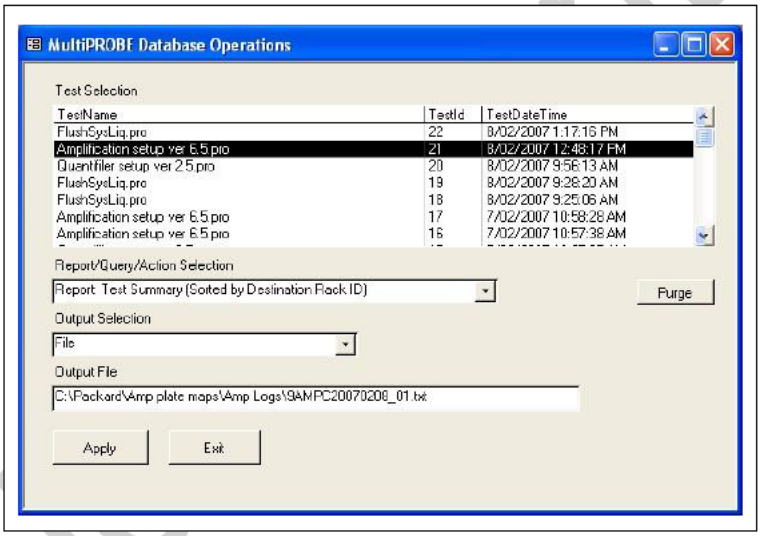
Finalising the MP II run

1. Discard the 12-channel plate used for Resin and Elution Buffer in the biohazard waste bin.
2. Empty the contents of the Amphyl wash station into the sink and rinse the troughs with nanopure H_2O upon completion of the run.
3. Remove all labware from the deck and clean with 5% TriGene™ followed by 70% ethanol, and setup for the next run if necessary.
4. Move the platemap in **C:\PACKARD\EXT PLATE MAPS** to the "Completed Extns" folder.

Importing the MP II log file into AUSLAB

1. To extract the MP II log file, click on the Microsoft Access icon in the WinPrep® main menu to open the MultiPROBE log database. Click on the relevant run Test ID in the Test Selection box. In the Report/Query/Action Selection dropdown menu, select "Report: Test Summary (Sorted by Destination Rack ID)" and in the Output Selection dropdown menu, select "File". Save the output file in *.csv format to **C:\PACKARD\EXT PLATE MAPS\EXT LOGS** with the same name as the AUSLAB batch ID and click "**Apply**".

Figure 4. The MultiPROBE log database for collecting MP II run information.



2. Open the log file and check for any errors that may have arisen during the Extraction process. Compare the listed errors to any that were encountered during the run. Report any critical errors to the line manager.
3. Copy the log file to **I:\EXTRACTION\EXT A MPII\LOGS or I:\ EXTRACTION\EXT B MPII\LOGS** for uploading to AUSLAB.
4. Log into the **AUSLAB Main Menu**.
5. Select **5.Workflow Management**.
6. Select **2. DNA Batch Details**.
7. Scan in the Extraction Batch ID barcode.
8. Press **[SF6]** ***Files***.
9. Press **[SF6]** ***Import Files***.
10. AUSLAB prompts "***Enter filename***"; enter the filename and extension and press **[Enter]**.
11. AUSLAB prompts "***Is this a result file Y/N?***" enter ***N*** and press **[Enter]**.
12. Press **[Esc]**.

Importing Extraction "Results" into AUSLAB

1. Log into the **AUSLAB Main Menu**.
2. Select **5. Workflow Management**.
3. Select **2. DNA Batch Details**.
4. Scan the Extraction batch ID barcode located on the worksheet.
5. Press **[SF6]** ***Files***.
6. Press **[SF6]** ***Import Files***.
7. AUSLAB prompts "***Enter filename***"; enter file name and extension and press **[Enter]**.
8. AUSLAB prompts "***Is this a results file y/n?***"; enter "**y**" and press **[Enter]**.
9. The file will be imported into AUSLAB and appear in the DNA file table.
10. Highlight entry and press **[Enter]**, for access to the DNA results table.
11. Page down through the table and check that all sample results have been imported.
12. Press **[SF8]** ***Table Sort Order***, this sorts the table so any samples that have failed Autovalidation are sorted to the top of the table.
13. Highlight the first entry that has failed and press **[Enter]**.
14. Confirm the reason for the failure by checking the **Processing Comment,** and return the sample to the correct next batch type dependant upon the type of Processing Comment – e.g. Processing comment of Microcon should see the sample returned to the Microcon outstanding allocations list.
15. Press **[Esc]** to exit back to the DNA results table.
16. Repeat steps **13-15** until all entries that failed Autovalidation have been checked.
17. Highlight any entries to be changed and press **[SF7]** ***Toggle Accept***
18. Press **[F7]** ***Complete Batch***, all of the samples with a ***Yes*** in the Accept column will be transferred to the outstanding Quant Casework or Quant Reference lists.
19. File the Extraction worksheet into the relevant folder in Room 6117.

8 SAMPLE STORAGE

Please refer to "*Analytical Sample Storage*" (QIS 24255) for how to store the old original 5 mL sample tubes, the DNA extract NUNC tubes, Slicprep with Basket and Axygen store plates.

9 VALIDATION

- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 9. Automation: Report on the Evaluation of DNA Extraction Chemistries." June 2007.
- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 11: Report on the Validation of the Automated Extraction Chemistry Kit using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Casework Platform." 2007.
- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 13: Report on the Verification of the Automated Extraction Chemistry Kit using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Casework Platform." 2007.

10 QUALITY ASSURANCE/ACCEPTANCE CRITERIA

- A Negative control (also known as the reagent blank) is included with each batch of extractions. This reagent blank is processed as a normal sample through to completion. If any results are obtained from this sample, either at the quantitation step or the Genescan analysis step, then the possible source of the contamination is investigated. The samples extracted with this control are thoroughly checked and repeated if possible.
- Positive and negative controls are included in each extraction batch as per table 4.

11 REFERENCES

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14. Promega, DNA IQ™ System -Small Casework Protocol. Promega Technical Bulletin #TB296 2006. Rev 4/06: p. 1-14.
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18. Vandenberg, N., van Oorchot., R. A. H., & Mitchell, R. J., An evaluation of selected DNA extraction strategies for short tandem repeat typing. Electrophoresis, 1997. 18: p. 1624-1626.

12 STORAGE OF DOCUMENTS

- All worksheets are stored in the Analytical area (Room 6117).

13 ASSOCIATED DOCUMENTS

QIS 17120 Operational Practices in the DNA Dedicated Laboratories

QIS 17171 Method for Chelex Extraction
QIS 17165 Receipt, Storage and Preparation of Chemicals, Reagents and Test Kits
QIS 23939 Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform
QIS 24469 Batch functionality in AUSLAB
QIS 24256 Sequence Checking with the STORstar Instrument
QIS 24255 Analytical Sample Storage

14 AMENDMENT HISTORY

Revision	Date	Author/s	Amendments
0	23 Oct 2007	B. Gallagher, T. Nurthen, C. Iannuzzi, V. Hlinka, G. Lundie, I Muharam.	First Issue

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DNA IQ™ Method of Extracting DNA from Blood and Cell Substrates

1 PURPOSE AND SCOPE

This method describes the routine automated DNA extraction of cell and blood samples on the PerkinElmer MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms using the Promega DNA IQ™ system. The manual method has been included as a back-up method should it be required.

This method applies to all Forensic Biology staff that is required to extract cell and blood samples.

Reference samples and Casework samples must be extracted separately. If Casework and Reference samples are to be extracted on the same instrument, the instrument must be decontaminated between operations.

2 DEFINITIONS

Samples	Samples awaiting DNA extraction
DNA Extracts	Samples that had DNA extraction processes performed
DNA IQ™ Resin	Magnetic Resin Beads used to bind DNA
MP II	MultiPROBE® II Platform
DTT	1,4 Dithiothreitol
Pro K	Proteinase K
SDS	Sodium Dodecyl Sulphate
TNE	Tris, NaCl and EDTA buffer
EDTA	Ethylenediaminetetraacetate
EP-A	Extraction Platform A – back wall platform
EP-B	Extraction Platform B – side wall platform

3 PRINCIPLE

Sample Pre-lysis

The Extraction Buffer used in the pre-lysis of cellular material contains Proteinase K (Pro K), TNE (Tris, NaCl, EDTA) and SDS. TNE acts as a basic buffer with EDTA chelating ions in solution. SDS is a detergent that lyses open cell membranes. Proteinase K is added to digest protein and cellular material that interferes with the DNA binding capacity of the resin. It is also added to rapidly inactivate enzymatic activity that could potentially degrade DNA (e.g.nucleases).

Proteinase K (also known as Endoproteinase K) is a fungal enzyme from *Engyodontium album* (formerly *Tritirachium album*). It is a stable S8 family serine alkaline protease that has broad specificity and is inactivated by diisopropylfluorophosphate, phenyl-methane sulfonyl fluoride and Hg^{2+} ions. As a highly active endopeptidase, it cleaves peptide bonds adjacent/next to the carboxyl group of N-substituted (blocked alpha amino) hydrophobic, aliphatic and aromatic amino acids. Therefore, it actively hydrolyses proteins, glycoproteins, peptides and esters of amino acids. The smallest peptide that can be hydrolysed with Proteinase K is a tetrapeptide.

DNA IQ™ Kit

The DNA IQ ™ kit is designed to purify DNA from a number of different substrates and has been optimised for trace DNA samples. An in house validation was performed using a modified version of the PerkinElmer automated protocol. The protocol has been modified to incorporate a number of work practices used in Forensic Biology FSS. These are:

- The use of the Slicprep™ 96 device (Promega) for removing substrate from lysate.

- The increase of extraction buffer volume to 500µL for use with the Slicprep™ 96 device.
- The increase of Lysis Buffer volume to 957µL proportional to the increase of Extraction Buffer volume, according to the manufacturer's instructions.
- Double Elution step, with an Elution buffer volume of 60µL for a final volume of 100µL.
- The use of NUNC Bank-It tubes for storage of final extracts.

Cell lysis is performed with Promega Lysis Buffer containing Dithiothreitol (DTT). 1,4 Dithiothreitol is a reducing agent used in extractions to break disulfide bonds of proteins. The Lysis Buffer used is a proprietary buffer containing chaotropic salts required for binding of DNA to the magnetic beads. According to the MSDS, the lysis buffer contains 50-75% guanidinium thiocyanate, < 2.5% EDTA, < 2% 3-[(3-Choalamidopropryl)dimethylammonio] propanesulfonic and < 2% polyethylene glycol tert-octylphenyl ether.

The basis of the DNA IQ™ kit is a magnetic bead resin which contains novel paramagnetic particles covered with silica. The magnetic bead resin usually has a DNA binding capacity of 100ng but the addition of Pro K will increase the binding capacity. Samples with small amounts of DNA are more efficient than samples with large amounts of DNA, and as a result a small sample size is critical to ensure efficient recovery of DNA.

The magnetic beads with silica have a negative charge at basic and near neutral pH. The Lysis Buffer changes the pH and salt concentration of the solution and the silica on the magnetic beads becomes positively charged which is then able to bind the DNA.

Several washing steps are employed in the protocol to remove inhibitors. The first washing step is performed with Lysis Buffer. This wash ensures the DNA is bound to the magnetic bead resin and washes out inhibitors. The next three washing procedures are with a 1xWash Buffer. This buffer contains an alcohol/aqueous mixture which ensures the DNA is not eluted during washing by keeping the DNA dehydrated, and aqueous phase washes out the inhibitor.

Elution buffer removes the DNA from the magnetic bead resin. The Elution Buffer changes the salt content. Heating the complex to 65°C allows the DNA to be released from the magnetic beads. The Elution Buffer is a low ionic strength buffer that reduces the affinity of the DNA for the silica that covers the magnetic beads, by re-hydration of the phosphate backbone.

The DNA IQ™ kit isolates DNA greater than 80bp, smaller DNA is removed selectively to prevent PCR inhibition.

MultiPROBE® II HT EX Plus with Gripper™ Integration Platform

Within Forensic Biology, blood and cell extractions are performed using 2 MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms (Extraction Platform A, EP-A) and (Extraction Platform B, EP-B) perform casework or reference samples.
Each platform uses a computer – controlled Cartesian X-Y-Z liquid handling system designed for the efficient automation of sample preparation. Liquid transfer is performed by an 8-tip System with VersaTip® and VariSpan™ options. The VersaTip® option allows the use of fixed and/or disposable tips (both clear and conductive). The VariSpan™ option permits variable probe spacing between each of the individual probes so that a wide range of labware such as micro plates, tubes, vials and reagent troughs can be accessed. Each sample probe is capable of independent motion in the Z direction due to independent Z drives.

The 8-tip System is also capable of multichannel liquid-level sensing by utilising Accusense™ technology. This technology works by each probe detecting a change in capacitance within the liquid. This capacitive mode of detection is also possible when using conductive disposable tips. Accusense™ also permits the detection of low or non-ionic polar solutions and solvents. Pipetting on the platforms is driven by 8 individually controlled syringes via the positive displacement of the system liquid (nanopure water) when transferring liquid.

The Gripper™ Integration on all the platforms (except for the Post – PCR MPII) allows for automated identification of labware via the scanning of barcodes and also allows for automated transfer of plates from one position on the deck to another. To increase deck space and capacity, all platforms include a left deck extension.

In this program a platemap is utilised to provide the necessary information for correct volumes and locations for pipetting. It also contains information regarding positions where pipetting must take place at various locations, the number and IDs of samples. Without a platemap the program will fail to work.

4 REAGENTS AND EQUIPMENT

4.1 Reagents

1. DNA IQ™ System Kit – 400 sample Kit
    - Resin
    - Lysis Buffer (LB)
    - 2x Wash Buffer (2xWB)
    - Elution Buffer (EB)
2. Tris/Sodium chloride/EDTA Buffer (TNE)
3. Proteinase K (Pro K) 20mg/mL
4. Dithiothreitol (DTT) 1M
5. 5% TriGene
6. 70% Ethanol
7. 1% Amphyl
8. 0.2% Amphyl
9. Isopropyl alcohol
10. AnalR 100 %Ethanol
11. 20% SDS
12. Decon® 90 solution
13. Nanopure H_2O

Table 1. Reagent storage locations.

Reagent	Device	Storage Location
Pro K	Freezer	Room 6120
DTT	Freezer	Room 6120
20% SDS	Shelf	Room 6122
Isopropyl alcohol	Shelf	Room 6122
TNE pH 8 Buffer	Shelf	Room 6122
DNA IQ™ Kit	Shelf	Room 6122
Amphyl (1% and 0.2%)	Shelf	Room 6127
Nanopure Water	Shelf	Room 6127
5% TriGene	Shelf	Room 6127
AnalR 100 %Ethanol	Shelf	Room 6127

Please see Table 2 for the volume of reagents for a full plate or half plate. See QIS 17165 (Receipt, Storage and Preparation of Chemicals, Reagents and Kits) for preparation of the TNE buffer. All reagents, except for the Lysis Buffer with DTT (in fume hood), can be made on the bench in Room 6122. DNA IQ reagents are prepared by staff performing the DNA IQ method.

Table 2. Table of reagent volumes.

Reagent (volume per sample)	Constituent (volume per sample)	Volume req'd for 96 Samples (mL)	Volume req'd for 48 Samples (mL)
Extraction Buffer (500 µL/sample	TNE buffer 462.5µL	54	27
	Prot K (20 mg/mL)25.0 µL	2.9	1.5
	SDS (20 %) 12.5µL	1.5	0.7
Lysis buffer (with DTT) (1.127mL/sample)	Lysis buffer (no DTT)	130	66
	DTT (add to Lysis buffer)	1.3	0.66
Lysis Buffer (with DTT) Reagent Trough	As above	125	63
DNA IQ RESIN Solution (50µL/sample)	Lysis buffer (with DTT) (from above) 43µL	6	3
	DNA IQ RESIN 7µL	1	0.5
DNA IQ 1X Wash Buffer (300µl/sample)	See Below for preparation	35	18
DNA IQ Elution Buffer (120µl/sample)	Use directly from Kit	14	8

NOTE: For batches not equal to either 96 or 48 samples samples, refer to Appendix Reagents Calculation Tables. Table 1 for batches of <48 samples and Table 2 for <96 (but >48)

Extraction Buffer

Prepare the buffer just before extraction in a 100mL sterile glass bottle according to the table 2. Use one aliquot of 20mg/mL Proteinase K (1.5mL) for a 48 sample run and two aliquots of Proteinase K for a 96 sample run. Remove aliquot/s of Proteinase K from the freezer, defrost, vortex and centrifuge before use. Ensure that the 20% (v/v) SDS is completely dissolved (clear) in the stock solution before making the extraction buffer. If not dissolved invert the container a few times and leave longer at room temperature.

Lysis Buffer with DTT

Lysis buffer is supplied with the kit. Lysis buffer with DTT is prepared at the start of each run. Remove the DTT from the freezer, defrost, vortex and centrifuge before use. Into a sterilised glass bottle, add 1.3mL of DTT to 130ml of Lysis buffer for 96 samples. If 48 samples are to be run, use 660µl of DTT to 66ml of Lysis buffer, again, made up in a sterile glass bottle. Make up the Lysis buffer with DTT within the Laminar Flow and ensure that full PPE is worn, including face shield. Warning: Lysis Buffer and DTT are toxic.

DNA IQ™ Resin

DNA IQ™ Resin is supplied with the kit. The resin is prepared at the start of each run in a 10mL sterile tube. Ensure the resin is properly mixed by vortexing before pipetting. Look for calculations in table 2 for the correct volumes of resin and lysis buffer (with DTT). In the 10mL tube, mix by inversion before adding to column 4 in the 12-channel reagent plate.

1X Wash buffer

2X Wash buffer is supplied with the kit. Once a new kit has been opened, add 35mL of ***AnalR*** Ethanol and 35mL of Isopropyl alcohol to the 2X wash buffer. Once the reagents have been added, label the lid and side of the bottle with "1X Wash buffer," initial and date.

4.2 Equipment

The following equipment (Table 3) and consumables (Table 4) are required for the DNA IQ extraction.

Table 3. Equipment used and location.

Equipment	Asset No.	Location
STORstar (B system)	10238493	6122
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (Ext A Platform)	10076438	6127
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (Ext B Platform)	10076437	6127
DPC Shaker (Ext A Platform)	N/A	6127
DPC Shaker (Ext B Platform)	N/A	6127
Automated Temperature Controller, Heat Block tiles and heat block adapters (Ext A Platform)	N/A	6127
Automated Temperature Controller, Heat Block tiles and heat block adapters (Ext B Platform)	N/A	6127
Eppendorf 5804 centrifuge	10238416	6127
Vortex	30087015	6127
Fridge	30433424	6127
Micro centrifuge	None	6127
Decapper	None	6127

Table 4. Consumables used for extraction

Consumables	Location
175uL Clear Non-Conductive Filter Tips – Pre-sterilized	6127
MβP Pure 1000uL Tips – Pre-Sterilized	6127
SlicPrep™ 96 device plate	6122
Axygen 2mL Deep Well storage plate	6127
12 Channel plate	6127
Nunc tubes	6120
Nunc Caps	6127
Sterile 50mL Falcon tubes	6122
Sterile 10mL tubes	6122
Autoclaved 100mL glass bottles	6122
Autoclaved 250mL glass bottles	6122
Aluminium sealing film	6127

5 SAFETY

As per the procedures in the QIS document "*Operational Practices in the DNA Dedicated Laboratories*" (QIS 17120), PPE is to be worn by all staff when performing this procedure.

The automated platforms, labware and associated equipment should be wiped with 5% TriGene™ followed by 70% ethanol before and after use.

While the MPII is running a procedure, refrain from placing any part of the body inside the cabinet. Either pause the program or use the emergency STOP button located at the front of the instrument before touching anything within the cabinet or on the deck surface.

Warning: Tris base, EDTA, SDS and Lysis Buffer are irritants. DTT is a reducing agent that destroys disulphide bonds. Chaotropic reagents such as guanidinium thiocyanate (GuSCN) are toxic. Do not breathe alcohol fumes. Handle carefully and wear appropriate PPE.

Lysis Buffer contains guanidinium thiocyanate which can be harmful if inhaled, swallowed or comes in contact with skin. Any left over Lysis Buffer with DTT is to be disposed of in a brown Winchester bottle in the fume hood. Never dispose down the sink. If spillage occurs, absorb with liquid-binding material (such as sand, kitty litter, etc) and dispose in a biohazard bin. Again, handle carefully and wear appropriate PPE, including face shield.

6 SAMPLING AND SAMPLE PREPARATION

Samples waiting to be extracted are stored in freezers as described in Table 5.

Table 5. Sample storage locations.

Sample type	Storage Device	Storage Location
Urgent/High/Medium Priority Samples	Freezer	6117-2
Medium Priority Samples	Walk in Freezer or Freezer in 6117	6109 or 6117-5
Low Priority Samples	Walk in Freezer or Freezer in 6117	6109 or 6117-5

QC samples

All extraction batches require two controls to be registered. These controls, UR numbers and positions are listed in Table 6.

Table 6. Extraction Quality Controls

QC Name	UR Number	Description
Negative Control	FBOT33	Negative Extraction control – Empty well
Positive Control	FBOT35	Positive extraction control – Known Donor dried blood swab

Registration of QC

1. Log into the **AUSLAB Main Menu**.
2. Select **1. Request Registration**.
3. Select **2. Full Reception Entry**.
4. Scan in barcode of control.
5. Enter the UR number as per Table 4 and press **[Enter]**.
6. Enter the appropriate Specimen type (e.g. Blood for blood control).
7. Request a **9PLEX** test, when prompted to enter the processing comment, enter **EXTP** (Positive extraction control) or **EXTN** (Negative extraction control).
8. Enter **LAB** in the Billing code field.
9. Press **[F7]** ***Save*** to save the Billing details.
10. Press **[F4]** ***Save twice*** to save the registration details.

N.B Quality controls should not have a DNA priority allocated at time of registration to ensure they are included in the top positions of a batch

Create the Extraction Batch

1. Log into the **AUSLAB Main Menu**.
2. Select **5. Workflow management**.
3. Select **1. DNA workflow table**.
4. Highlight the appropriate Extraction batch type and press **[F5]** ***Batch Allocation***.
5. Press **[F6]** ***Create batch***.
6. Press **[F8]** ***Print menu***.
7. Press **[F6]** ***Print Batch label***. (print 7)
8. Press **[F7]** ***Print Sample Label***. (print 3 sets)
9. Press **[F8]** ***Print Worksheet***. (print 2)
10. Press **[SF5]** ***Main menu***.
11. Press **[SF11]** ***Print***.
12. Press **[SF6]** ***Accept batch***.
13. Press **[Pause/Break]** to exit to the **Main Menu**.
14. Obtain worksheets **(FBLASER3)** and labels **(FBLABEL13-16)** from the Analytical Section printing bench **(Room 6117)**.

Locating Samples

To locate samples refer to "*Analytical Sample Storage*" (QIS 24255).

Checking Samples

Check that appropriately sized portions of sample (eg swab, fabric, cigarette butts) have been submitted. If samples are not sized correctly they are to be sub-sampled please refer to *"Examination of Items"* (QIS 17142)
Label 1.5mL tubes removed from inside the original 5mL tube with sample labels if required. Label empty Nunc tubes ready for sequence checking.

Sequence Check the Sample substrates and Nunc Bank-It™ tubes

To sequence check sample substrates and storage tubes please refer to method "*Procedure for the Use of the STORstar unit for automated sequence checking*" (QIS 24256).

ENSURE the Slicprep™ 96 device is labelled, with the AUSLAB Batch ID label on the left side of the plate and the barcode on the right hand side of the plate.

ENSURE the Nunc tube rack is labelled with the AUSLAB Batch ID and barcode on the front of the plate.

7 PROCEDURE

Automated setup of DNA IQ extractions in 96-deep well format is performed using the MultiPROBE® II PLUS HT EX EP-A and EP- B platforms located in Room 6127.

Refer to "Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform" (QIS 23939) for instructions on the use and maintenance of the MultiPROBE® II PLUS HT EX platforms.

Summary of DNA IQ EXTRACTION winprep program (v 1.3)

1. ***Lysis of the biological material on solid support:*** Add prepared Extraction Buffer (500µL) to Slicprep plate wells. Cover the Slicprep plate & Spin baskets with Aluminium seal and incubate 45 min @ 37 °C. (this occurs at steps 8-12 of the protocol)

2. ***Remove the Slicprep plate & Spin baskets:*** add the collar and centrifuge for 2 min. Remove the collar and discard it. Remove the Spin baskets part and keep it in a clean container. Return the Slicprep plate to the deck. (this occurs at step 14 of the protocol)

3. ***Binding of paramagnetic resin to DNA and further Lysis:*** add Resin solution (50µL) and Lysis Buffer (957µL). Automated mixing and shaking @ room temperature for 5 min. (this occurs at steps 17-22 of the protocol)

4. ***Removing lysis reagents:*** Slicprep plate is moved to the PKI Magnet to separate beads. Removing of supernatant (1600µL) without disturbing resin, dispense this solution in the storage plate. (this occurs at steps 25-27 of the protocol)

5. ***Washing of the resin-DNA complex:*** To remove any inhibitors in solution.
The first wash is with Lysis buffer (125µL), shaking @ room temperature for 1 min. The plate is moved to the PKI Magnet and the supernatant is removed into the storage plate.

The next three washes are with 1X Wash buffer (100µL), shaking @ room temperature for 1 min. During each wash cycle, the plate is moved to the PKI Magnet and the supernatant is discarded. (this occurs at steps 30-68 of the protocol)

6. ***Removing any excess of 1X Wash buffer:*** air dry @ room temperature for 5 min. (this occurs at step 69 of the protocol)

7. ***Elution of DNA from the Resin-DNA complex:*** Add Elution buffer (60µL) and incubate @65 °C for 6 minutes (3 min no shaking and 3 min shaking). The plate is moved to the PKI Magnet. The eluted solution (supernatant) is removed to the NUNC tubes. Step **7** is repeated twice. (this occurs at steps 71-92 of the protocol)

8. ***Flushing of capillaries:*** The capillaries are washed with Amphyl and nanopure water.

Preparation of Reagents prior to extraction

1. Defrost Prot K and DTT

2. Refer to table 2 for reagent volumes to make up the required amount of Extraction Buffer, Lysis buffer (with DTT) and Resin solution. Also measure the required amount of 1X Wash buffer.

3. Record the Lot numbers of all the reagents used onto the AUSLAB worksheet.

Setting up the EP-A or EP-B MPIIs

These steps are to be carried out in the Automated extraction Room (Room 6127)

4. Turn on the instrument PC.

5. Log onto the network using the **Robotics** login.

6. Double click the WinPrep® icon on the computer desktop (Figure 1).

Figure 1 The WinPrep® icon.

7. Log onto the WinPrep® software by entering your username and password, then press **"Enter"**.

8. Ensure the **System Liquid Bottle is FULL** before every run and perform a Flush/Wash.

9. Ensure that the daily/weekly start-up has been performed before running any program. If WinPrep® has been closed or been idle for a long period of time initialise the MP II platform as described in QIS 23939.

10. Open the Extraction setup MP II test file in WinPrep® by selecting:
    - **File**
    - **Open**, navigate to **C:\PACKARD\MULTIPROBE\BIN\QHSS PROTOCOLS**
    - Select "**DNA IQ Extraction_Ver1.3.mpt.**"
    - Click the "**Open**" button

11. Check the whole program there are no bold fonts (e.g. plates may lose gripability and the program will not run). See the line manager.

12. Decontaminate the required labware by wiping with 5% TriGene followed by 70% ethanol before placing the labware on to the designated grid positions as outlined in the virtual deck view within WinPrep® (Figure 2).

- The white WALLAC Isoplates (catalogue #1450-514) that are used to support the SlicPrep™ 96 device plate must be placed into positions **E13**, **D16** and **C19**.

- Ensure that the PKI Magnet at **F16** is stable in its insert. This is critical so that beads and DNA are not lost during pipetting.

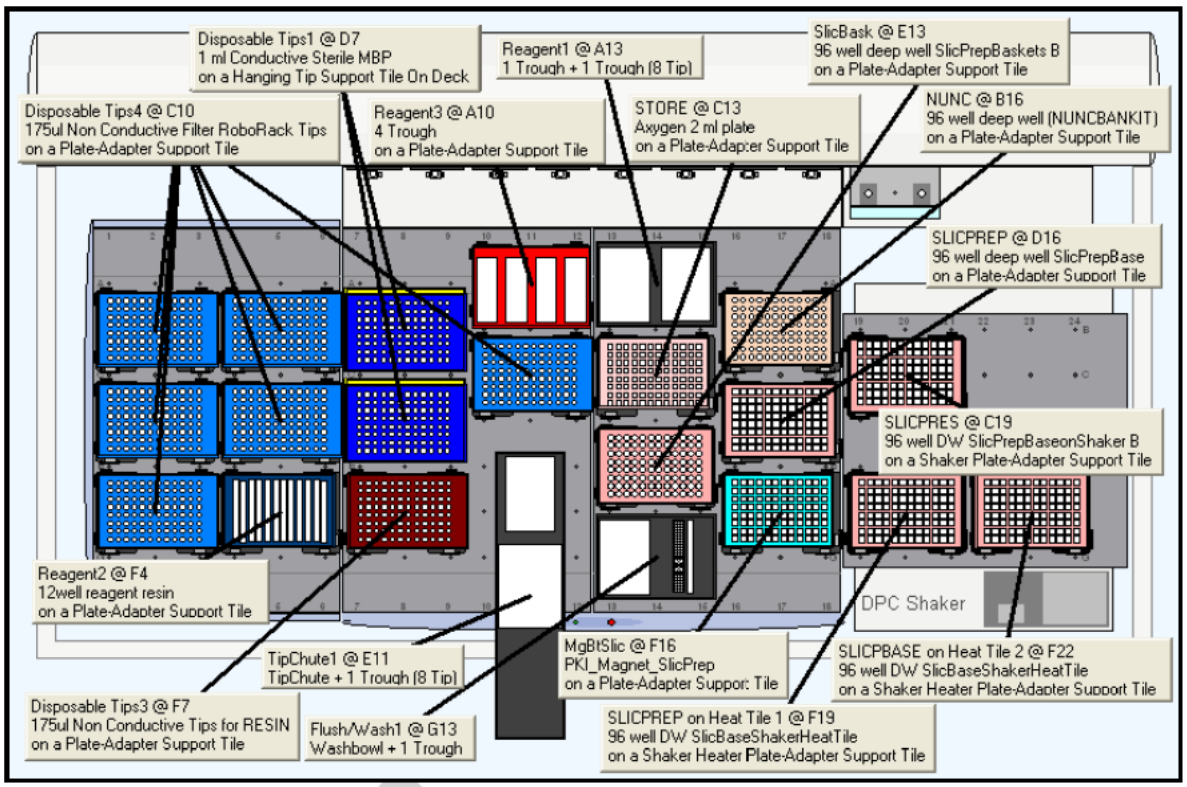


Figure 2. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

13. Ensure that the DPC Shaker and Heater Controller Box are switched on.
    For EP-A: Tile 3 should be at F19 (50^{0}C), Tile 1 at F22 (85^{0}C).
    For EP-B: Tile 1 should be at F19 (50^{0}C), Tile 2 at F22 (85^{0}C).
    Note: Press the start/stop button twice at the front of the DPC Shaker to ensure that it displays zero on the screen.

14. To the Amphyl wash station at **A10**, add fresh 1% Amphyl to the trough on the far left hand side, 0.2% diluted Amphyl to the middle left and place an empty reagent tough in the middle right position of the station. The nanopure water goes to position **G13** into a 160mL trough in the Flush-Wash station.

15. Pour the required amounts of Extraction Buffer and Lysis Buffer into the labelled 150mL reagent troughs. Place Lysis Buffer on the left hand side and the Extraction buffer on the right hand side of the 2 trough holder located in position **A13**.
    Note: Ensure that full PPE is worn, including face shield when handling these reagents

16. **Nunc tube rack**: Check that is the same Auslab batch number as the platemap to be run. Ensure that the barcode of the first (A1) and last tube (H12) are the respective ones. Attach batch label and batch barcode label to front side of rack. Add B1-Lite

generated "**NUNC**" barcode to the right side of the nunc tube rack. Then place nunc rack into position **B16**

17. On an Axygen 2ml deep well Storage plate, label the left side of the plate with both the Batch ID and barcode. Label the right side of the plate with a B1-Lite generated "**STORE**" barcode. Then place in position **C13**.

18. **Slicprep™ 96 device**: Gently remove septa mat from Slicprep™ 96 device and check that substrates are at the bottom of the Spin baskets, if not push them down with a sterile disposable tip and place the Slicprep™ 96 device into position **E13**.

19. In I drive from Extraction folder open the required plate map. Check that the plate map is complete. If there are blanks (samples that have been removed), make all volumes to 0. If it is not a full batch, delete the rows of blank samples at the end of the platemap, then save the changes. Copy the plate map to the following file path: **C:\PACKARD\EXT PLATE MAPS**

20. After ensuring that all the necessary labware has been positioned in the correct grid position as displayed within WinPrep®, click the "**EXECUTE TEST**" button. While the test is loading, record all run information in the Run Log book.

21. Click "**Reset Tip Boxes**" and ensure that the number of tips displayed in this window match those as available on the deck platform. Fill new tips if necessary. Click "**Close**" to accept the tip count, followed by clicking "**Next**"

22. Select the correct platemap by browsing to **C:\PACKARD\EXT PLATE MAPS**. Ensure that the platemap selected matches the batch ID affixed to the 96-well Slicprep™ 96 device in position **D16.** Once this has been done, click "**Start**", to continue.

23. After the barcodes have been read, a user prompt will appear as a reminder to:
    "**Ensure**
    1. Shaker and heat box are on.
    2. Deck has been populated correctly.
    3. The Lysis buffer is on the left side and Extraction buffer is on the right at A13."
    Click "**OK**" to continue.

24. Once the extraction buffer has been added to the plate, a message will appear waiting for the heating tile to reach 50^0C (real temp 37^0C). When current temperature reaches 50^0C click "**Continue**".

25. The next prompt that appears will request the following:
    "Cover Slicprep with the Aluminium sealing film, then place in position F19. Press "OK."

26. After shaking, a User Prompt will appear with the following directions:
    "**Remove plate, add white plastic collar and centrifuge 5mins at 3021rpm, then in the cabinet, remove the spin basket part and place it in the empty 1 ml tip container**."
    Place the Slicprep™ 96 device into the plate centrifuge and ensure the correct balance plate is used. Once the plate has been centrifuged, carry the plate to the hood and remove the basket of the Slicprep™ 96 device, storing the basket in an empty 1mL tip box, discard the Collar. Complete the step by clicking "**OK**".
27. Add the Resin to the 12 Channel plate. Pipette mix thoroughly. Then dispense 50uL of resin into each well of the Slicprep™ 96 device.

28. Place the 12 channel plate into position **F4** then add the Elution buffer to the plate by splitting the amount of elution buffer in half between channels 11 and 12.

29. Place the Wash buffer in the far right hand side of the 4 well reagent trough holder (Amphyl wash station at **A10)**

30. The next User prompt will appear with the following directions:
"**Place the Slicprep in position D16. Ensure wash buffer has been added. Manually add 50uL of Resin. Ensure Elution Buffer has been added."** Press **"OK"** when steps 23-25 have been performed.

31. Once the lysis and wash steps have been completed, a message will appear waiting for the heating tile to reach 85^0C (real temp 65^0C). **DO NOT PRESS CONTINUE** it will continue automatically when temperature has reached 85^0C.

32. After the first elution step when the plate is moved to the PKI Magnet, a User message will appear requesting:
"**Check Nunc tubes are uncapped at position B16**
Push down the Slicprep on the PKI Magnet then press OK."
Note: Allow the plate to cool slightly before pushing down on all four corners of the plate to lock it into place on the PKI Magnet.

33. After the second elution step, the above prompt will appear again. **Note:** Allow the plate to cool slightly before pushing down on all four corners of the plate to lock it into place on the PKI Magnet

34. Once the program is completed, a final User Message prompt appears asking to:
"**Remove all the plates starting with the Slicprep plate, place the Spin Basket into the Slicprep plate.**
Cover the Storage plate with the aluminium sealing film."
Recap the NUNC tubes
Once all plates are removed from the deck and sealed, place into small clipseal plastic bags. Click "**OK**" to proceed to the Amphyl wash step to decontaminate the system tubing.

Finalising the MP II run

35. Discard the 12-channel plate used for Resin and Elution Buffer in the biohazard waste bin.
36. Remove Lysis buffer with DTT (wear face shield) and dispose of left over reagent into the glass bottle used previously. Bring this bottle to the room 6122 and dispose in the brown Winchester bottle located in the fume hood.
37. Empty the contents of the Amphyl wash station into the sink and rinse the troughs with nanopure H_2O upon completion of the run.
38. Remove all labware from the deck and clean with 5% TriGene™ followed by 70% ethanol, and setup for the next run if necessary.
39. Remove the tip chute and funnel, rinse with warm tap water to help loosen and remove any residue inside the chute before cleaning with 5% TriGene and 70% ethanol. Hang the chute to dry on the rack beside the sink. If running another extraction straight after, replace the tip chute with another spare, clean chute.
40. Move the platemap to **C:\PACKARD\EXT PLATE MAPS** to the "**Completed Extractions**" folder.

Recording Reagent Details and other information in AUSLAB

41. To record reagent lot numbers, log into the **AUSLAB Main Menu.**
42. Select **5.Workflow Management**.
43. Select **2. DNA Batch Details**.
44. Scan in the Extraction Batch ID.
45. Press **[F6]** ***Reagents***.
46. Press **[SF8]** ***Audit***.
47. Press **[F5]** ***Insert Audit Entry***, enter the lot number details, operator name and Extraction platform the batch was run on and press **[Enter]**.

Importing the MP II log file into AUSLAB

48. To extract the MP II log file, click on the Microsoft Access icon in the WinPrep® main menu to open the MultiPROBE log database.
49. Click on the relevant run Test ID in the Test Selection box. In the Report/Query/Action Selection dropdown menu, select "Report: Test Summary (Sorted by Destination Rack ID)"
50. In the Output Selection dropdown menu, select "File". Save the output file as *.csv format to **C:\PACKARD\EXT PLATE MAPS\EXT LOGS** with the same name as the AUSLAB batch ID and click "**Apply**". (refer to figure 4. below)

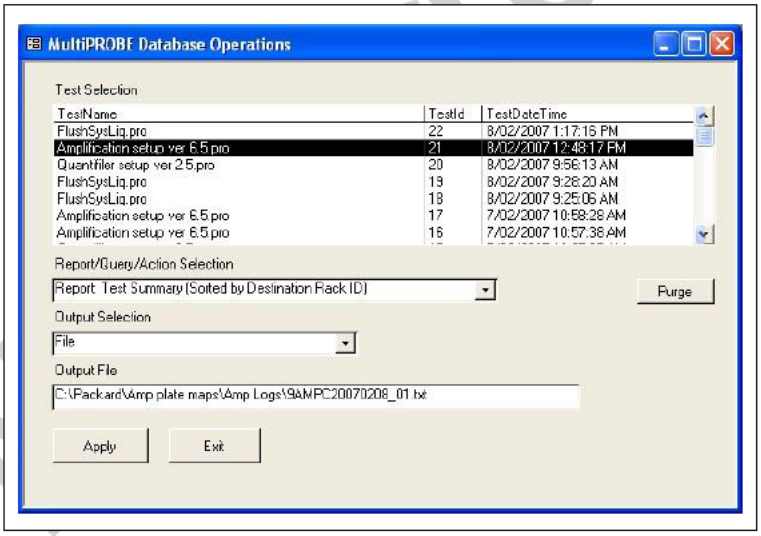


Figure 4. The MultiPROBE log database for collecting MP II run information

51. Open the log file and check for any errors that may have arisen during the Extraction process. Compare the listed errors to any that were encountered during the run. Report any critical errors to the line manager.
52. Copy the log file to **I:\EXTRACTION\EXT A MPII\LOGS or I:\ EXTRACTION\EXT B MPII\LOGS** for uploading to AUSLAB.
53. Log into the **AUSLAB Main Menu**.
54. Select **5.Workflow Management**.
55. Select **2. DNA Batch Details**.
56. Scan in the Extraction Batch ID barcode.
57. Press **[SF6]** ***Files***.
58. Press **[SF6]** ***Import Files***.
59. AUSLAB prompts "***Enter filename***"; enter the filename and extension and press **[Enter]**. (e.g. I:\EXTRACTION\EXT A MPII\Logs\CWIQEXT20071115_01.csv)
60. AUSLAB prompts "***Is this a result file Y/N?***" enter ***N*** and press **[Enter]**.

61. Press **[Esc]**.

Importing Extraction "Results" into AUSLAB

62. Log into the **AUSLAB Main Menu**.
63. Select **5. Workflow Management**.
64. Select **2. DNA Batch Details**.
65. Scan the Extraction batch ID barcode located on the worksheet.
66. Press **[SF6]** ***Files***.
67. Press **[SF6]** ***Import Files***.
68. AUSLAB prompts "***Enter filename***"; enter batch name and extension and press **[Enter]**.(e.g. CWIQEXT20071115_01.txt)
69. AUSLAB prompts "***Is this a results file y/n?***" enter "**y**" and press **[Enter]**.
70. The file will be imported into AUSLAB and appear in the DNA file table.
71. Highlight entry and press **[Enter]**, for access to the DNA results table.
72. Page down through the table and check that all sample results have been imported.
73. Press **[SF8]** ***Table Sort Order***, this sorts the table so any samples that have failed Autovalidation are sorted to the top of the table.
74. For all samples that have failed check the **Processing Comments,** by entering into the sample.
75. **a)** If processing comments state sample is to be sent to another batch type **other** than quant. Return the sample to the correct next batch type – e.g. microcon, nucleospin and pooling
    b) Press **[Esc]** to exit back to the DNA results table.
    c) Do not toggle accept.
76. **a)** If processing comment does not state next step for sample the sample will be processed as normal.
    b) Press **[Esc]** to exit back to the DNA results table.
    c) Highlight any entries to be changed and press **[SF7]** ***Toggle Accept***
77. Press **[F7]** ***Complete Batch***, all of the samples with a ***Yes*** in the Accept column will be transferred to the outstanding Quant Casework or Quant Reference lists.
78. File the Extraction worksheet into the relevant folder in Room 6117.

8 SAMPLE STORAGE

Please refer to "*Analytical Sample Storage*" (QIS 24255) for how to store the old original 5 mL sample tubes, the DNA extract NUNC tubes, Slicprep with Basket and Axygen store plates.

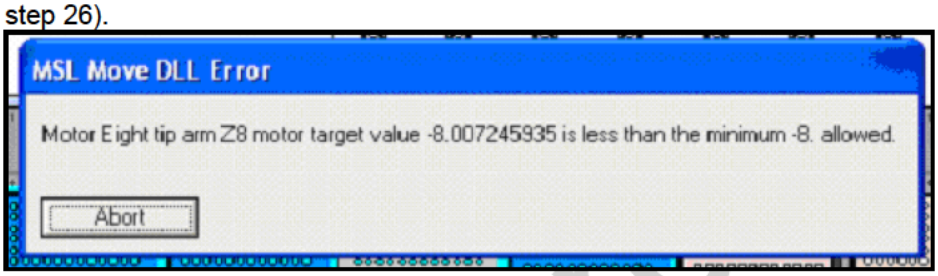
9 TROUBLESHOOTING

1. If the barcode reader is not reading the barcodes of the Nunc tube rack, or the Slicprep Plate or the Store plate, manually scan the appropriate barcodes.
2. When reading the Nunc tube rack barcode, if the Gripper is not picking up or placing the Nunc tube rack properly on the deck, just manually place the rack properly on the plate adapter support tile.
3. When reading the Store plate barcode, if the Gripper is not picking up or placing the Store plate properly on the deck, just manually place the plate properly on the plate adapter support tile.
4. When reading the Slicprep plate barcode if the Gripper is not picking up the plate properly :
    a. if the plate was not properly placed on the plate adapter support tile with the Wallac Isoplate, just manually place the plate properly.
    b. if the plate was properly placed on the plate adapter support tile with the Wallac Isoplate on it: it means that the gripper needs to be initialised. Abort the

run, Initialise the instrument and restart the run. If problem persists, shutdown the MPII and PC, restart and then initialise the whole instrument. Otherwise, contact your line manager.

c. Calibrate relevant labware using the SlicPrep Calibration plate. This has preset standardised positions that need to be the same on all labware where the Slicprep plate is being moved. The same plate is used on both extraction platforms A and B.
d. Check the calibrations against the run program DNAIQGripperTest.pro. This program moves the Slicprep across all the labware the gripper moves across. Start with the Slicprep at D16.

5. In steps 18 or 26, if a message is stating that the instrument is having a motor problem when picking up 1 mL tips and the only option is to Abort, abort, initialise and open program version **1.3a** (if the problem is in step 18) or version **1.3b** (if the problem is in step 26).



As the program will start the gripper will pick up the plates, it is not necessary that the Nunc tube rack is in position (B16), only ensure that it is reading the correct barcode. It is **important not** to place the Slicprep in the original position (E13) as the Slicprep plate has the Spin basket part removed (ie keep at D16), ensure it will scan the correct batch barcode. The Store plate remains in the original position. If the problem persists even after restarting, replace the rack of disposable 1 mL conductive sterile MBP tips for a new one.

6. If the program has already started step 18 and the previous message is appearing, you need to abort. Initialise the instrument and open program version **1.3a.**
Go to the plate map in C:\PACKARD\ EXT PLATE MAPS and change volume to 0 for all the samples that the Lysis Buffer have been dispensed (Column 6), ensure that the number of samples where the Lysis buffer was added is the same as the ones where the volume needs to be changed.
Save the changes and use this plate map for the restart of the program. Please read troubleshooting 5 for barcode reading of plates.
If the problem persists even after restarting, replace the rack of disposable 1 mL conductive sterile MBP tips for a new one.

7. If the program has already started step 26 and the previous message is appearing, you need to abort. Initialise the instrument and open program version **1.3b**.
Go to the plate map in C:\PACKARD\ EXT PLATE MAPS and change volume to 0 in all the samples that the Lysis Buffer and Ext buffer have been removed (Column 9), ensure that the number of samples where the solution was removed is the same that the ones the volume need to be changed.
Save the changes and use this plate map for the restart of the program. Please read troubleshooting 5 for barcode reading of plates.
If the problem persists even after restarting, replace the rack of disposable 1 mL conductive sterile MBP tips for a new one.

8. If a disposable tip gets stuck on the 8 tip arm during disposal of tips a user message will appear. Remove and press retry and then continue.

9. If the message:

has appeared, press OK and the program will be aborted automatically. Check that all the connections to the instrument (shaker, heater and computer) are properly plugged in. If everything is OK, you need to close WinPrep, shut down the instrument, shaker, heater and PC. After 2 min restart everything. Once Winprep has been opened, reinitialise the instrument and start the program (check version number according to which step the message has came up). Please read troubleshooting 5 for barcode reading of plates.

10 VALIDATION

- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 9. Automation: Report on the Evaluation of DNA Extraction Chemistries." June 2007.
- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 11: Report on the Validation of the Automated Extraction Chemistry Kit using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Casework Platform." 2007.
- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 13: Report on the Verification of the Automated Extraction Chemistry Kit using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Casework Platform." 2007.

11 QUALITY ASSURANCE/ACCEPTANCE CRITERIA

- A Negative control (also known as the reagent blank) is included with each batch of extractions. This reagent blank is processed as a normal sample through to completion. If any results are obtained from this sample, either at the quantitation step or the Genescan analysis step, then the possible source of the contamination is investigated. The samples extracted with this control are thoroughly checked and repeated if possible.
- Positive and negative controls are included in each extraction batch as per table 4.

12 REFERENCES

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18. Vandenberg, N., van Oorchot., R. A. H., & Mitchell, R. J., An evaluation of selected DNA extraction strategies for short tandem repeat typing. Electrophoresis, 1997. 18: p. 1624-1626.

13 STORAGE OF DOCUMENTS

All worksheets are stored in the Analytical area (Room 6117).

14 ASSOCIATED DOCUMENTS

QIS 17120 Operational Practices in the DNA Dedicated Laboratories
QIS 17142 Examination of Items
QIS 17171 Method for Chelex Extraction
QIS 17165 Receipt, Storage and Preparation of Chemicals, Reagents and Test Kits
QIS 23939 Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform
QIS 24255 Analytical Sample Storage
QIS 24256 Sequence Checking with the STORstar Instrument
QIS 24469 Batch functionality in AUSLAB

15 AMENDMENT HISTORY

Revision	Date	Author/s	Amendments
0	23 Oct 2007	B. Gallagher, T. Nurthen, C. Iannuzzi, V. Hlinka, G. Lundie, I Muharam.	First Issue
1	12 Dec 2007	M Harvey, C Iannuzzi, A McNevin	Reviewed and updated after initial training

16 APPENDIX

16.1 Reagents Calculation Tables

1. **Table for less than 48 samples** (note difference is in DNA IQ RESIN Solution)

Lysis-DTT buffer		Volume (in mL)
Lysis buffer	(Nx1.35)+0.75	
DTT (1M)	Lysis Buffer/100	
Extraction Buffer		
TNE buffer	Nx0.56	
Prot K (20 mg/L)	Nx0.03	
SDS (20 %)	Nx0.015	
DNA IQ RESIN Solution		
LYSIS buffer	0.054x(N+8)	
DNA IQ RESIN	0.009x(N+8)	
DNA IQ 1X Wash buffer	Nx0.36	
DNA IQ Elution buffer	Nx0.144	

N = number of samples on batch including positive and negative controls, round up to nearest multiple of 8)

2. **Table for more than 48 samples** (note difference is in DNA IQ RESIN Solution)

Lysis-DTT buffer		Volume (in mL)
Lysis buffer	(Nx1.35)+0.75	
DTT (1M)	Lysis Buffer/100	
Extraction Buffer		
TNE buffer	Nx0.56	
Prot K (20 mg/L)	Nx0.03	
SDS (20 %)	Nx0.015	
DNA IQ RESIN Solution		
LYSIS buffer	0.054x(N+16)	
DNA IQ RESIN	0.009x(N+16)	
DNA IQ 1X Wash buffer	Nx0.36	
DNA IQ Elution buffer	Nx0.144	

N = number of samples on batch including positive and negative controls, round up to nearest multiple of 8)

16.2 Manual method for extraction using DNA IQ™

16.2.1 Sampling and Sample Preparation

Samples waiting to be extracted are stored in freezers as described in Table 3.

Table 3. Sample storage locations.

Sample type	Storage Device	Storage Location
Urgent/High/Medium Priority Samples	Freezer	6117-2
Medium Priority Samples	Walk in Freezer	6109
Low Priority Samples	N/A	

QC samples

All extraction batches require two controls to be registered. These controls, UR numbers and positions are listed below in Table 4.

Table 4. Extraction Quality Controls

QC	UR Number	Extraction types
Neg Control	FBOT33	All
QC swab (blood)	FBOT35	Blood

1. Log into the **AUSLAB Main Menu**.
2. Select **1. Request Registration**.
3. Select **2. Full Reception Entry**.
4. Scan in barcode of control.
5. Enter the UR number as per Table 4 and press **[Enter]**.
6. Enter the appropriate Specimen type (e.g. Blood for blood extraction).
7. Request a **9PLEX** test, when prompted to enter the processing comment, enter **EXTP** (Positive extraction control) or **EXTN** (Negative extraction control).
8. Press **[F7]** Enter **LAB** in the Billing code field.
9. Press **[F4]** ***Save*** to save the Billing details.
10. Press **[F4]** ***Save*** to save the registration details.

N.B Quality controls should not have a DNA priority allocated at time of registration to ensure they are included in the top positions of a batch

Create the Extraction Batch

15. Log into the **AUSLAB Main Menu**.
16. Select **5. Workflow management**.
17. Select **1. DNA workflow table**.
18. Highlight the appropriate Extraction batch type and press **[F5]** ***Batch Allocation***.
19. Press **[F6]** ***Create batch***.
20. Press **[F8]** ***Print menu***.
21. Press **[F6]** ***Print Batch label***. (for the deep well plate)
22. Press **[F7]** ***Print Sample labels***. (print four sets of labels for all extractions)
23. Press **[F8]** ***Print Worksheet***.
24. Press **[SF5]** ***Main menu***.
25. Press **[SF11]** ***Print***.
26. Press **[SF6]** ***Accept batch***.
27. Press **[Pause/Break]** to exit to the **Main Menu**.
28. Obtain worksheets (FBLASER3) and labels (FBLABEL13-16) from the Analytical Section printing bench (Room 6117).

Locating Samples

Determine the storage locations of the required samples using the Batch Creation table/Batch details table print out. The columns ***Rack*** and ***Pos*** respectively identify the rack and the grid location where the sample has been stored. Remove the samples from the storage rack and place in an orange rack (12x8).

When all samples have been located:

1. Log into the **AUSLAB Main Menu**.
2. Select **2. Sample Processing**.
3. Select **7. Search Sample storage**.
4. Scan in the sample barcode that is affixed to the sample tube.
5. Press **[F6]** ***Remove Sample***.
6. AUSLAB prompts "***Are you sure you want to remove XXXX-XXXX? (Y/N)***", Enter **Y** and press **[Enter]**.
7. AUSLAB prompts "***Please enter remove comment***", No comment is required. Press **[Enter]**.
8. Press **[Scroll lock]** to clear.
9. Repeat steps **5 - 8** until all of the samples have been removed from their rack.

Sequence Check the tubes

1. Thaw samples at room temperature and label 1.5mL sample tubes.
2. Sequence check the tubes.
3. Add the sequence check details into AUSLAB.
4. Log into **AUSLAB Main Menu**.
5. Select **5. Workflow Management**.
6. Select **2. DNA Batch Details**.
7. Scan in the appropriate extraction batch ID barcode.
8. Press **[F5]** ***Sequence Check***.
9. Scan in the appropriate extraction batch ID barcode.
10. Press **[Pause/Break]** to exit to **Main Menu**.

16.2.2 Procedure

1. Enter the number of samples to be extracted (including controls) into the "No of Samples" column of the DNA IQ Reagent Calculations Table to calculate the volumes of each reagent to be measured out for the extraction. Aliquot regents into either 5ml tubes or 50ml Falcon tubes. **Note:** The volume of Lysis buffer calculated includes the volume used in the resin-lysis solution

2. Turn on the Eppendorf Thermo mixer and set the temperature to 37ºC.

3. Remove 1.5ml tube and retain the 5mL tube. Prepare the Spin baskets by placing a DNA IQ™ Spin basket into a 1.5mL Microtube. Label the spin baskets (for every tube except Ext. control), 2mL SSI tubes and Nunc storage tubes (for every sample) with the sample barcodes. Have a second operator perform a sequence check of all tubes. This person must Press **[F5] Sequence Check** against the batch in AUSLAB

4. Using the Reagents table, prepare Extraction Buffer, Lysis buffer with DTT, & Resin Solution. Reagents need to be prepared fresh before each run.

5. Add 300 µL of Extraction buffer to each tube. Vortex each tube before incubating the tubes at 37°C on the Thermomixer at 1000 rpm for 45 minutes.

6. Remove the tubes from the Thermo mixer and add to a rack, increase the temperature on the Thermomixer to 65°C (for use in the Elution steps).

7. Transfer the substrate from the original tube to a DNA IQ™ Spin Basket using autoclaved twirling sticks. Centrifuge the Spin basket for 2 minutes at room temperature at its maximum speed. Once completed, remove the spin basket & retain in the original 5ml tube. Ensuring minimal contamination and transfer the extract to a labelled 2mL SSI sterile screw tube.

8. Transfer the remaining extract from the original tube to the corresponding 2mL tube. Vortex the tube gently.

9. Add 550 µL of Lysis Buffer to each tube.

10. Into a separate, clean 2mL SSI tube, aliquot the required amount of lysis buffer for the Resin solution. Ensure that the DNA IQ™ Resin solution has been thoroughly mixed by vortexing the resin bottle before adding the required resin volume to the lysis buffer. Pipette mix the solution to clear the tip of any lingering resin beads. Mix the solution by inverting the tube to prevent bubbles forming.

11. Add 50µL of DNA IQ™ Resin-Lysis solution into each tube. Invert the resin-lysis tube at regular intervals to keep the resin suspended within the solution to ensure uniform results.

12. Vortex each tube for 3 seconds at high speed before placing the tubes in the Multitubeshaker set at 1200 rpm to incubate at room temperature for 5 minutes.

13. Remove from the Multitubeshaker and vortex the tubes for 2 seconds at high speed before placing the tubes in the magnetic stand. Separation will occur instantly.

Note: If resin does not form a distinct pellet on the side of the tube, or if the pellet has accidentally mixed with the solution while in the stand, vortex the tube and quickly place back in the stand.

14. Carefully remove all of the solution in the tube into the original 1.5mL tube (i.e. the tube which originally contained the substrate), ensuring that the resin is not disturbed from its place on the side of the tube.

Note: If some resin is drawn up in tip, gently expel resin back into tube to allow re-separation.

15. Add 125µL of prepared Lysis Buffer and vortex for 2 seconds at high speed. Return the tubes to the magnetic stand and allow for separation to occur. Once separation has occurred again remove the Lysis Buffer into the original 1.5mL tube (i.e. the tube which originally contained the substrate).

16. Add 100µL of prepared 1X Wash Buffer and vortex for 2 seconds at high speed. Return tube to the magnetic stand and once separation has occurred remove and discard all Wash Buffer.

17. Repeat Step 16 another two times for a total of three washes. Ensure that all of the solution has been removed after the last wash.

18. Wipe down a Biohazard hood with bleach followed by ethanol. Uncap the tubes, placing the lids inside down onto a clean rediwipe in consecutive order and place the tubes in the same order into a clean plastic rack. Air-dry the resin in the hood for 15

minutes at Room temperature. Do not dry for more than 20 minutes, as this may inhibit removal of DNA during the elution phase.

19. Once the resin is dry, replace the lids on the corresponding tubes and remove from the hood. Add 50µl of Elution Buffer to each of the samples by carefully pipetting the liquid to the side of the tube, above the pellet. Do not mix.

20. With the lids on, incubate the tubes in the Thermomixer at 65°C for 3 minutes. After the three minutes are up, continue to incubate for a further 3 minutes shaking at 1100 rpm.

21. Remove the tubes and vortex for 2 seconds at high speed. Immediately place the tube in the magnetic stand while hot to ensure maximum DNA yield during elution.

22. Carefully transfer the DNA containing supernatant to the corresponding labelled Nunc tubes.

23. Remove tubes from the magnetic stand and add carefully another 50 µL of Elution Buffer above the magnetic pellet.

24. Repeat step 30 to 32. The final volume after this elution should be approximately of 95 µL of DNA solution.

25. DNA can be stored at 4°C for short-term storage or at -20 or -70°C for long-term storage.

16.2.3 Sample storage

1. Log into **AUSLAB Main Menu**.
2. Select **2. Sample Processing**.
3. Select **6. Sample Storage**.
4. Scan in Rack barcode.
5. Press **[SF5]** ***Fill Rack***.
6. Scan in sample barcode and place in rack in scanned position.
7. Repeat for all samples.
8. Press **[Esc]**.
9. Press **[Pause/Break]** to return to the **Main Menu**.
10. Select **3. Patient Enquiry**.
11. Scan in Rack barcode.
12. Tab down to the next blank ***DNA Batch No*** field and press **[F2]** ***Edit***.
13. Scan in the Batch ID of the samples stored.
14. Press **[Pause/Break]** to return to the **Main Menu**.

PPN 8842157

MultiPROBE II Liquid Handling

Forensic Workstation

Application Guide

Automated DNA IQ™ System for Mixed Casework Sample DNA Isolation

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2200 Warrenville Road
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PPN 8842157

Automated DNA IQ™ System for Casework Samples

Paramagnetic Particle Method for Forensic DNA Isolation

This is a description of the MultiPROBE® II Workstation protocol used to automate DNA isolation from high volume forensic casework samples using a modified DNA IQ System from Promega. The new method, developed by the Centre of Forensic Sciences (CFS) in Toronto, Ontario, was designed to process a range of samples in 96-well plate format, including blood-stained paper and cloth, cigarette butts, gum, FTA punches and swab swipes (Komonski et al, 2004). Promega's standard methods are primarily designed for DNA extraction from dry blood stains and swabs (see Reference Section). Modifications were made to increase the extraction efficiency for all samples types, including swabs and stains. Major changes to the Promega method are to (1) decrease the extraction/lysis temperature, and (2) replace the initial extraction using the kit lysis buffer with a digestion step using Proteinase K-SDS at 37 °C. Following low temperature extraction, the Promega kit Lysis Buffer (2 volumes) plus DNA IQ resin is added to the cell lysates to capture genomic and mitochondrial DNA. The normal kit purification process is then followed for the rest of the protocol. After ethanol washing, the bound DNA is eluted at 65 °C and transferred to a clean polypropylene 96-well collection plate. A smaller elution volume (25 uL) is used. All steps are carried out using standard deep well plates and microplates. This new method using Proteinase K-SDS extraction at low temperature is very similar to that used by Promega to isolate DNA from hair and tissue samples (Promega TB307, Mandreker et al, 2002). At CFS, different "contact" casework sample types from the same crime scene are processed concurrently in the same source plate. This method is highly amenable to automation. This magnetic particle-based method can be used for DNA isolation from all forensic sample types, including database swabs and blood stains.

MultiPROBE II Workstation Features

The workstation used for this application consists of the basic MultiPROBE II PLUS HT EX with Gripper™ Integration Platform, DPC MicroMix® 5 Shaker and Automated Heater Controller Option with two custom heat transfer block inserts (see **Appendix A** for MultiPROBE II part list). The MultiPROBE II Workstation offers the following features for automated forensic DNA purification:

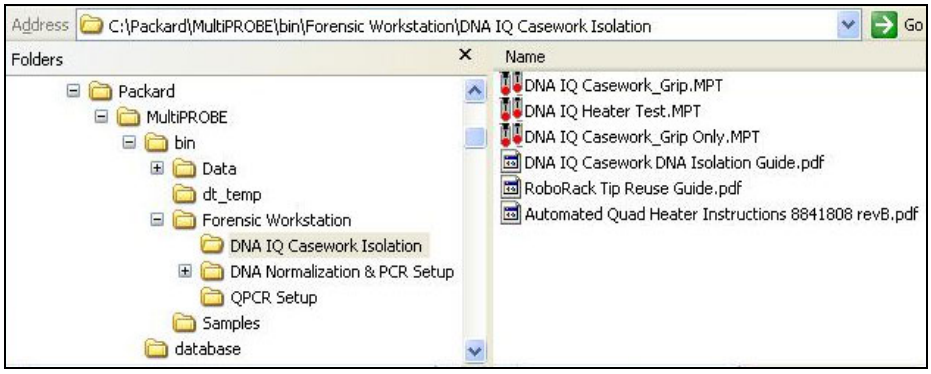
- ✓ Walk-away protocol automatically carries out all steps needed for forensic DNA isolation using the DNA IQ System with typical yields of 1-100 ng per sample volume of 25 uL.
- ✓ Simple, reliable, high throughput and cost-effective procedure reuses disposable tips.
- ✓ Paramagnetic particles are captured using MagnaBot® 96 Magnetic Separation Device (Promega Cat #V8151) and MagnaBot Spacer. Its 24 magnetic pins bind particles to sides of each well in seconds, allowing easy removal of the waste fluid.
- ✓ Integrated shaker for cell lysis and DNA extraction increases throughput and yields.
- ✓ Integrated gripper moves the sample processing microplate to and from the shaker to the magnet during the wash steps.
- ✓ MultiPROBE II quad heater automates a 37 °C heating step to extract DNA from samples in deep well plates using a custom heat transfer block. A second heater tile automates the 65 °C elution step in a microplate using a second custom heat transfer block.
- ✓ Processing time of ~135 min for a 96-sample plate includes a 30 min low temperature extraction/lysis step.
- ✓ WinPREP® protocol is optimized to prepare forensic DNA that is ready-to-use for downstream quantification (e.g., real-time QPCR, AluQuant® and other kits) and PCR analysis (e.g, Profiler Plus®, PowerPlex® and other kits).

Automated Software Installation

The MultiPROBE II Forensic Workstation installation uses two CD install disks. Together, both disks contain all the files needed to run various automated forensic applications using WinPREP v206, v229 or v238 software. This application has been validated using an 8-Tip configuration. However, the installation CD contains WinPREP templates that can be used with both 4-Tip and 8-Tip MultiPROBE II configurations. For additional information, see the **Forensic Workstation Software Installation Instructions** (P/N 8842153).

The install process automatically detects whether the system has gripper software installed. Since this DNA IQ application was carried out with a gripper, to install these WinPREP tests requires that the Gripper Integration Platform option be installed before installing this software.

1. To begin the installation, insert the **Forensic Workstation Software** disk (P/N 7800612) in the CD Drive. Follow the prompts. Select the installation folder by clicking "OK" for the default folder (last WinPREP application installed), or "browse" to install in another folder.
2. When the "**Select Application**" prompt appears, check the desired protocol group box. Select the **DNA IQ Casework** protocol box. For this application, WinPREP tests, Application Guides, labware, and performance and other files needed to run the application will be installed in the MultiPROBE/bin directory and Forensic Workstation subfolders.
3. A prompt will appear to insert the **MultiPROBE II Options Software** disk (P/N 7800604). Follow prompts to complete the installation. The install process automatically places the appropriate WinPREP files in the MultiPROBE/bin/Forensic Workstation subfolder in the bin directory. Option-specific tests and other files are placed in the main MultiPROBE/bin directory.



Application-specific files will be installed in different directories as listed below:

Packard/MultiPROBE/bin folder:

- Gripper Position.pos file
- DPC Shaker Option (WinPREP .mpt test and files)
- Automated Heater Controller Option (WinPREP .mpt test and files)
- Various .rak and FW_.prf files

Packard/MultiPROBE/bin/Forensic Workstation/DNA IQ Casework folder:

- DNA IQ Casework_Grip.mpt (WinPREP test)
- DNA IQ Casework_Grip Only.mpt (WinPREP test)
- DNA IQ Heater Test.mpt (WinPREP test)

- DNA IQ Casework Isolation Guide (pdf)
- RoboRack™ Tip Reuse Guide (pdf)
- Automated Quad Heater Instruction (PPN 8841808 RevB pdf)

4. To run the test, copy and paste the DNA IQ Casework WinPREP template into the MultiPROBE/bin directory. It is best to save the test with a new name, such as the date, before running. To create a **read-only** version of the test, see **Customization** Section.
5. Do not modify the original tests or files.

Manual Software Installation

All Application Guides (pdf format) are also contained in labeled folders on the **Forensic Workstation Software install** disk. To install these files, click and drag into appropriate folders on computer.

All MultiPROBE II options are individually contained on the **MultiPROBE II Options Software** disk. To install any single option, open the folder and click the "exe" set up file. Follow the prompts to install each option.

Hardware Installation

Current Dell computers with Windows XP© operating systems that are used with MultiPROBE II systems usually have one serial port and 6-8 USB ports. If more than one option using RS232 communication is required for an application, a USB to serial port connector or adapter is required. See the "**USB Serial Adapter Installation Instructions"** for more information.

This application uses two options requiring RS232 communication, the DPC Shaker and the Automated Heater Controller. One **USB to Serial Adapter** (PPN 0101073) is included in the MultiPROBE II Forensic Workstation DNA Isolation Options (See **Appendix A** Part List). This includes PPN 7002307 (8-Tip Domestic), 7002309 (8-Tip International), PPN 7002308 (4-Tip Domestic), and 7002310 (4-Tip International) configurations. If more options are added to the workstation, for example a plate stacking device or a second heater, another USB to Serial Adapter is needed for each RS232 connection.

If communication error messages appear when running a test, check the computer's Device Manager to ensure that the comport assignments for the devices in the WinPREP tests match the actual physical connections. Go to "My Computer/Properties/Hardware/Device Manager/Ports (COM & LPT) to see a list of all devices connected.

For the DNA IQ Casework protocol, the following setup is recommended:

- The heater controller option should be connected via a RS232 cable to Serial Port 1.
- The shaker option should be connected via the **USB to Serial Adapter** connector and shaker cable to the first USB Port (4; usually upper left I/O port).
- For each additional option added to the MultiPROBE II workstation, order another USB to Serial Adapter (see Appendix A part list). To install new option user scripts in a WinPREP template, use "ctrl-alt-windows-i" to browse to appropriate option test with the required scripts.
- If using the **Multi-Comport Option** for RS232 port communication (e.g., shaker, heater), "2" is added to the actual comport number in the WinPREP template. Connect the heater to serial port #1. Connect the shaker to Multi Comport #2 (~ comport #4 in a WinPREP test).

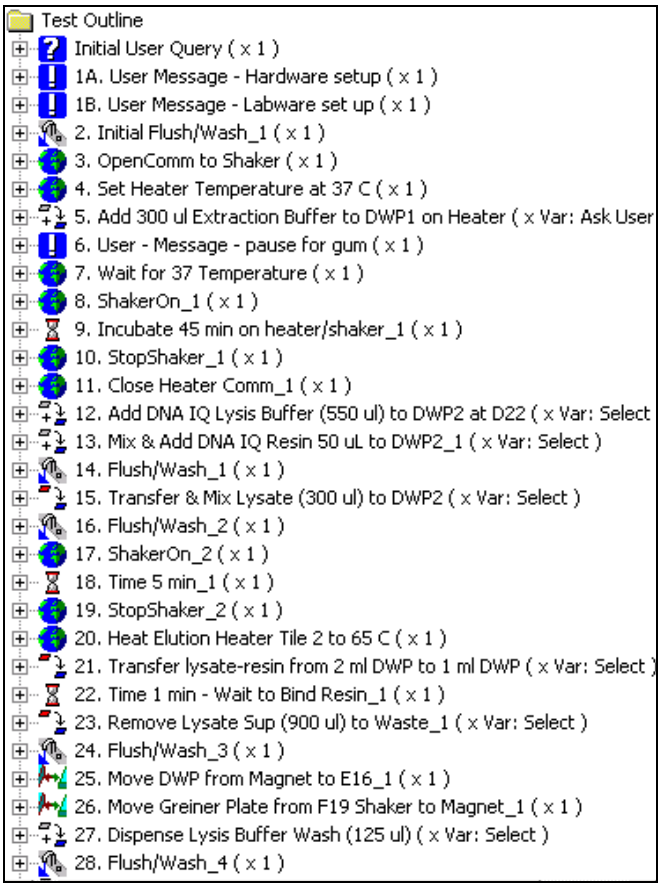
PPN 8842157

Instructions for DNA Purification from Forensic Casework Samples

The following MultiPROBE-operated protocol was created for the Ministry of Public Safety and Security, Centre of Forensic Sciences, Toronto, ON, Canada. See **Appendix A** for a list of MultiPROBE II parts used for this application. See **Appendix B** for a list of other protocol components. The protocol was adapted from the method described by Komonski et al, 2004. See also Promega TB296, TB297, and TB307 (**Appendix C**). For trouble-shooting chemistry issues, please refer to TB 296 and TB297 or call Promega Technical Support.

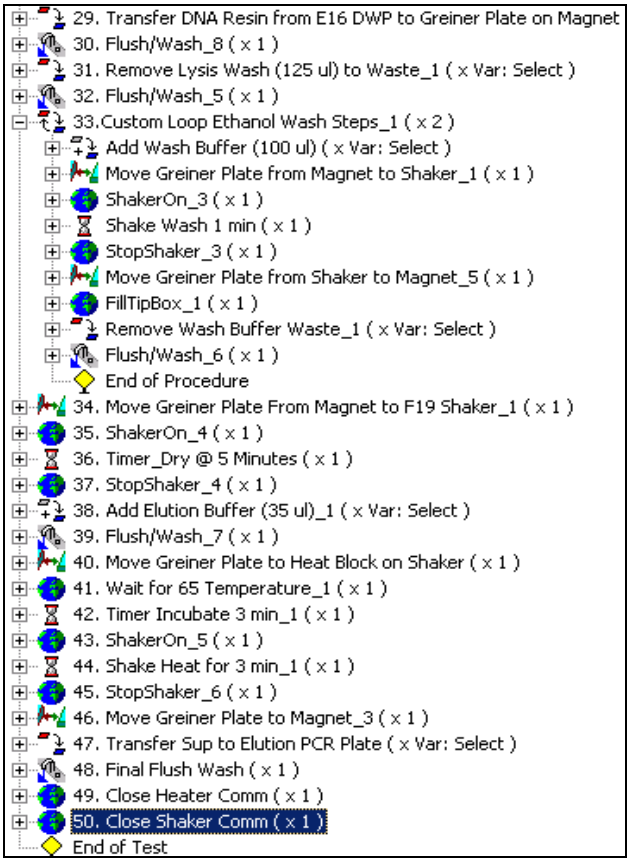
Protocol Overview

Figure 1A and B summarizes the WinPREP steps used to process up to 96 purified forensic samples with the automated DNA IQ System Casework protocol:



- User Query - Select N# samples to process.
- User Messages – deck setup.
- Auto-set sample plate on heat tile to 37 °C.
- Add 300 uL extraction buffer to sample plate on 37 °C heater tile (1).
- Incubate 45 min with heating & shaking.
- Add DNA IQ Lysis Buffer & DNA IQ resin to mixing plate on shaker.
- Transfer sample extracts to Mixing Plate.
- Incubate and shake 5 min.
- Start pre-heating elution heat tile (2).
- Transfer lysates-resin mix from mixing plate to deep well plate on magnet.
- Wait 1 min to bind resin to magnet.
- Aspirate supernatant to waste.
- Move deep well plate from magnet.
- Move clean microplate to magnet.
- Add LB Wash to DNA-bound resin in deep well plate.

Figure 1A. Part 1 of the WinPREP test outline and summary of the DNA IQ Casework Protocol steps.



- Transfer resin mixture to microplate to continue wash steps.
- Aspirate wash supernatant to waste.
- Loop Ethanol Wash Steps (repeat n = 2).
  - Add Wash Buffer.
  - Move plate to shaker.
  - Shake to mix.
  - Move plate to magnet.
  - Aspirate wash to waste.
  - Reuse tips.
- Move plate to shaker.
- Dry 5 min with shaking.
- Add 35 uL elution buffer
- Move to 65 °C heater tile.
- Incubate 3 min at 65C.
- Incubate 3 min more at 65 °C with shaking.
- Move plate to magnet.
- Transfer DNA to PCR collection plate.

Figure 1B- Continued. Part 2 of the WinPREP test outline and summary of the DNA IQ Casework Protocol steps.

A detailed procedure using mixed dried forensic samples and the Promega DNA IQ System is as follows:

A. Forensic Casework Sample Preparation

- For swabs, cut off shafts close to swab.
- For chewing gum, cut into 2-3 small pieces. DO NOT USE BIG PIECES. Gum samples typically contain large amounts of DNA. Note: Do not heat gum above 37 °C as it melts and will leave strings of goo on deck during transfer steps.
- For blood stains, cut a 3x3 mm piece of paper, cloth or tissue or use 2-3 small 2 mm punches from FTA® cards.
- For cigarette butts, cut a 1 cm piece of cigarette butt filter paper into 4-6 pieces.
- For fabric or tissue, cut a 1 cm piece into 2-3 pieces.
- Place samples in a deep well V-bottom Innovative Microplate® sample plate.

B. MultiPROBE II Instrument Setup

The system used at CFS to validate this protocol is a MultiPROBE II PLUS HT EX system with Gripper Integration Platform, WinPREP v229 software. To enter the appropriate hardware configuration, open the WinPREP "**DNA IQ Casework.mpt**" test template. On the WinPREP menu bar, go to Utilities/Setup/Instrument/Settings. Check appropriate system and subsystem boxes (see **Figure 2**). For the MultiPROBE II Expanded System deck configuration, select "Expansion module" for the left side and "DPC Shaker" for the right side. The enabled subsystems should match the options selected during the install – the Gripper and the Eight tip arm (HT option) are shown activated here.

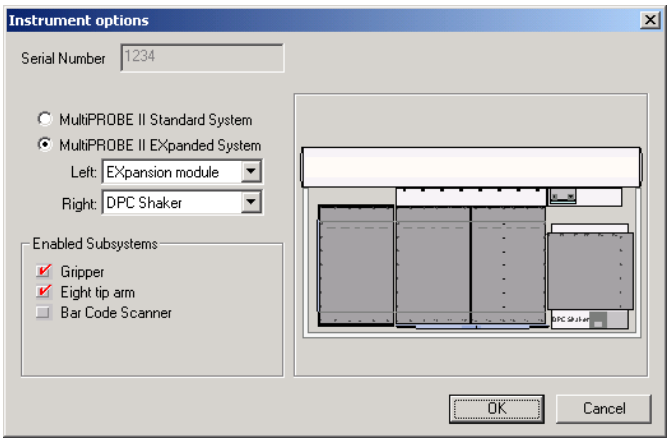


Figure 2. Instrument option setup used for the DNA IQ Casework protocol.

The DNA IQ Casework protocol requires installation of the VersaTip™ Plus option. The 1 mL Syringe option gives the best performance due to the large number of high volume transfers used in this protocol. This option consists of 8x 1000 uL syringes installed on the MultiPROBE II HT EX system. To enter the appropriate pipet arm settings configuration, open the WinPREP **"DNA IQ Casework.mpt"** test template. Go to Utilities/Setup/Eight tip arm/Arm Settings on the WinPREP menu bar. Enter **1000** (or 500 if 500 uL syringes are installed) into the syringe size box and select "VersaTips**"** as shown in **Figure 3**.

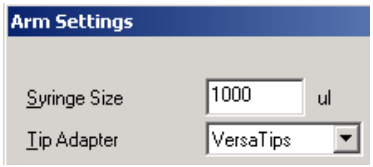


Figure 3. Arm settings used with 1000 uL syringe installation.

C. Preparation of Solutions

1. Proteinase K-SDS Extraction Buffer (CFS ProK-SDS recipe):

   For 1.2 N samples, prepare fresh Proteinase K-SDS Extraction buffer in TNE. Mix Well.

1X Buffer:	277.5 uL of TNE
	15 uL Proteinase K (20 mg/mL)
	7.5 uL 20% SDS
	300 uL

2. Promega DNA IQ System Kit Reagent Preparation:

- Prepare DNA IQ Lysis Buffer for 1.2 N samples x 725 uL in 50 mL sterile conical tubes. Each sample requires 725 uL (600 uL for lysis step and 125 uL for wash step). Add 2.6 uL of 0.39 M stock DTT to every 100 uL of Lysis Buffer used.
- Prepare DNA IQ resin in Lysis buffer with DTT. For N samples, mix stock resin well to suspend, transfer 1.2N x 7 uL to 15 mL sterile conical centrifuge tube. Add 1.2N x 43 uL Lysis Buffer with DTT to resin in tube and mix well.
- Prepare Wash Buffer for 1.1 N samples. Add ethanol and isopropanol according to kit directions.
- Measure out 1.2 N x 35 uL Elution Buffer (nuclease-free water).

D. MultiPROBE II Deck Layout and Test Configuration for DNA IQ Casework Protocol

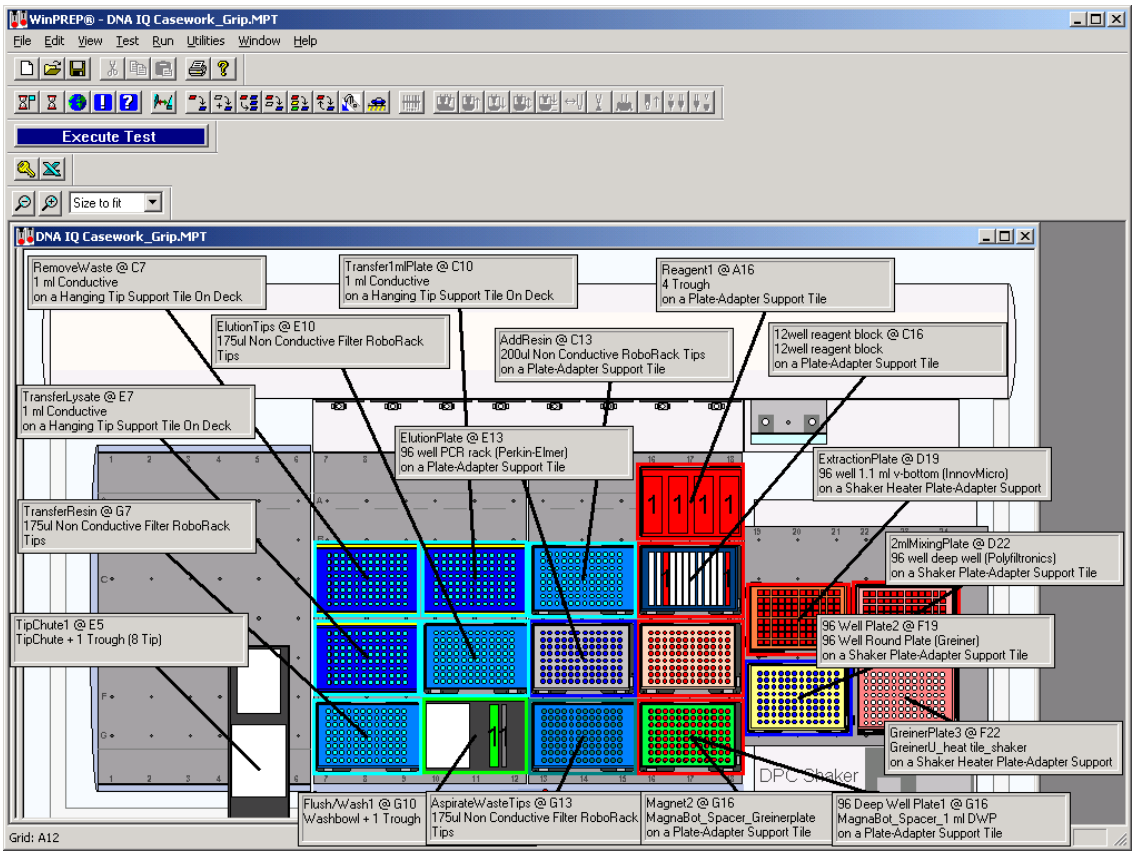


Figure 4. WinPREP template and deckview for DNA IQ Casework protocol used at CFS Toronto. A new coated Disposable Tip Slide can be placed at G4 to replace the standard "TipChute + 1 Trough (8 Tip)" labware definition shown at E5 here.

E. Automated DNA Isolation from Forensic Samples

1. Open the WinPREP "**DNA IQ Casework_Grip.mpt**" test template (see **Figure 4**).
2. If running the protocol for the first time:
   - **Check rak file definition for each labware type**. This includes processing plates, reagent troughs, and disposable tip racks on all deck modules. Ideally, aspirate tip Z-height should be just off well bottom (~1-2% of total well height). If height is incorrect, tip could aspirate above sample liquid or hit the bottom, plugging tip. Check labware definition (rak file) for all plates. After centering X and Y tip positions, check well top and bottom Z-height positions. Correct if needed. Note: VersaTip stinger cannot go to bottom of most PCR plate wells – due to flared probe end. Edit both plate types used on the MagnaBot: 1 mL Nunc deep well plate and U-bottom Greiner microplate.
   - **Check gripper positions**. Run DNA IQ System Casework_Grip Only.mpt template to verify gripper positions. Edit if needed.
   - **Check heater function**. Run DNA IQ Heater.mpt template to check that communication is set up correctly. During a water run, insert temperature probe into sample wells to verify temperature is within 2 ^{o}C.
   - **Perform a water run.** Substitute equivalent clear tips for any conductive tips used (can't do with1 mL tips). Pause after each aspirate and dispense step in each step for at least the first column in a plate to ensure that all tips have aspirated liquid evenly. Check Z height of tip in bottom of well for each aspirate step. If tip hits bottom of well, increase the % well height as needed. Check that all wells have same amount of liquid after each reagent addition or liquid transfer step.
   - **Check shaker amplitudes during water run**. The MicroMix 5 DPC Shaker amplitudes vary somewhat among instruments. If water run samples splash, decrease amplitude. If shaker appears weak, increase amplitude by 1 or 2.
3. If running the first WinPREP test of the day, run the Flush/Wash routine (see WinPREP menu bar: Utilities/Diagnostic Tests/Flush and Wash test). Check that syringes and tubing are free of bubbles.
4. Click open "User Message" and check the set-up directions.
5. Place labware, extraction buffer and reagents on MultiPROBE II deck as shown in **Figure 4**.
   - MPII Flush/Wash bowl at G10.
   - MPII Tip Chute + 1 Trough (8 Tip) at E5. If using the "Disposable Tip Slide", place at deck position G4 using the locking pin to secure. See **Disposable Tip Slide Guide** (P/N 8842155). See also p24 of this guide for deckview using the Disposable Tip Slide.
   - MagnaBot with Spacer at G16 on Plate-Adapter Support Tile.
   - Clean Greiner 96-well U-bottom Processing Plate at F19 on Shaker Plate-Adapter Support Tile.
   - Heater Tile 1 at D19 on Shaker Heater Plate-Adapter Support Tile.
   - 96V Deep Well Heat Transfer Block on Heat Tile 1 at D19.
   - Heater Tile 2 at C22 on Shaker Heater Plate-Adapter Support Tile.
   - 96U Heat Transfer Block on Heat Tile 2 at C22.
   - Clean deep well Mixing Plate (2 mL square well Whatman® plate) at D22 on Shaker Plate-Adapter Support Tile.
   - Clean deep well processing plate (1 mL Nunc®) at G16 on MagnaBot with Spacer on Plate-Adapter Support Tile.
   - Clean PCR Collection Plate on Plate-Adapter Support Tile at E13 on Plate-Adapter Support Tile.
   - 3 boxes 175 uL Clear Filter RoboRack™ Tips at G13, E10, G7 on Plate-Adapter Support Tiles.

- 3 boxes 1 mL Conductive Tips in Hanging Tip Racks at C7, E7 and C10.
- 1 box 200 uL Clear RoboRack Tips at C13 on Plate-Adapter Support Tile.
- Empty Plate Adapter Tile at E16 (ignore deckview - it maps plate used later in procedure).
- Innovative Microplate®12-Channel Trough on Plate-Adapter Support Tile at C16.
  - DNA IQ resin in column 3.
  - Elution Buffer in column 12.
- 4-Position Trough Support at A16 on Plate Adapter Support Tile.
  - Add 1.2 N x 300 uL Extraction Buffer to far right tray (col. 4).
  - Add 1.2 N x 600 uL or 60 mL of Lysis Buffer to 60 mL trough at 2nd from right position (col. 3). Maximum volume is 60 mL.
  - Add 1.2 N x 125 uL of Lysis Buffer to 60 mL trough at 3rd from tray (col. 2).
  - Add 1.2 N x 300 uL of Wash Buffer to 60 mL trough at far left left tray (col. 1).

6. Place forensic samples in 1.4 mL Innovative Microplate V-bottom square-well top deep well plate at D19 on heat transfer block on Shaker Heater Tile 1.
7. Press "**Execute Test**" button on WinPREP template.
8. When run is over, collect purified DNA sample plate at E13 for downstream analysis (quantification and normalization, hybridization, PCR or DNA sequencing analysis).

Automated Heating Steps

Two heating steps are used for the automated DNA IQ Casework Application: (1) extraction-lysis step at 37 °C at start of the protocol, and (2) DNA elution step late in the protocol. Figures 5-7 below show the Automated Heater Controller Option (PPN 7601824) parts used.

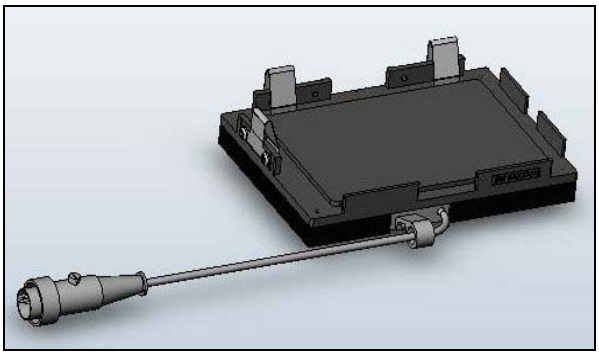


Figure 5. Automated Heater Tile fits on main deck or shaker deck.

PPN 8842157

Custom Heat Transfer Blocks

Forensic DNA Extraction Step

96 V-Bottom Deep Well Plate Heat Transfer Block on Heater Tile 1

The 1.4 mL V-bottom disposable plate (1.1 mL working volume) used during the Proteinase K-SDS extraction step is shown positioned above the 96V deep well heat transfer block (PPN 5083083). This assembly is used to incubate the forensic samples in extraction buffer at 37 °C while shaking for 30-60 min. The custom MultiPROBE II heat block fits on the basic quad heater tile and holds the plate securely while simultaneously shaking and heating.

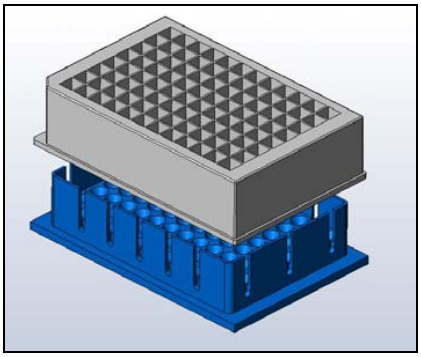


Figure 6A. 96-well 1.4 mL sample deep well plate on DWP Heat Transfer Block.

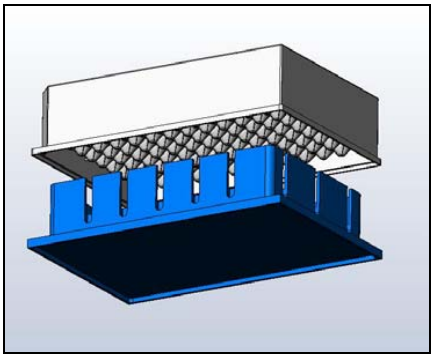


Figure 6B. Bottom view of assembly.

Forensic DNA Elution Step

96 U-Bottom Heat Transfer Block on Heater Tile 2

The 96-well Greiner U-bottom Processing Plate is heated at 65 °C while shaking during the elution step. A custom heat transfer block (PPN 5080165) positions this plate when placed in the heater tile on the shaker deck.

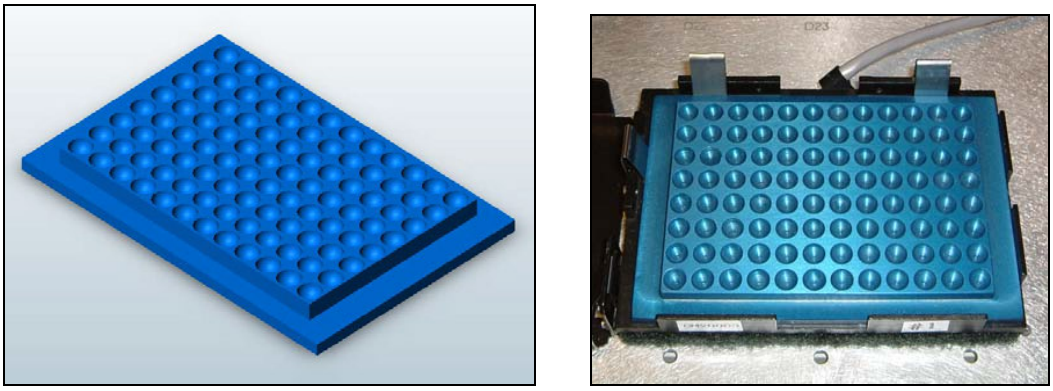


Figure 7. 96U Heat Transfer Block (left) used to heat sample plate on Heater Tile (right).

The assembly is shaken vigorously during the heating step. The elution heater tile and plate assembly is gripper-compatible due to the addition of gripper-compatible springs at the rear of the heater tile.

MagnaBot Assembly and Automated Processing

Overview

At the start of the DNA IQ Casework protocol, the MagnaBot with Spacer assembly is placed on a standard plate adapter support tile at workstation deck position G16. Two processing plates are used sequentially during the casework sample protocol: a deep well plate and a microplate. **Figure 8** shows the round-bottom microplate used for the later washing and elution steps.

Figure 8. MagnaBot Assembly. Shown is the 24-post magnet with spacer and Greiner U-bottom plate at deck position G16.

Extraction, Lysis and DNA Binding

After the DNA extracts are transferred to a 2 mL deep well plate on the shaker, the DNA IQ resin and 2 volumes of DNA IQ Lysis Buffer are added and mixed for 5 min to allow binding of DNA to the paramagnetic resin. The particles are attracted to the magnetic pins and, after a few seconds, collect on the sides of the well. This allows the pipettor to efficiently aspirate the lysate waste fluid. The first wash buffer is added to the particles and the mixture is then transferred to the Greiner processing microplate on the magnet. The resin again binds to the sides of the well, and the waste supernatant is removed. This process is repeated for 2-3 more wash steps. For each reagent addition, the plate is moved to the shaker using the gripper. Reagent is added and the plate shaken for 1 min. The gripper then moves the plate back to the magnet to aspirate the waste step.

Washing and Elution

For the elution step, the microplate is moved off the magnet to the shaker to dry for 5 min. The pipettor then adds the elution buffer with shaking. The gripper moves the plate to the heat tile for incubation with shaking at 65 °C. After heating the plate is moved back to the MagnaBot and the pipettor transfers the eluted DNA to a polypropylene elution PCR plate on the deck.

Summary: DNA IQ Casework Protocol Uses Two MagnaBot Processing Plates

DNA IQ 1 mL Deep Well Processing Plate

- Used to concentrate large volume sample lysates (900 uL: 300 uL of Extraction Buffer plus 2 volumes or 600 uL of Lysis Buffer).
- The 24-post Promega MagnaBot is compatible only with specific 1 mL deep well plates. They include Nunc (cat# 20445), Marsh (cat# AB 0564) and Beckman plates.

DNA IQ U-bottom Processing Microplate

- Used to continue washing and elution steps with smaller volumes of wash (100 uL) and elution buffers (25-35 uL).

- Shallow processing microplate reduces liquid level sensing errors and potential cross-contamination due to tips plate wells.
- The 24-post Promega MagnaBot is compatible only with specific round bottom microplates. They include Promega Cat# A9161 and Greiner Cat# 650261.

DNA IQ System – Mixed Casework Samples: WinPREP Test Protocol Details

A description of each step used for the automated DNA IQ System application using forensic mixed casework samples is shown below. The procedure numbers correspond to the same numbered step of the WinPREP protocol. For an abbreviated schematic of the automated steps used for the forensic DNA protocol, see **Appendix D.**

Before running the complete WinPREP DNA IQ protocol for the first time, run the "**Evaluate test**" listed under the "Run" menu to ensure the template is operational. For shaker steps, start with amplitude 5 and increase, if possible. The elution step needs highest shaker settings possible without splashing. This protocol uses Liquid Level Sensing (LLS) for several of the aspirate steps.

Processing begins with the forensic samples placed in a deep well plate on heater tile 1 on the DPC Shaker deck.

1. Press**"Execute Test**" once sample plate, reagents and labware are placed on deck. See Test Outline Comments tab for more detail.
   - See "**User Query"** window. Select number of samples to be run - if less than 96. Check that tip boxes are full.
   - See **User Messages** to check labware, heater tile and reagent layout.
2. **Initial Flush/Wash**.
3. **Open Shaker Comm Port.** Communication with the DPC Shaker is initiated via the RS-232 port of the MultiPROBE II computer. The test is set to use comport 5 (Multi-Comport #3).
4. **Set Heater Temperature to 37 °C.** Opens communication for Heater Tile 1 on Shaker deck. The test is set to use Comport 3 (Multi-Comport #1). Begins heating Heater Tile 1 assembly. **Note**: Set temperature of 39 °C is used in order to reach a sample temperature of 37°C.
5. **Add 300 µL Extraction Buffer to deep well plate on DPC Shaker deck.** Reagent Step. ProK-SDS solution is multi-dispensed x4 to each forensic sample in the deep well plate at D19 on Heater Tile 1. Fixed Tips. Aspirate with LLS from trough 4 at A16, dispense at 102% well height.
6. **User Message – Gum Samples**. Pause to add 200 uL more of Extraction Buffer to gum samples. Press Ok when ready to continue. Gum expands at 37 °C. Extra volume prevents sticking to tips. This step can be eliminated for protocols without gum samples.
7. **Wait for 37 °C Temperature.** Thermal sensor relays current sample temperature and waits until heater tile reaches set temperature of 39 °C. Test automatically continues 2 min after set temperature is reached or user can click "continue" to proceed immediately.
8. **Start Shaker**. Custom step automatically selects the DPC Shaker Form (42) and Amplitude (5) used to extract DNA from forensic samples.
9. **Incubate 45 min on Heater-Shaker.** Heating and shaking continues for 30-60 min. This can be reduced or increased depending on the age and type of dried sample.
10. **Stop Shaker.**
11. **Close Heater Comm.** Shut down heater communication to clear program for Heater Tile 1.
12. **Add 550 µL of DNA IQ Lysis Buffer to Mixing Plate.** Reagent Step. Kit Lysis Buffer is multi-dispensed x3 to 2 mL Whatman plate on Shaker Plate Adapter Tile at D22. Fixed Tips. Aspirate with LLS from trough

3 at A16, dispense at 102% well height. 2 volumes Lysis Buffer are added per 1 volume of extraction buffer.

13. **Mix & Add DNA IQ Resin (50 uL).** Reagent Step. Using 8x 200 uL disposable filter tips, resin is pre-aspirate mixed for 6 cycles (aspirate at 3%, dispense at 30% well height) in 12-channel trough column 3. Use 200 uL Conductive RoboRack Tips .with "WaterWaste 200 uL DT_FW.prf" performance files. Replicate-dispense x3 into 2 mL Mixing Plate on shaker at D22. Use 1 column of 8 tips per procedure. Aspirate at 3%, dispense at 80% well height. Discard tips. Total sample resin-lysate volume in Mixing Plate= 900 uL.
14. **Flush/Wash_1.**
15. **Transfer 300 uL Lysate with Mixing to DWP**. Single liquid transfer with post-dispense mix step. Blowout mode. Samples are transferred from Extraction Plate to Mixing Plate using 1 mL Conductive Tips with WaterBlowout 1 mL DT_FW.prf file, followed by vigorous tip mixing. Aspirate using LLS and dispense at 80% well height into Mixing Plate. Mix Volume 450 uL x3 cycles (aspirate at 10%, dispense to 40% well height). Discard tips.
16. **Flush/Wash_2.**
17. **Shaker On**. Amp 5, Form 42.
18. **Timer 5 min**. Incubate to allow DNA to bind to resin.
19. **Stop Shaker**.
20. **Set Heater Elution Temperature to 65 °C**. Open communication to Heater Tile 2 at C22 on Shaker deck. Set temperature used here is 70 °C in order to reach a sample temperature of 65 °C. Begins heating Tile 2 Assembly to 70 °C. The test is set to use Comport 3 (Multi-Comport #1).
21. **Transfer 900 uL Samples from Mixing Plate to 1 mL Nunc Plate on Magnet**. Single liquid transfer with pre-aspirate mix step. Waste Mode. 1 mL Conductive Tips used with WaterWaste 1 mL DT_FW.prf performance file. Pipet mix 6 cycles (aspirate at 5%, dispense at labware default) and transfer 900 uL lysate-resin mixture to 1 mL Nunc deep well plate (round-bottom) on MagnaBot at G16. Aspirate at 1%, dispense at 80% well height. Discard Tips.
22. **Timer 1 min**. Wait for resin to bind to sides of Processing DWP wells on magnet.
23. **Aspirate Supernatant from DWP Plate on Magnet to Waste**. Single liquid transfer step. Blowout Mode. Transfer lysate waste from processing DWP on magnet at G16 to Flush/Wash bowl at G10. Use 1 mL Conductive Tips with WaterBlowout 1 mL DT_FW.prf file. Aspirate using LLS, dispense at labware default. Discard tips.
24. **Flush/Wash 3.**
25. **Move Plate.** Gripper moves 1 mL Nunc DWP on magnet at G16 to E16 support tile.
26. **Move Plate.** Gripper moves Greiner Plate from Shaker F19 to magnet at G16.
27. **Add 125 uL Lysis Wash Buffer to Processing DWP**. Reagent Step. Use fixed tips, waste mode, and replicate dispense x4 into plate at E16. Aspirate with LLS from trough 2 at A16, dispense at 102% well height.
28. **Flush/Wash 4.**
29. **Mix & Transfer 125 uL DNA-Resin to Greiner Processing Plate**. Single liquid transfer step with pre-aspirate mix. Blowout Mode. Transfer Resin-DNA suspension using 175 uL Clear Filter RoboRack Tips at G7 with "WaterBlowout 175 uL DT_FW.prf" file. Mix resin x3 cycles in Lysis Buffer at E16 and transfer 140 uL mixture from 1 mL DWP at E16 to Greiner plate on magnet at G16. Save Tips.
30. **Flush/Wash 5.**
31. **Aspirate Waste from Greiner plate on magnet**. Single liquid transfer step. Blowout Mode. Transfer waste from Greiner Processing Plate at G16 to Flush/Wash bowl at G10. Reuse 175 uL clear filter tips at G7. Aspirate at 1% of well height, dispense at labware default. Discard tips.
32. **Flush/Wash 6.**

33. **Custom Loop - Ethanol Washes (x2)**. Carry out 2 washes using parent/child procedure. A third wash can be added by changing number of samples from 2 to 3. Use Reset Well Map at Start of procedure.
    - **Add 100 uL Wash Buffer**. Reagent Step. Fixed tips. Select "stand-alone" execution mode. The ethanol-isopropanol mixture is multi-dispensed into the Processing Plate to wash the DNA bound to the particles. Aspirate from trough 1 at A16 using LLS.
    - **Move Plate.** Gripper moves Greiner Plate from magnet at G16 to F19 on Shaker.
    - **Start Shaker**. Shake plate using Form 44 and Amplitude 4.
    - **Timer**. Time shaker for 1 min.
    - **Stop Shaker**.
    - **Move Plate.** Gripper moves Greiner Plate from Shaker F19 to magnet at G16.
    - **User Program - FillTipBox..** MSL script function. Fill in exact name of tip box to be reset for this step as shown here.

      MSL_DeleteTipFile("AspirateWasteTips");
    - **Aspirate Waste from Greiner plate on magnet**. Single liquid transfer step. Blowout Mode. Select "stand-alone" execution mode. Aspirate 105 uL wash waste from Processing Plate at G16 to Flush/Wash bowl at G10.
      - **User Program** – ResetGetTipWellMap. MSL script function. Operation Code "G". Operation Number "1". AssemblyChange "No".
      - **Get Tip** - Reuse 175 uL filter tips at G13.
      - **Transfer Group**. Aspirate at 1% of well height, dispense at labware default. Open "Dispense Parameters", select "Advanced" tab, select Reset Well Map to "Start of Procedure".
      - **Drop Tip** – tip box at G13.
    - **Flush/Wash 7**.
34. **Move Plate.** Gripper moves Greiner Plate from magnet at G16 to F19 on Shaker to dry resin off the magnet.
35. **Start Shaker**. Form 20, Amp 4.
36. **Dry Plate.** Air dry for 5 min off the magnet on shaker to evaporate alcohol.
37. **Stop Shaker.**
38. **Add 35 uL of Elution Buffer to Binding Plate.** Reagent Step. Elution reagent in 12-channel trough at C16 (column 12) is replicate dispensed x3 using default aspirate height and 175 uL filter tips at E10. Dispense at 90% well height. Reuse tips.
39. **Flush/Wash 8.**
40. **Move Plate.** Gripper moves Greiner Plate from Shaker F19 to Heater Tile at C22.
41. **Wait for 65 °C temperature**. Test waits for set temperature to reach 70 °C and then will automatically continues 2 min later or you can click "Continue".
42. **Timer 5 min**. Time for 3 min at 65 °C on heater.
43. **Start Shaker**. Shake plate using Form 44 and Amplitude 3.
44. **Timer 5 min**. Time shaker for 3 min with heating at 65 °C.
45. **Stop Shaker**.
46. **Move Plate.** Gripper moves Greiner Plate from Shaker Heater at C22 to magnet at G16.
47. **Transfer DNA.** Single Liquid transfer step. Transfer 35 uL eluted DNA supernatant from Greiner plate at G16 to PCR Collection Plate at E13. Re-use 175 uL filter tips at E10. Aspirate at 1%, dispense at 10% well height. Discard tips. Actual purified DNA volume recovered will vary between 25-30 uL.

48. **Final Flush/Wash.**
49. **Close Heater Comm**.
50. **Close Shaker Comm Port.**

Workstations with and without the Gripper™ Integration Platform

Templates with Gripper. The DNA extraction protocols normally are carried out using the Gripper. Before running the complete protocol for the first time, edit all labware positions used by the gripper.

Templates without Gripper. The DNA IQ Casework template cannot be opened unless the Gripper Integration platform option box is checked during the WinPREP software installation. Modify the template by substituting a **User Message** step to replace each gripper **Move Plate** step. Include instructions with each user message to identify the correct labware deck position on the deck for each manual get and put labware step. During the modified test, each "User Move Plate" step will show a pause window. User will click "OK" when ready to proceed to the next step in the procedure.

Purified DNA Sample Analysis.

Before carrying out DNA typing analysis, purified casework DNA samples are usually quantified using real-time QPCR analysis (Richard et al, 2003; ABI Quantifiler™ Kit), Quantiblot™ Human DNA Quantitation Kit or Promega's AluQuant® Human DNA Quantitation procedure. Each procedure uses ~1-2 µL of DNA.

The MultiPROBE II Forensic Workstation can also be used to automate the quantitation assays and carry out DNA normalization and dilution of samples to a fixed concentration. Finally, the MultiPROBE system can carry out DNA typing reaction PCR setup in 96- or 384-well formats using the Promega Powerplex®, ABI Profiler™ or ReliaGene STR kits. At the Centre of Forensic Science (Toronto, ON), 1 ng of DNA is amplified in a 9600 thermal cycler using the AmpFlSTR® Profiler Plus™ Amplification Kit (Applied Biosystems, Foster City, CA). For more details, see Komonski et al, 2004.

Disposable Tips

Disposable Tip Usage

For each plate of casework DNA samples processed, currently 3 boxes of 1 mL tips are used with the MagnaBot device. If using a magnet designed to hold 2 mL deep well plates, one of the 1 mL tip boxes is eliminated. The protocol also requires 3 boxes of 175 uL non conductive tips.

Minimizing Disposable Tip Usage

The reagent addition step "**13. Mix & Add DNA IQ Resin (50 uL)**" uses 8x 200 uL Conductive tips per procedure. Go to the Flush/Wash tab and select "Per Procedure" parameter under the get tip & drop tip subsection.

Tip Reuse

The RoboRack boxes of disposable tips are reused 2-3 times in the DNA IQ protocol using the "**ResetDropTip**" MSL script in the **Drop Tip/Advanced/Post-Step Function** parameter. Map the tip box labware to the "Drop Tip" step rather than to the Tip Chute. The ethanol wash steps require other custom user scripts to reuse tips in the parent/child loop procedure. For more details, see also the **RoboRack Tip Reuse Guide.pdf** on the install disk.

Performance Files

The performance files for disposable tips used with the Forensic Workstation applications have been modified (as indicated by _FW designation) so that 3 syringe sizes are selected: 250, 500 and 1000 uL. However, this does not change the fact that each of these prf files has been optimized for a defined syringe size, volume and other parameters. Go to Utilities/Performance Set Library/Edit on menu bar to see properties of the new and original prf files.

The automated DNA IQ Casework protocol uses several disposable tip box types and performance files as listed below:

- **1mL Conductive Tips.** Use with WaterBlowout 1 mL DT_FW.prf performance file.
- **175 uL Clear Filter RoboRack Tips.** Use with "WaterBlowout 175 uL DT_FW.prf" file.
- **200 uL Conductive RoboRack Tips.** Use with "WaterWaste 200 uL DT_FW.prf" and "WaterBlowout 200 uL DT_FW.prf " performance files.

Disposable Tip Slide

A new Teflon®-coated Disposable Tip Slide (see **Figure 9**) is included with the MultiPROBE II Forensic Workstation. See **Disposable Tip Slide Guide** (P/N 8842154) in the MultiPROBE/bin/Forensic Workstation/Additional Guides directory folder. The rak file for this labware piece is included on the Forensic Workstation install CD and is installed automatically in the **"**TipChute" category of the WinPREP labware library.

For the DNA IQ Casework application, the slide can be substituted for the standard "TipChute + 1 Trough (8-Tip)" labware placed at deck position E5 in (see **Figure 4**). To make this change, place the coated slide at MultiPROBE II deck position G4. Check that the "Disposable Tip Slide" labware .rak file is correctly mapped to deck position G4 in the WinPREP test.

Protocol Customization

Optimizing Elution Temperature

For the elution step using the automated heater tile, set temperature used for the sample processing plate is 70 °C in order to achieve a sample temperature of 65 °C in the well. The standard heater tiles can be heated to a maximum of 70 °C. Custom heater tiles are available that can achieve temperatures of 95-100 °C.

Elution Plate

Other elution plates can be substituted for the 96-well PCR plate at deck position E13. We have also used 96-well Greiner V-bottom polypropylene microplates for the elution transfer step. Select any of the plate definitions in the WinPREP labware library to replace the "ElutionPlate" at E13.

Low Sample Recovery

If processing samples that have very low amounts of DNA, substitute a Greiner 96 Well U-bottom polypropylene sterile microplate (Greiner Cat# 650261) for the Promega kit processing and collection plates.

Creating "Read Only" Tests

To prevent a WinPREP test from being altered, the test can be converted to a "Read-Only" format. Go to the folder (MultiPROBE/bin directory) containing the target WinPREP .mpt test and click to highlight. Go to Menu bar and select File/Properties/General/Attributes. Click the "Read-Only" box and "Apply" to save.

Cross-contamination Analysis

Samples and buffer blanks are placed in alternating wells in a checkerboard or zebra pattern before processing. One such example is shown in the schematic below.

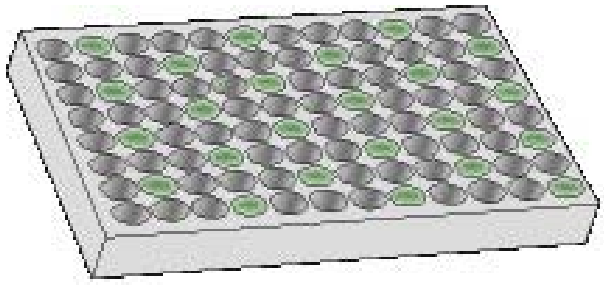


Figure 9. A sample checkerboard scheme used to analyze for samples cross-contamination. Other schemes, "zebra" or "X" patterns, etc., can also be used.

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DNA IQ Precautions and Troubleshooting

- If communication error messages appear when running a test, check that the commport assignments for the devices in the WinPREP tests match the actual physical connections. Go to **My Computer/Properties/Hardware/Device Manager/Ports (COM & LPT)**. The Device Manager window lists all devices connected to the computer. For the DNA IQ Casework protocol, the heater option should be connected via a RS232 cable to **Serial Port 1** and the heater controller connector. The shaker option should be connected via the **USB to Serial Adapter** connector and shaker cable to the first **USB Port 4** (usually upper left I/O port). If different com ports are chosen, be sure that the port number listed in the device manager matches the com port number selected in the WinPREP test for that option. See also the "**USB Serial Adapter Installation Instructions"** for more information.

- To prevent contamination with nucleases or other biological material, use disposable gloves to handle labware and reagents, etc.

- Be sure to add fresh or newly thawed DTT to the Lysis Buffer just before starting the protocol.

- To increase throughput, use fixed tips to dispense denaturing, chaotropic reagents such as Extraction Buffer, Lysis Buffer and alcohol-containing wash buffer. Use disposable filter tips to dispense nuclease-free water or to transfer any sample or waste solution.

- For all reagent wash steps, maintain a dispense height of 102% above the well bottom to prevent cross-contamination of samples. The aspirate height uses normal liquid level sensing parameters.

- Gum samples require special handling. Use small amounts of gum. If too big a piece is placed in well, the disposable tip will stick and drag a gooey trail of gum during transfer step. Add 200 uL more Proteinase K-SDS Extraction Buffer to gum samples before extraction at 37 °C. Transfer 300 uL after extraction as for other samples.

- Do not use the Promega SV DNA Purification Buffers with this protocol.

- Do not put Wash Buffer in tray on deck until just before run because alcohol will evaporate.

- The amplitude of the Micromix DPC shaker can vary between instruments and may need to be adjusted. Start using amplitude 5 and then increase or decrease as needed. Avoid splash-out while providing vigorous mixing of samples.

- The DNA IQ resin settles very quickly and requires tip mixing before aspirate step. Tip mixing the resin particles in a pyramid bottom trough prior to dispensing is more effective for uniform suspension than shaker mixing.

- Add wash reagents to processing plate off the magnet for better mixing. Use both pipet mixing and shaker mixing steps for more effective washing and elution.

- If DNA yields from samples are too low, use polypropylene microplates for the processing (Greiner 96-well U-bottom polypropylene sterile microplate, Cat# 650261) and elution steps. The Promega kit plates A9161 are polystyrene and can absorb DNA.

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Routine Maintenance

- Run Flush/Wash routine each day before running PCR Setup test.
- Check the tip alignment weekly by running the Tip Align test (see Utilities\Diagnostic Tests\Tip Align section of menu bar).
- Use de-aerated water that is molecular biology-grade nuclease-free water (Milli-Q™, NANOpure® DIamond™ or similar) for the system liquid.
- Follow the recommended cleaning procedures in the MultiPROBE II User Manual.

Appendix A

MultiPROBE II Part List Used for DNA IQ System Casework Application

Forensic DNA Isolation Workstation Components: 8-Tip Configuration

MultiPROBE II HT EX with Computer (8-Tip)

Gripper Integration Platform Option

500 uL **OR** 1 mL HT Syringe Assembly (8-Tip) – 1 mL syringes recommended for casework samples

7002307 MPII Forensic DNA Isolation Option, 8-Tip Domestic
OR
7002309 MPII Forensic DNA Isolation Option, 8-Tip International

Includes the following:

- Left Expansion Module
- Kit, VersaTip Plus HT RoboRack (8-Tip)
- DPC MicroMix® 5 Shaker (Right EX)
- Tile, Troughs, MultiPROBE II HT (qty 1)
- Hanging Tip Rack (qty 1)
- 4 Trough Reagent Holder, 60 mL
- Automated Heater Controller (Quad)
- 96-U Heat Transfer Block (PPN 5080165)
- 1.4 mL 96V-bottom DWP Heat Transfer Block (PPN 5083083)
- 96-PCR Heat Transfer Block (PPN 5080163)
- Disposable Tip Slide
- USB to Serial Adapter (for Shaker Option)

(Note: Order 500 uL syringes, not 1000 uL syringes, if instrument is also to be used to carry out casework DNA isolation application).

Forensic DNA Isolation Workstation Components: 4-Tip Configuration

The DNA IQ Casework Extraction application can also be run using a MultiPROBE II 4-Tip system by ordering the following parts:

MultiPROBE II EX with Computer (4-Tip)

7002308 MPII Forensic DNA Isolation Option, 4-Tip Domestic
OR
7002310 MPII Forensic DNA Isolation Option, 4-Tip International

Additional MultiPROBE II Accessories

7607537	Hanging Tip Rack (qty 1)
6000655	1 mL Conductive Tips, MBP, Qty 960
6008103	HT 60 mL Troughs (qty 25)
6000663	Reagent Troughs, 150 mL (qty 25)
6000685	175 uL Clear Filter RoboRack tips, Qty 960
6000681	200 uL Clear Filter RoboRack tips, Qty 960
0101073	USB to Serial Adapter - needed for other options using RS232 communication

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Appendix B

Promega Products Part List

- DNA IQ™ System, 400 samples Cat #DC6700

Each kit contains reagent sufficient for 400 isolations. Includes:

3 mL	Resin
150 mL	Lysis Buffer
79 mL	2X Wash Solution
50 mL	Elution Buffer
2	Protocols (Casework and Database)

- DNA IQ™ System, 100 samples Cat #DC6701

Each kit contains reagent sufficient for 100 isolations. Includes:

0.9 mL	Resin
40 mL	Lysis Buffer
30 mL	2X Wash Solution
15 mL	Elution Buffer
2	Protocols (Casework and Database)

- MagnaBot 96 Magnetic Separation Device (Cat #V8151)
- MagnaBot Spacer, ¼ inch Foam (Cat #Z3301)

Other Components Needed by User

- Ethanol 95%, RNase-free (120 mL per 96 well plate)
- Isopropanol
- 1M Dithiothreitol (DTT)
- Extraction Buffer or Proteinase K solution (SDS, TNE buffer, Proteinase K at 20 mg/mL)
- Innovative Microplate 12-channel reagent block #S30019
- Innovative Microplate 1.4 mL V-bottom polypropylene deep well plate # S30026
- Whatman 2 mL square deep well plate (Cat#7701-5200)
- Greiner 96-well U-bottom polypropylene microplate, sterile (Cat# 650261) to process low DNA amounts
- ABI PRISM® 96-well Optical Reaction Plate w/ Barcode, Code 128 #4306737
- ABI PRISM® Optical Adhesive Covers #431971
- Greiner 96-Well V-Bottom PP Microplates (Cat # 651201, can be used for sample elution)

Appendix C

Publications

For more information on MultiPROBE II-automated DNA IQ purification protocols, see:

PerkinElmer

- Tack, L. and T. Grunst. 2002. Automated Forensic DNA Isolation from Blood using Promega's DNA IQ System and the MultiPROBE II Forensics DNA Workstation. Poster: SAFS Fall Annual Meeting, Sept 30-Oct 3, 2002, Bioloxi, MS.
- Biondi, M., C. Palaski, C. Tomsey, and L. Tack. 2003. Forensic Sample Processing using a Robotic Workstation: Automated Paper-Based Spotting of Whole Blood Convicted Offender Samples and High Throughput DNA Isolation for STR Analysis. 14th International Symposium on Human Identification. Poster Abstract: Sept 29-Oct 2, 2003, Phoenix, AZ.
- Tack, L. 2003. MultiPROBE II Forensic Workstation: Automated DNA Isolation from Database Samples Using the Promega DNA IQ System. PerkinElmer Application Note: AAFS 55th Annual Meeting, Feb18-21, 2003, Chicago IL.
- Tack, L. 2004. Automating Casework and Database Applications with the MultiPROBE II Forensic Workstation. Forensic DNA Technology Workshop, May 19-20, 2004, Toronto ON.

Other

- Promega Technical Bulletin #TB296: "DNA IQ™ System – Small Sample Casework Protocol". 6/02
- Promega Technical Bulletin #TB297: "DNA IQ™ System – Database Protocol". 6/02
- Promega Technical Bulletin #TB307. Tissue and Hair Extraction Kit (for use with DNA IQ™) Protocol.
- Mandrekar, P., L. Flanagan and A. Tereba. 2002. Forensic Extraction and Isolation of DNA from Hair, Bone and Tissue. *Profiles in DNA* 5(2): 11-13.
- Turner, C., R. Weispfenning, E. Vincnet, K. Huston and J. Bessetti. 2003. Genomic DNA purification from cigarette butts and buccal swabs using the DNA IQ™ System. Promega Application Note #AN106.
- P. Mandrekar, L. Flanagan and A. Tereba. 2003. Extraction and Isolation of DNA from Blood Cards and Buccal Swabs in a 96-well Format. Promega Technical Article #AN102. 2/03.
- Richard, M.L., R Frappier, and J. C. Newman. 2003. Developmental validation of a real-time quantitative PCR assay for automated quantification of human DNA. J. Forensic Sci. 48(5):1041-1046.
- Komonski, D., A. Marignani, M. L. Richard, J. Roger H. Frappier and J. C. Newman. 2004. Validation of the DNA IQ™ System for use in the DNA Extraction of High Volume Forensic Casework. Can. Soc. of Forensic Sci. J., Jun. in press.

Web Sites

www.perkinelmer.com

www.promega.com (browse to "Automated Methods")

Trademarks

Appendix D

Protocol Schematic: DNA Isolation from Forensic Casework Samples

WinPREP DNA IQ Casework DNA Purification

Set Heat Tile 1 to 37 °C
Add 300 uL Extraction Buffer to Sample Plate
Automated Shaking
Heat at 37 °C for 30-60 min
Add 550 uL Lysis Buffer to Mixing Plate on Shaker
Add/Mix 50 uL DNA IQ Resin in Lysis Buffer to Mixing Plate
Transfer/Mix 300 uL Sample Extracts to Mixing Plate
Incubate 5 min with Shaking
Set Heat Tile 2 to 65 °C
Mix/Transfer Lysate-Resin Mixture to DWP on Magnet
Incubate 1 min & Aspirate Waste
Move DWP to Deck
Move Greiner Plate to Magnet
Add/Tip Mix 125 uL Lysis Buffer Wash to DWP
Transfer Resin To Greiner Plate on Magnet
Aspirate Waste from Greiner Plate on Magnet
Add 100 uL Wash Buffer to Greiner Plate on Magnet
Move to Shaker & Shake 1 min
Move to Magnet & Aspirate Waste
Repeat Wash Buffer Steps x1
Move Plate to Shaker
Air Dry 5 Min
Add/Tip Mix 35 uL Elution Buffer
Move to Heater Tile 2 on Shaker
Read Temperature 65 °C
Shake and Heat at 65 °C for 5-10 min
Move to Magnet
Transfer DNA to Collection Plate

Figure 10. Schematic for Automated DNA IQ Casework Protocol. List of WinPREP steps used to automate purification of DNA from forensic samples using the DNA IQ Casework Sample DNA Purification Protocol.

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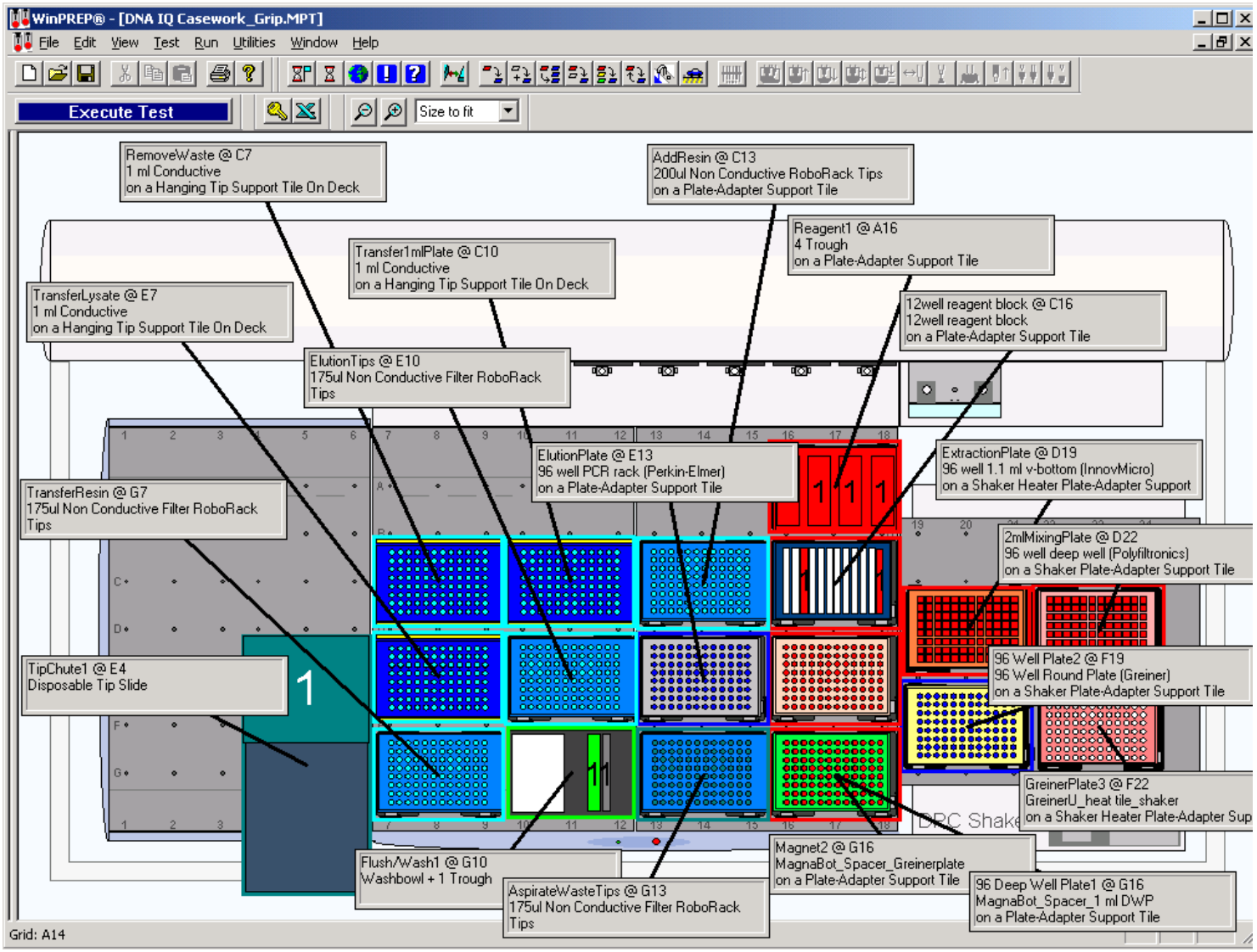
WinPREP® - [DNA IQ Casework_Grip.MPT]
File Edit View Test Run Utilities Window Help
Execute Test
Size to fit
RemoveWaste @ C7
1 ml Conductive
on a Hanging Tip Support Tile On Deck
AddResin @ C13
200ul Non Conductive RoboRack Tips
on a Plate-Adapter Support Tile
Reagent1 @ A16
4 Trough
on a Plate-Adapter Support Tile
Transfer1mlPlate @ C10
1 ml Conductive
on a Hanging Tip Support Tile On Deck
TransferLysate @ E7
1 ml Conductive
on a Hanging Tip Support Tile On Deck
12well reagent block @ C16
12well reagent block
on a Plate-Adapter Support Tile
ElutionTips @ E10
175ul Non Conductive Filter RoboRack
Tips
ElutionPlate @ E13
96 well PCR rack (Perkin-Elmer)
on a Plate-Adapter Support Tile
ExtractionPlate @ D19
96 well 1.1 ml v-bottom (InnovMicro)
on a Shaker Heater Plate-Adapter Support
TransferResin @ G7
175ul Non Conductive Filter RoboRack
Tips
2mlMixingPlate @ D22
96 well deep well (Polyfiltronics)
on a Shaker Plate-Adapter Support Tile
TipChute1 @ E4
Disposable Tip Slide
96 Well Plate2 @ F19
96 Well Round Plate (Greiner)
on a Shaker Plate-Adapter Support Tile
GreinerPlate3 @ F22
GreinerU_heat tile_shaker
on a Shaker Heater Plate-Adapter Sup
Flush/Wash1 @ G10
Washbowl + 1 Trough
AspirateWasteTips @ G13
175ul Non Conductive Filter RoboRack
Tips
Magnet2 @ G16
MagnaBot_Spacer_Greinerplate
on a Plate-Adapter Support Tile
96 Deep Well Plate1 @ G16
MagnaBot_Spacer_1 ml DWP
on a Plate-Adapter Support Tile
Grid: A14

PerkinElmer MultiPROBE II Robotics
Validation for Crime Scene Samples

Introduction

Forensic Biology frequently encounters casework samples from high volume crime cases that included swabs with little DNA present. These are difficult to process due to the low amount of DNA in comparison to samples typically processed using automated systems, such as whole blood samples in paternity testing or a diagnostic laboratory. This validation is concerned with an automated extraction process for use in forensic casework samples.

The MultiPROBE II Forensic Workstation from PerkinElmer Life and Analytical Science was chosen from tender and is used to automate DNA isolation from casework samples using a modified DNA IQ System from Promega. The method was developed by the Centre of Forensic Science (CFS) in Toronto, Ontario, Canada and was designed to process a range of samples in 96 well format, including blood stained paper and cloth, cigarette butts, gum, FTA punches, and swab swipes. Modifications were made by CFS to the Promega method to increase the overall extraction efficiency.

These included:

- Decrease the extraction/lysis temperature
- Replace the initial extraction using the kit lysis buffer with a digestion step using Proteinase K-SDS at 37ºC.
- Addition of an extraction step using proteinase K-SDS at 37ºC to replace the high temperature incubation in the kits lysis buffer
- Decreasing the number of resin washes
- Decreased elution volume.

Following low temperature extraction, the Promega kit Lysis buffer (x2 volumes) plus DNA IQ resin is added to the cell lysate to capture genomic and mitochondrial DNA. The normal kit purification process is then followed for the rest of the protocol. After ethanol washing, the bound DNA is eluted at 65ºC and transferred to a clean polypropylene 96 well collection plate. The DNA sample is eluted in 35µl of Elution Buffer. All steps are carried out using standard deep well plates and microplates.

The workstation used for this application consists of the basic MultiPROBE II Plus HTEx with Gripper integration Platform, DPC MicroMix 5 Shaker and Automated Heater Controller Option with two custom heat transfer block inserts.

The MultiPROBE II system automates all liquid handling, magnetic separation, heating and shaking/mixing steps used in the DNA IQ based protocol, minimizing user error and increasing productivity. The MultiPROBE II workstation can routinely extract 48 samples in approximately 2.5 hours with minimal hands on time by the forensic staff. This results in a walk away, automated extraction method that is efficient, reliable, sensitive and accurate process.

WinPREP Software

WinPrep is the MultiPROBE II software program where you can define and run tests and control the instrument.

DNA IQ System Kits

Chelex is the current method of DNA extraction in Forensic Biology. The extraction basically involves lysing the cells open (with Proteinase K) to release the DNA from the nucleus. Chelex resin is used in the procedure from the time the cells are lysed. Chelex is a styrene divinylbenzene copolymer containing paired iminodiacetate ions that act as chelating groups in binding to polyvalent metals. Chelex removes metal ions from specimens, leaving behind the purified DNA.

Chelex is a simple, rapid DNA extraction method that does not involve organic solvents. However it can leave amplification inhibitors and is not easily performed by automation. The Promega DNA IQ kit can be easily adapted for robotic platforms, namely the MultiPROBE II by PerkinElmer.

Promega's DNA IQ System kit allows automation of the paramagnetic resin used during the extraction process that purifies the DNA without requiring extensive washing to remove the lysis reagent. This system is designed to rapidly purify small quantities of DNA, approximately 100ng or less and it is claimed to become more efficient with samples containing less than 10ng DNA, such as trace DNA. During the extraction process, the resin binds the DNA and then becomes bound to a magnetic stand allowing for easy removal of all liquids, inhibitors and cellular debris without centrifugation.

The resin does not appear to affect any downstream applications such as quantitation and profiling with Applied Biosystem's Quantifiler Human DNA and Profiler Plus Amplification kits. In addition, Promega have stated that no clogging of capillaries or any effect on the lifetime of the capillaries when using samples that have been purposely contaminated with resin has occurred during capillary electrophoresis. This is most likely due to the fact that the DNA IQ Resin has a neutral charge and can not be loaded onto the capillary during electrokinetic injection of DNA. However, if trace amounts of resin are present in the eluted DNA, the resin can be removed by placing the sample on the magnetic stand and transferring the supernatant to a new tube.

The DNA IQ System –Small sample Casework Protocol is specifically designed for samples that contain less than 100ng of DNA. DNA extracted using this method will be of varying concentration as the amount of resin added is in excess.

Types of sample from which DNA has been isolated from using the DNA IQ System.

- Blood (fresh and frozen EDTA, Citrate, Heparin, ACD)
- Blood Stains (from FTA paper, cotton, denim, soil, leather, surface to swab)
- Buccal (Cotton, rayon, FTA paper)
- Hair
- Tissues (fresh and fixed)
- Cigarette Butts
- Toothbrushes
- Envelopes
- Urine
- Bone.

Figure 1. DNA IQ Casework Extraction Protocol – Original Protocol.

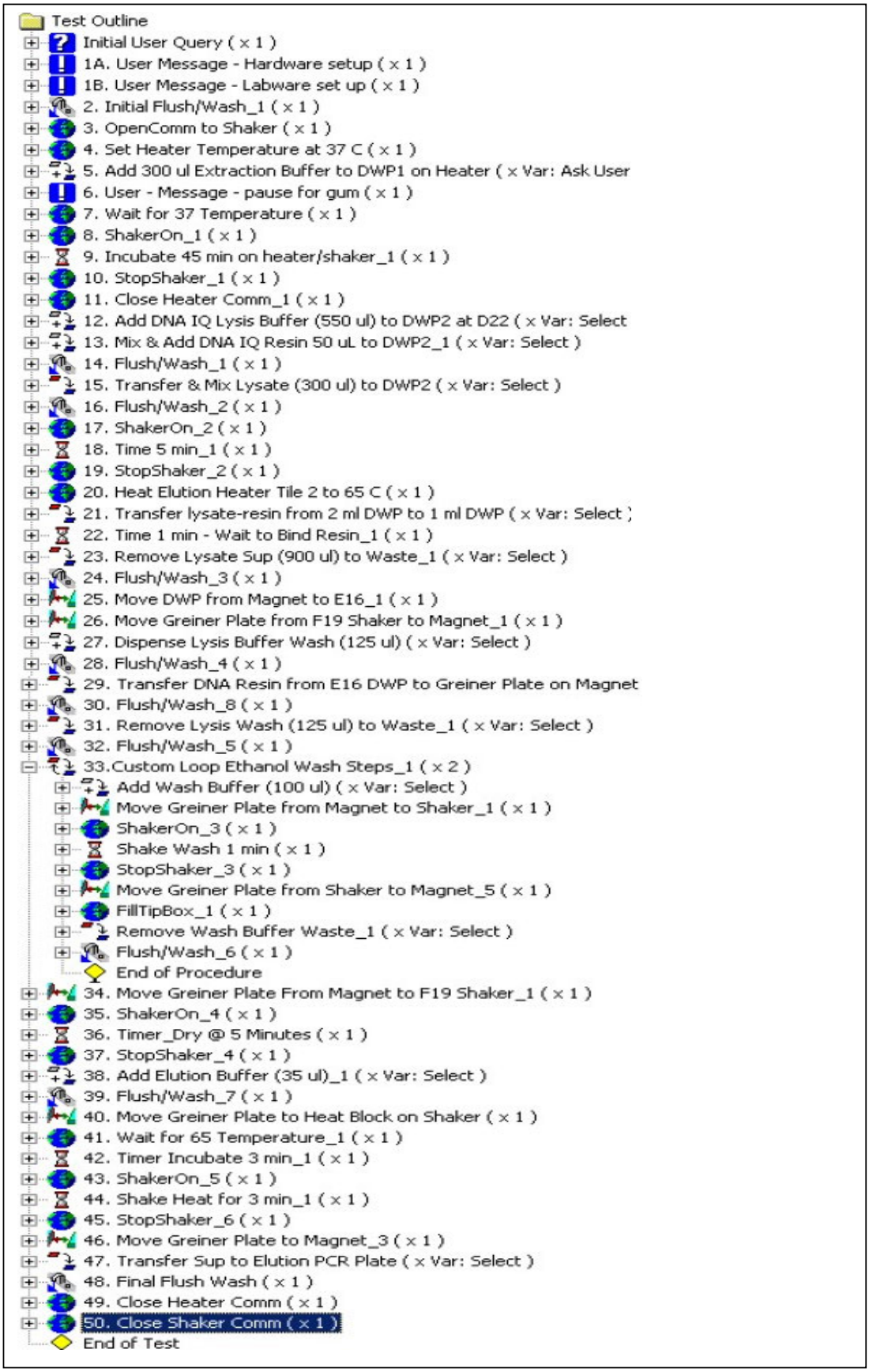

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Figure 2. PathWest DNA IQ Casework Extraction Protocol – Final Protocol -Amended

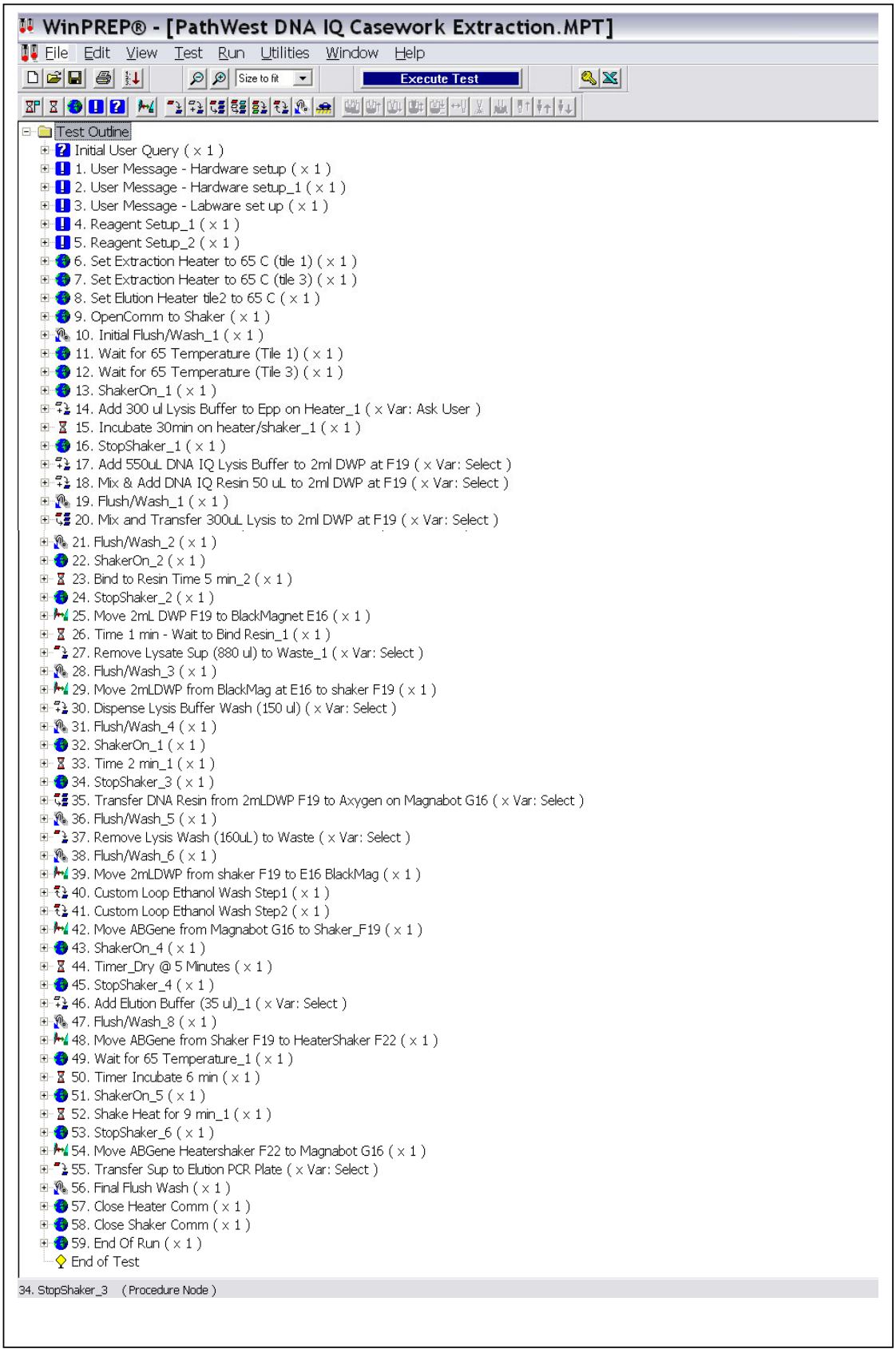
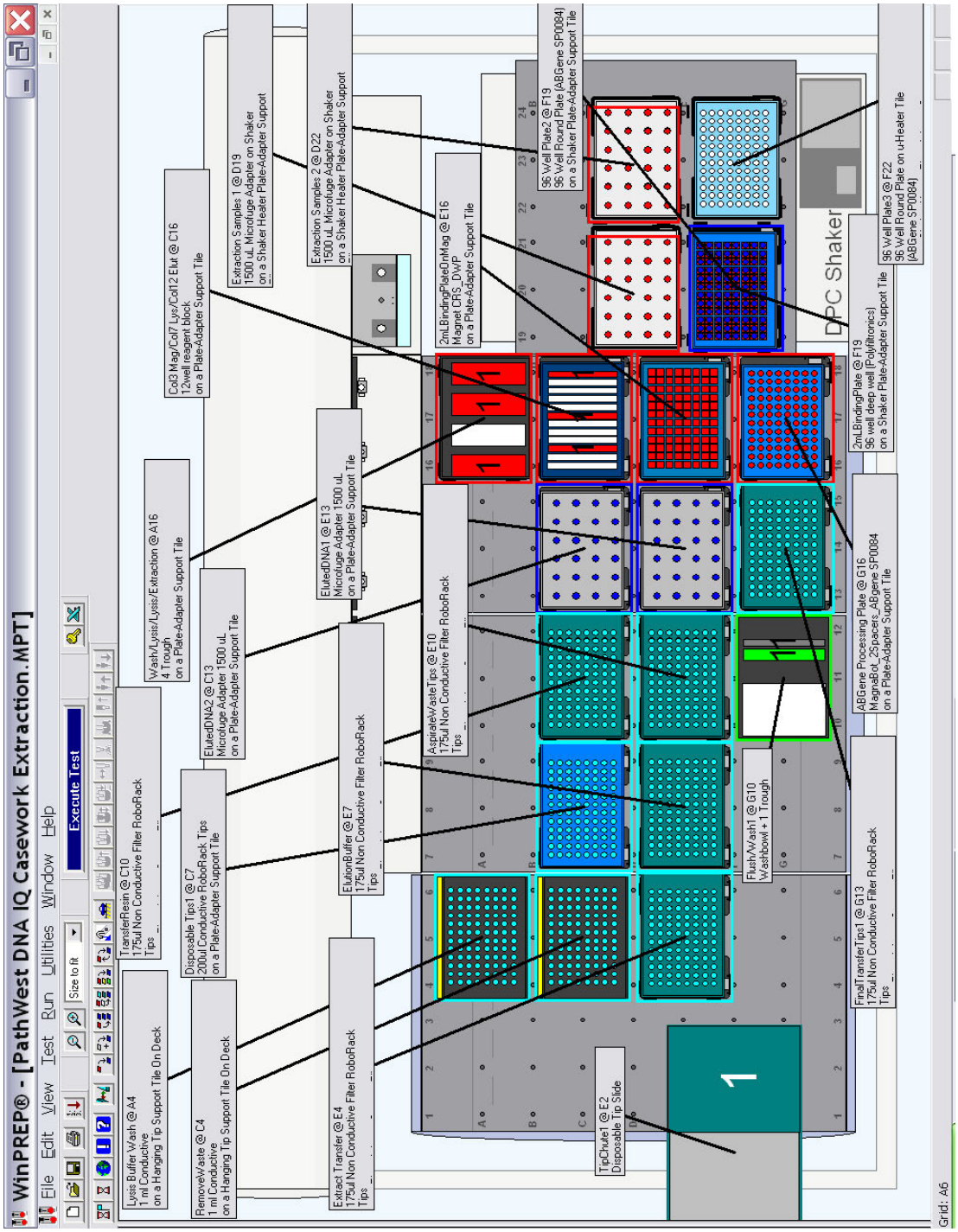


Figure 3. MultiPROBE II Deck Layout and Test Configuration for PathWest DNA IQ Casework Extraction Protocol



Internal Extraction Controls

For each extraction run performed two internal controls are utilised, a positive control and a negative control (blank). A positive internal extraction control has been utilised involving 1µl of EDTA blood from a female staff member with a known profile that has been aliquoted onto a Copan Swab. This control ensures that the extraction has been completed successfully, all reagents were effective, and incubations were performed at a suitable temperature.

Both the Positive and Negative internal extraction controls acts as a contamination control, to ensure that there has been no cross contamination from sample to sample.

These controls are placed randomly throughout the extraction run.

For the purpose of this validation 1µl LST Positive Control was utilised. The known profile can be seen in table 1 below.

Table 1. Known DNA profile of the Internal Extraction Positive Control.

Sample	D3S1358	vWA	FGA	Amel	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820
1µl LST Pos Ctl	14, 16	16, 20	24, 24	X, X	11, 13	28, 30	14, 17	11, 12	12, 13	8, 11

Materials and Methods

DNA Samples

- All DNA samples extracted on the MultiPROBE II robotics platform consisted of various volumes of EDTA blood from staff members with known profiles aliquoted onto sterile Copan swabs.
- One female staff sample with known profile was utilised to optimise the DNA extraction method at 1µl, 5µl and 10µl.
- Sample tracking samples consisted of 10µl of EDTA blood of known profiles from 38 different staff members.
- Mixture experiments consisted of combining EDTA blood in various volumes from one male and one female staff member that were either very similar or very different in DNA profiles. Cell counts were obtained on these samples to compare.
- Overloaded samples consisted of saturating a sterile Copan swab with EDTA blood from staff members (approximately 70-80µl of blood was added to Copan swabs)

Consumables

The following is a list of consumables used on the MultiPROBE II Liquid Handling System.

- DNA IQ System – Small Sample Casework Protocol (Promega Cat # DC6700)
- 95-100% Ethanol (BDH Cat# 10107.7Y)
- Isopropyl Alcohol (AJAX Fine Chemicals Cat # A425)
- 1M DTT (BDH Cat# 441494N)
- Proteinase K (Merck Cat#VL398168513)
- Microfuge tubes 1.5ml (Promega Cat # V1231)
- 96-deep well Whatman plate (Whatman Cat# 7701-5200)
- 96-well AB Gene plate (AB Gene Cat# AB-0796)
- Innovative Microplate 12 Trough (Innovative Microplates Cat# S30019)
- Mircopipette Tips
    - 175µl Non Conductive Tips (PerkinElmer Cat# 6000685)
    - 200µl Non Conductive Tips (PerkinElmer Cat# 6000681)
    - 1000µl Hanging Tips (PerkinElmer Cat# 6000655)
- Lysol (PerkinElmer Cat# 6000652)

Extraction Methods

Promega DNA IQ Extraction Kit

Unless otherwise stated all extraction methods were performed as per the manufacturer's instructions.

Chelex Extraction Method

The Chelex DNA extraction method was performed as per current Forensic Biology method (FBDNA005).

Automated Extraction Method

Unless otherwise stated all automated extraction methods followed the PathWest DNA IQ System for Casework DNA Isolation as per the program installed on the MultiPROBE II Robotics platform by PerkinElmer.

Quantitation

All samples were quantitated using the Applied Biosystems Quantifiler™ Human DNA Quantification Kit (P/N 4343895) on the ABI Prism 7500 according to the current Forensic Biology Method (FBPCRS003, FBRT001, FBRT002).

AmpFlSTR Profiler Plus Amplification Kit Genotyping Assay.

Short Tandem Repeat (STR) analysis was performed for a selection of samples. Analysis was completed using the standard Forensic Biology Amplification method (FBPCRS001) followed by profiling on a 3100 Genetic Analyser (FBPCR021). Results were analysed with GeneScan Software V 3.7 and Genotyper software V3.7.

Decontamination

All liquid handling steps are concluded with a flush/wash step. This involves an adjustable volume of fluid (degassed sterile water) flushing through the system, including the lines and fixed tips into the waster bottle. A flush/wash is done at the start and completion of each extraction run. An eight hour with lysol decontamination of the robotic platform is performed weekly.

Experiments

The following Experiments were performed in this Validation

- Comparison of Differing extraction methods.
    - DNA IQ Systems Kit
    - Chelex
- Comparison of Robotics Platform and Manual Extraction Methods
    - Manual Extraction Method
    - Robotic Extraction Method
- Various methods of extraction DNA from Swabs
    - Liquid samples only
    - Removal of swab heads from shaft
- Differing incubation temperatures
- Differing lysis solution
- Sample Tracking
- Contamination
    - Zebra Crossing
    - Horizontal Crossing
    - Checkerboards
- Reproducibility
- Overloaded samples
- Mixtures.
- Minimum input DNA.
- Re-elution of Extracts
- Performing overnight extractions

Experiment 1 Comparison of Differing Extraction Methods.

Experiment A

Promega DNA IQ™ System

Aim

The aim of this experiment is to establish whether the DNA IQ System kits are a suitable commercially available kit to use in regards to ease of use, and DNA recovery.

Method

Each of the sample swab head were cut off the shaft and dissected into 6-8 pieces and placed into a sterile microfuge tube. Once the sample was stored in a microfuge tube the Promega DNA IQ™ System (P/N DC6700) – small sample casework protocol was used as per kit instructions for Cotton Swab Material using the Promega MagneSphere® Technology Magnetic Separation Stand (P/N Z5342). No changes were made to this method.
This method was performed manually using the magnetic separation stand.

Samples used:

2x staff members EDTA blood was aliquoted onto sterile Copan™ swabs at various volumes 1µl, 5µl and 10µl. Each sample was performed multiple times (5x 1µl, 5x 5µl and 5x 10µl samples) over several extraction runs by different staff members.

The following samples were extracted by the Promega DNA IQ method.

Table 2. Sample Storage codes for samples extracted using the Promega DNA IQ method.

Staff	DNA Storage Codes
GRT	V095B7 – V095D2
LST	V095D3 – V095E8
GRT	V095E9 – V095G4
LST	V095I9 – V096A4
GRT	V098A8 – V098C3
LST	V097B7 – V097D2

Quantitation

All samples were quantitated using the Applied Biosystems Quantifiler™ Human DNA Quantification Kit (P/N 4343895) on the ABI Prism 7500 according to the current Forensic Biology Method (FBPCRS003).

Profiling

A selection of extracts were profiled using Applied Biosystems Profiler Plus Amplification Kit (P/N 4303326) on the 3100 Genetic Analyser as per the current Forensic Biology method (FBPCR021).

Results

The mean DNA concentration results for samples extracted using the Promega DNA IQ extraction kit were tabulated in the table below for each volume of EDTA blood extracted. Total final eluate volume is 35µl DNA.

Table 3. Mean DNA yields from Promega DNA IQ kits for various samples.

Staff	Volume	Mean	Std Dev	Total DNA (ng)
LST	1µl	0.8449	0.3715	**29.5**
LST	5µl	2.642	0.76664	**92.5**
LST	10µl	4.47133	2.3145	**156.5**
GRT	1µl	1.64627	0.65552	**57.6**
GRT	5µl	6.00267	3.52097	**210.1**
GRT	10µl	9.6493	6.64197	**337.7**

Experiment B

Chelex DNA Extraction Method

Aim

The aim of this experiment is to establish a baseline level of DNA concentrations to compare against other extraction methods.

Method

All samples were extracted using the current Forensic Biology DNA Extraction-Casework Method (FBDNA005) with 5% Chelex (BioRad P/N 143-2832). No changes were made to this method.

The following samples were extracted using the current Forensic Biology Casework DNA method:

Table 4. Sample Storage codes for samples extracted using the current Forensic Biology method using Chelex.

Staff	DNA Storage Codes
LST	V100H3 – V100I8
GRT	V101G3 – V101H8
GRT	V103B1 – V103C1
GRT	V104J10 – V105B10

Results

The mean DNA concentration results for samples extracted using the current Forensic Biology method were tabulated in the table below for each volume of EDTA blood extracted. Total final eluate volume is 200µl DNA.

Table 5. Mean DNA yields from Chelex Extraction method for various samples.

Staff	Volume	Mean	Std Dev	Total DNA (ng)
LST	1µl	0.1154	0.1368	**23.1**
LST	5µl	1.1184	0.4434	**223.7**
LST	10µl	2.858	0.621	**571.6**
GRT	1µl	0.407	0.138	**81.5**
GRT	5µl	1.565	0.505	**313.1**
GRT	10µl	4.038	1.247	**807.6**

Conclusion

Table 6. Comparison of total DNA yields for Chelex Extraction and DNA IQ Extraction methods.

Staff	Volume	Chelex Extraction Total DNA (ng)	DNA IQ Extraction Total DNA (ng)
LST	1µl	**23.1**	**29.5**
LST	5µl	**223.7**	**92.5**
LST	10µl	**571.6**	**156.5**
GRT	1µl	**81.5**	**57.6**
GRT	5µl	**313.1**	**210.1**
GRT	10µl	**807.6**	**337.7**

From the above results it can be seen that the DNA IQ method of extraction does produce slightly lower yields of DNA than our current chelex extraction method. However it must also be noted that DNA IQ guarantees to obtain up to 100ng of DNA per sample. Samples expecting to give a greater yield than this may have reduced efficiency in extraction. It can be seen that Promega DNA IQ performs effectively with lower DNA yields such as trace DNA in which this system will be predominantly used for. The greater the volume of blood, the lower the yield of DNA per µl of blood.

When using our current methods of profiling samples, 0.5ng to 1.25ng of total DNA is required to perform subsequent downstream sample processes. From this we can conclude that the DNA IQ method of extraction provides sufficient yields of DNA for all analysis tests currently performed in Forensic Biology.

Experiment 2 Comparison of Robotics Platform and Manual Extraction Methods

Aim

The aim of these experiments is to compare how efficient the MultiPROBE II robotic platform is in comparison to manual extraction techniques of the same method in relation to final DNA yield and time efficiency.

Experiment A Manual plate.

Method

The initial starting swab was cut off the shaft and cut into 6-8 pieces and placed into a microfuge tube. Samples were extracted manually as per the protocol that was installed on the robotics platform by PerkinElmer named DNA IQ 2 Magnet Casework Extraction Method. All pipetting steps (aspirating and dispensing) were performed offline on the bench by staff and the 96-well magnetic tile was also utilised by staff offline. The heater tile on the robotic platform was used for all incubation stages and shaking steps were performed online using the DPC shaker.

The following samples were extracted using the DNA IQ 2 Magnet Casework Extraction method manually by staff.

Table 7. Sample Storage codes for samples extracted using the DNA IQ 2 Magnet Casework Extraction method manually by staff.

Staff	DNA Storage Codes
LST	V096A5 – V096B10
GRT	V096C1 – V096D6
GRT	V096D7 – V096F2
LST	V096F3 – V096G8
LST	V097J2 – V098A7
GRT	V098C4 – V098D9

Results

The mean DNA concentration results for samples extracted using the PerkinElmer DNA IQ 2 Magnet Casework Extraction method manually by staff were tabulated in the table below for each volume of EDTA blood extracted. Total final eluate volume is 35µl DNA.

Extracts were quantitated and a selection of extracts from this experiment were profiled. These experiments were predominantly performed to optimise the DNA extraction method to obtain maximum DNA yield.

Table 8. Total DNA yields obtained by the Manual Extraction Offline using the DNA IQ 2 Magnet Casework Extraction Method

Staff	Volume	Mean	Std Dev	Total DNA (ng)
LST	1µl	0.0741	0.0486	2.6
LST	5µl	0.15979	0.1279	5.5
LST	10µl	0.18715	0.0789	6.6
GRT	1µl	0.10726	0.09892	3.8
GRT	5µl	0.13637	0.06515	4.8
GRT	10µl	0.2003	0.115122	7.0

Experiment B Automated Robot

The initial starting swab was cut off the shaft and cut into 6-8 pieces and placed into a microfuge tube. Samples were extracted as per the protocol that was installed on the platform by PerkinElmer named DNA IQ 2 Magnet Casework Extraction Method. The robotics platform performed all steps including all pipetting, dispensing, mixing, incubating and shaking.

The following samples were extracted using the DNA IQ 2 Magnet Casework Extraction method on the MultiPROBE II.

Table 9. Sample Storage codes for samples extracted using the automated DNA IQ 2 Magnet Casework Extraction method.

Staff	DNA Storage Codes
GRT	V097D3 – V097E8
LST	V097H6 – V097J1
LST	V098D10 – V098F5
GRT	V098H2 – V098I7

Results

Samples were quantitated using Applied Biosystems Quantifiler Human DNA Quantitation Kit. The mean DNA concentration results for samples extracted using the fully automated DNA IQ 2 Magnet Casework Extraction method were tabulated in the table below for each volume of EDTA blood extracted. Total final eluate volume is 35µl DNA.

Several extracts from this experiment were profiled. These experiments were predominantly performed to optimise the DNA extraction method to obtain maximum DNA yield.

Table 10. Total DNA yields obtained by the Automated DNA IQ 2-magnet casework extraction method.

Staff	Volume	Mean	Std Dev	Total DNA (ng)
LST	1µl	0.1717	0.1343	6.01
LST	5µl	0.34289	0.19906	12.00
LST	10µl	0.7592	0.3931	26.57
GRT	1µl	0.24462	0.17917	8.56
GRT	5µl	0.42307	0.27081	14.81
GRT	10µl	0.9556	0.628636	33.45

Conclusion

Table 11. Comparison of the total DNA yields (ng) for the Manual versus Automated extraction methods.

Staff	Volume	Manual Total DNA (ng)	Fully Automated Total DNA (ng)
LST	1µl	2.6	6.01
LST	5µl	5.5	12.00
LST	10µl	6.6	26.57
GRT	1µl	3.8	8.56
GRT	5µl	4.8	14.81
GRT	10µl	7.0	33.45

From the table above we can see that the fully automated robotics platform has given greater DNA yields than the manual extraction technique of the same method. However both methods are significantly lower in DNA yield when compared to the DNA IQ manual kit insert method and our current forensic biology method using Chelex. These results can be seen in the Table 12 below.

Comparison of results from Experiment One and Two.

Table 12. Comparison of the Final DNA volumes (ng) for all methods tested.

		Chelex	DNA IQ Kit Systems	2-Magnet CW Extn – Manual	2-Magnet CW Extn – Automated	Expected Results with DNA IQ
LST	1µl	23.1	29.5	2.6	6.0	6-10
	5µl	223.7	92.5	5.5	12.0	30-50
	10µl	571.6	156.5	6.6	26.6	60-100
GRT	1µl	81.5	57.6	3.8	8.6	6-10
	5µl	313.1	210.1	4.8	14.8	30-50
	10µl	807.6	337.7	7.0	33.4	60-100

There were no significant differences in profiles. DNA samples extracted using these methods of extraction, were analysed on the 3100 Genetic Analyser. Some extracts showed allelic imbalance for all extraction methods especially the robot fully automated methods. DNA extracts from these methods were repeated for amplification to eliminate any possible post extraction problem. The allelic imbalance was improved and the results showed no significant difference between the different types of extraction methods tested. All DNA profiles for all methods showed peak heights of approximately 400-800 RFU.

Although there is minimal difference in profile peak heights obtained, the current 2-Magnet method on the MultiPROBE II is giving significantly lower DNA yields when compared to the current forensic biology casework DNA extraction method and the Promega DNA IQ manual extraction method. As the robotics platform will be utilised to extract trace DNA from samples maximum DNA yields are required to be obtained. Further experiments will be performed to optimise DNA yield utilising the MultiPROBE II.

It has been noted that the 96 well heater tile does not properly fit the AB Gene 96 well plates. A correct replacement heater tile is still on order from PerkinElmer. It is unknown how much heat loss is occurring using the existing tile in the incubation stages.

Graph 1. Graphical representation of the total DNA yield of the1µl LST positive control for differing extraction methods for several extractions.

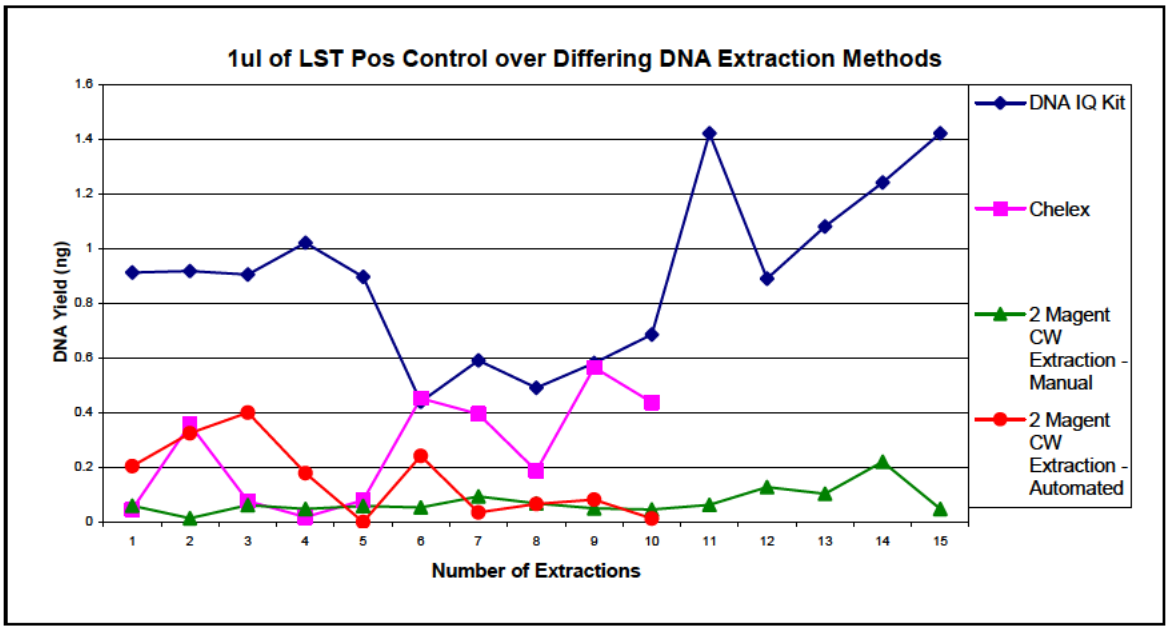


Figure 4. 1µl LST Positive Control extraction by our current Chelex Forensic Biology method

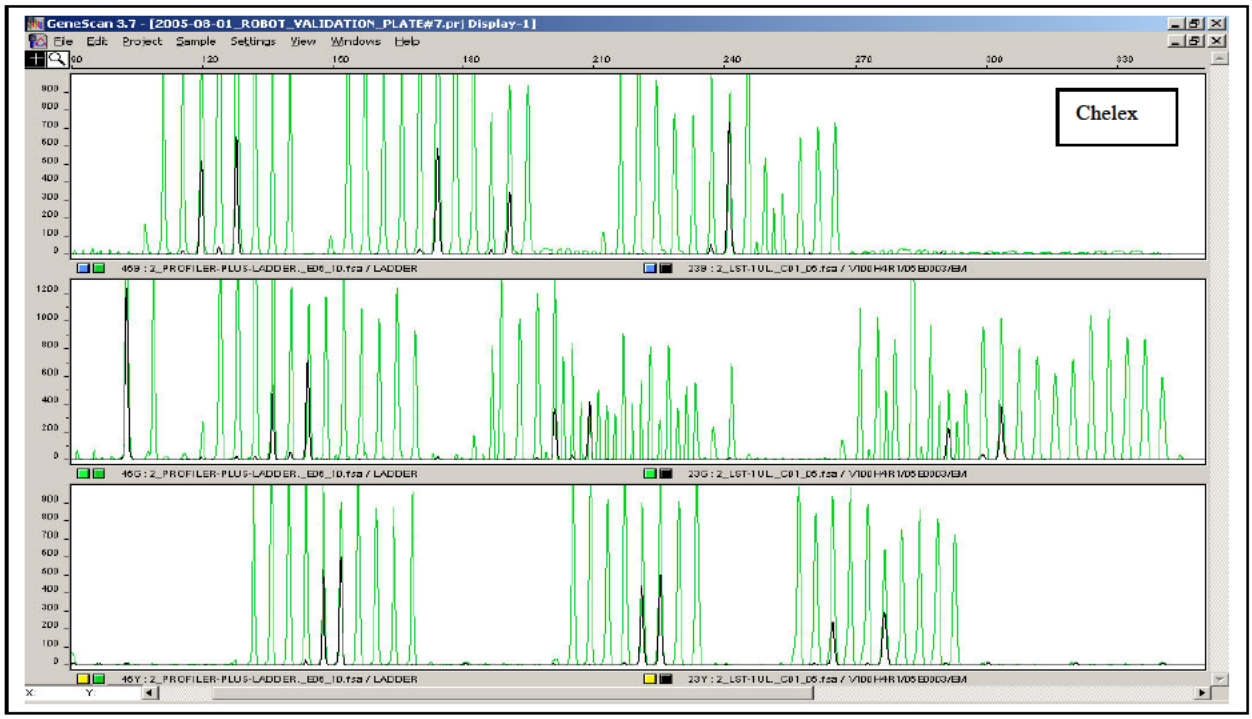


Figure 5. 1µl LST Positive Control extraction by Promega DNA IQ Kit

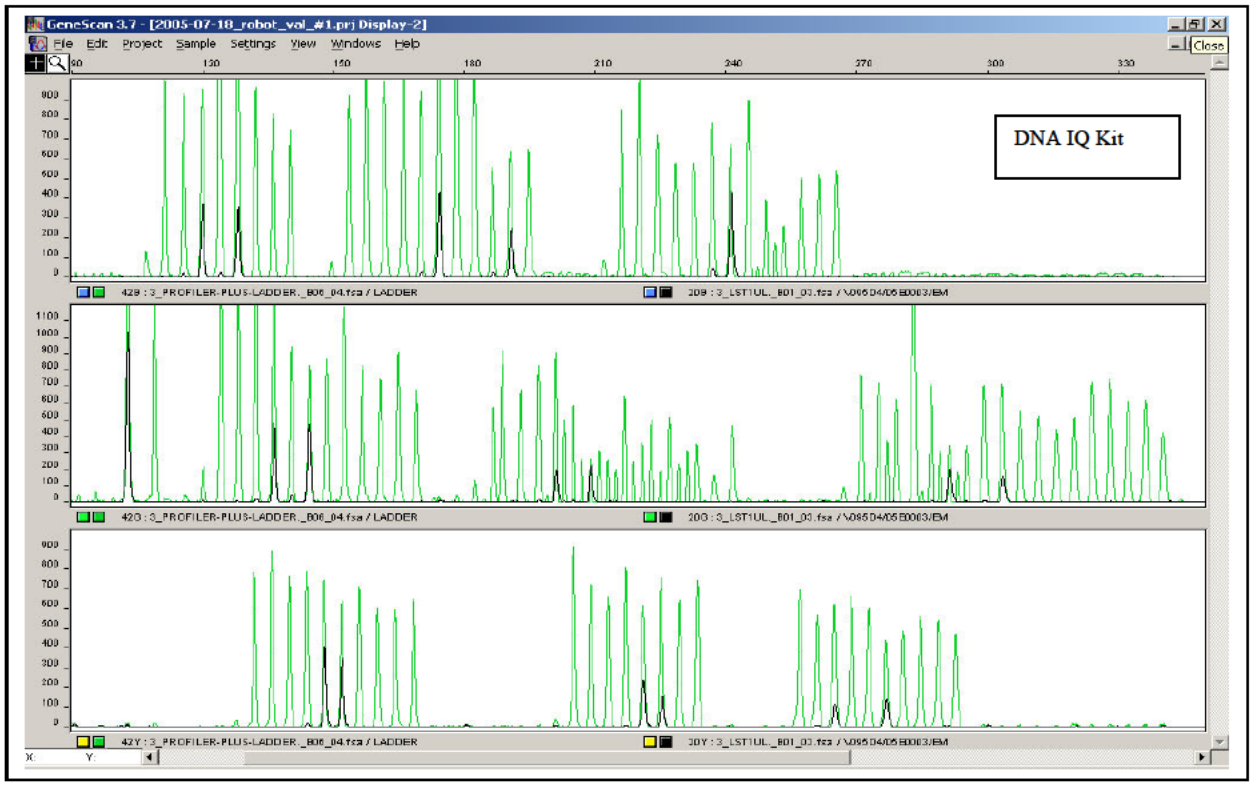


Figure 6. 1µl LST Positive Control extraction by 2 Magnet Casework DNA fully Automated.

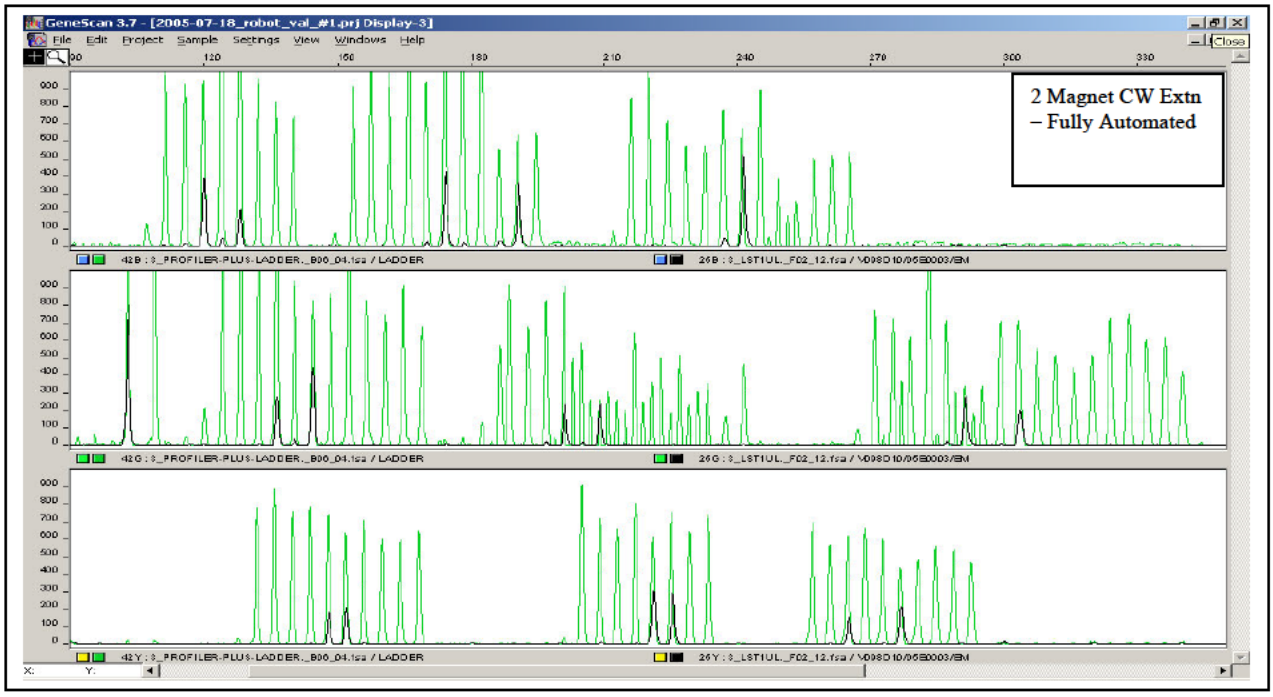
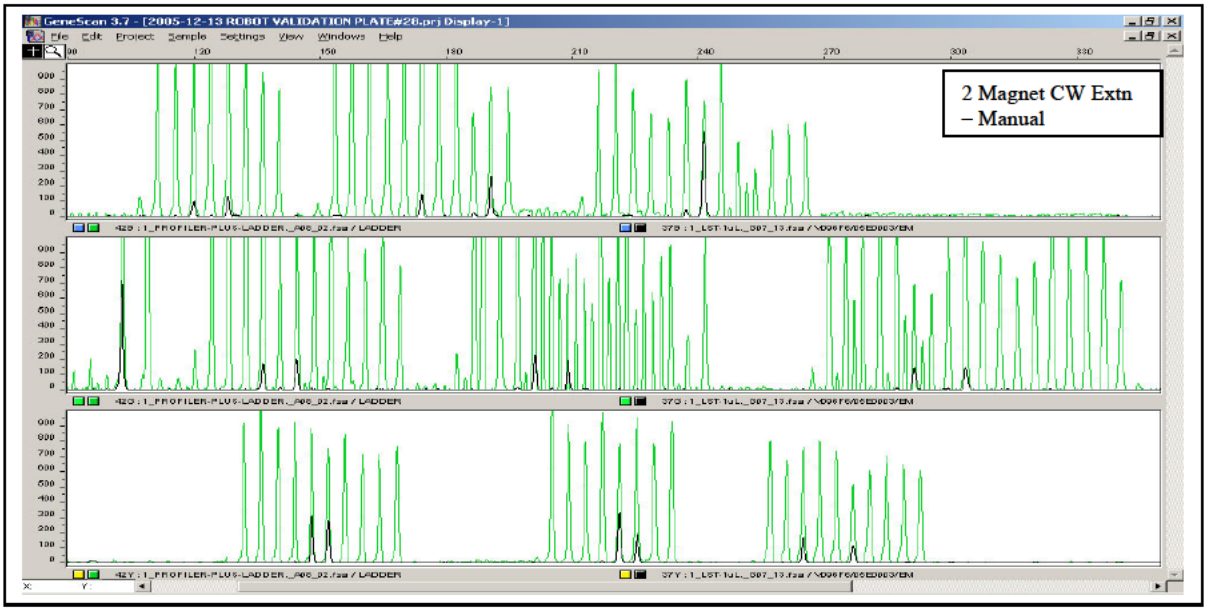


Figure 7. 1µl LST Positive Control extraction by 2 Magnet Casework DNA – Manual Extraction



Experiment 3 Various methods of extracting DNA from swabs.

Experiment A Liquid Sample Swabs

Aim

The aim of these experiments was to trial different initial starting techniques for obtaining the sample swab head into a microfuge tube that is then placed onto the robotic platform for extraction.

Method

The sample swab head was cut off the shaft and dissected into 6-8 pieces and placed into a microfuge tube containing extraction buffer (as per method volume). This was then incubated at 56ºC for 2 hours in heat blocks. After incubation the extraction buffer and swab pieces were removed, spin baskets (Promega P/N V1221) were added to the microfuge tube and the extraction buffer with sample swab were added into the spin basket. Samples were not vortexed during this incubation period. The microfuge tubes were centrifuged for 2 mins at 1300rpm. The spin baskets containing the swab were discarded and the microfuge tube containing liquid only was added to the robotic platform. Samples were extracted as per the protocol that was installed on the robotics platform by PerkinElmer named DNA IQ 2 Magnet Casework Extraction Method from Step 15.

The following samples were extracted using the DNA IQ 2 Magnet Casework Extraction method on the MultiPROBE II from a liquid starting material.

Table 13. Storage Codes for samples extracted from a liquid starting material.

Staff	DNA Storage Codes
LST	V098F6 – V098H1
GRT	V098I8 – V099A3
GRT	V100A9 – V100C4

Results

Samples were quantitated using Applied Biosystems Quantifiler Human DNA Quantitation Kit. The mean DNA concentration results for samples extracted using the fully automated DNA IQ 2 Magnet Casework Extraction method were tabulated in the table below for each volume of EDTA blood extracted. Total final eluate volume is 35µl DNA.

Extracts from this experiment were not profiled. These experiments were predominantly performed to optimise the DNA extraction method to obtain maximum DNA yield.

Table 14. Total DNA yields obtained by the Automated DNA IQ 2-magnet casework extraction method.

Staff	Volume	Mean	Std Dev	Total DNA (ng)
LST	1µl	0.2163	0.1525	7.57
LST	5µl	0.3198	0.38218	11.19
LST	10µl	0.5406	0.5841	18.92
GRT	1µl	0.351	0.16301	12.29
GRT	5µl	0.5811	0.31369	20.34
GRT	10µl	0.932	0.549824	32.62

Conclusion

It was noted that this experiment was extremely laborious and the use of spin baskets had an increase concern in possibility increasing contamination. The Promega MagneSphere® Technology Magnetic Separation Stand was also very difficult to use with an increased possibility of creating contamination.

Although the DNA yields obtained with this method were comparable to the automated 2-magnet casework extraction method, it was decided with the number of disadvantages of this technique and an increased possibility of contamination to not investigate this variation of the extraction any further.

Experiment B Swab heads cut off the shaft

Aim

The aim of these experiments was to trial different initial starting techniques for obtaining the sample swab head into a microfuge tube that is then placed onto the robotic platform for extraction.

Method

The swab head was cut off at the shaft and placed whole into a microfuge tube. Samples were extracted as per the protocol that was installed on the robotics platform by PerkinElmer named DNA IQ 2 Magnet Casework Extraction Method. The robotic platform performed all stages of this extraction method, including pipetting, dispensing, mixing, incubating, shaking.

The following samples were extracted using the DNA IQ 2 Magnet Casework Extraction method on the MultiPROBE II.

Table 15. Storage Codes for samples extracted by removal of swab heads.

Staff	DNA Storage Codes
LST	V099E1 – V099F6

Results

Samples were quantitated using Applied Biosystems Quantifiler Human DNA Quantitation Kit. The mean DNA concentration results for samples extracted using the fully automated DNA IQ 2 Magnet Casework Extraction method were tabulated in the table below for each volume of EDTA blood extracted. Total final eluate volume is 35µl DNA.

Extracts from this experiment were not profiled. These experiments were predominantly performed to optimise the DNA extraction method to obtain maximum DNA yield.

Table 16. Total DNA yields obtained by the Automated DNA IQ 2-magnet casework extraction method.

Staff	Volume	Mean	Std Dev	Total DNA (ng)
LST	1µl	0.723	0.3345	25.31
LST	5µl	1.5215	0.67097	53.25
LST	10µl	2.65725	0.6671	93.0

Conclusion

The initial run was successful in this method, however in subsequent runs several disadvantages to this method were noted. The robotic probes with this method crashed into the plastic shaft of the cotton head on the swab, creating error messages and possible damage to the Teflon tips resulting in the run to be aborted. The disposable tips were prone to getting stuck into the swab head and on occasion the swabs were transferred from the microfuge tube to the 96 deep well plate, creating possible contamination concerns.

Although the DNA yields obtained from this method were excellent in comparison to the 2-Magnet Casework DNA extraction method, the disadvantages with the increased possibility of contamination resulted in any further analysis and investigation of this method to be aborted.

Experiment 4 Differing Incubation Styles.

Experiment 4A-D.

It was noticed that the 96well plates supplied by AB Gene (P/N AB-0796) do not seat properly on the heater tile. A replacement part was requested to be custom made and is on order. This was noticed in the installation of the robotics platform by PerkinElmer Liquid Handling Specialist Tanja Albers. The current heater tile is suspected of giving reduced DNA yields in comparison to the expected yield with DNA IQ kit due to possible poor heat transfer. The Elution Buffer is added in order to release the DNA from the resin. The efficiency of the elution hinges upon the temperature at which materials are heated. If the sample is not heated sufficiently, the yields of DNA obtained will be lower than expected. Experiments 4A-D are performed with incubations offline in an incubator and/or heat blocks in place of the heater tile.

Experiment 4A. Manual Extraction with Final Incubation Performed Offline.

Aim

The aim of this experiment is to ensure optimal DNA yields from samples by performing the final 65ºC incubation stage offline in an incubator. This experiment was performed manually by staff utilising the robotic heating tile and DPC shaker.

Method

The initial starting swab was cut off the shaft and cut into 6-8 pieces and placed into a microfuge tube. Samples were extracted manually as per the protocol that was installed on the platform by PerkinElmer called DNA IQ 2-Magnet Casework Extraction Method.

All pipetting steps (aspirating and dispensing) were performed offline on the bench by staff and the 96-well magnetic tile was also utilised by staff offline. The heater tile on the robotic platform was used for the initial 56ºC incubation stage only and shaking steps were performed online using the DPC shaker. The final 65ºC incubation for 15 minutes was performed offline in a Binder (Crown Scientific) incubator. Gentle agitation was performed manually, approximately every 1-2mins for 5secs.

The following samples were extracted using the DNA IQ 2 Magnet Casework Extraction method manually by staff with the final 65ºC incubation stage performed offline in an incubator.

Table 17. Sample Storage codes for samples extracted using the DNA IQ 2-Magnet Casework Extraction method manually with the 65ºC incubation performed offline.

Staff	DNA Storage Codes
GRT	V101H9 – V101J4
GRT	V104I4 – V104J8

Results

Samples were quantitated using Applied Biosystems Quantifiler Human DNA Quantitation Kit. The mean DNA concentration results for samples extracted using the manual DNA IQ 2-Magnet Casework Extraction method with the final incubation offline were tabulated in the table below for each volume of EDTA blood extracted. Total final eluate volume is 35µl DNA.

Several extracts from this experiment were profiled. These experiments were predominantly performed to optimise the DNA extraction method to obtain maximum DNA yield.

Table 18. Total DNA yields obtained by the Manual DNA IQ 2-magnet casework extraction method with the final 65ºC incubation offline.

Staff	Volume	Mean	Std Dev	Total DNA (ng)
GRT	1µl	0.2839	0.2054	9.94
GRT	5µl	0.80479	0.53556	28.17
GRT	10µl	0.52326	0.2771	18.31

Experiment 4B. **Automated Extraction with Final Incubation Performed Offline.**

Aim

The aim of this experiment is to ensure optimal DNA yield from samples by performing the final 65ºC incubation stage offline in an incubator. This experiment was an automated extraction utilising the MultiPROBE II system.

Method

The initial starting swab was cut off the shaft and cut into 6-8 pieces and placed into a microfuge tube. Samples were extracted as per the protocol that was installed on the platform by PerkinElmer named DNA IQ 2-Magnet Casework Extraction Method. The robotics platform performed all steps including all pipetting, dispensing, mixing, initial incubating and shaking. However the final 65ºC incubation for 15 minutes was performed offline in a Binder incubator by staff. Gentle agitation was performed manually, approximately every 1-2mins for 5secs.

The following samples have been extracted using the DNA IQ 2 Magnet Casework Extraction method on the MultiPROBE II with the final incubation step offline in a 65ºC incubator.

Table 19. Storage Codes for DNA extracts performed with final incubation offline.

Staff	DNA Storage Codes
GRT	V104E3 – V104F8
GRT	V101E7 – V101G2

Results

Samples were quantitated using Applied Biosystems Quantifiler Human DNA Quantitation Kit. The mean DNA concentration results for samples extracted using the Automated DNA IQ 2 Magnet Casework Extraction method with the final incubation offline were tabulated in the table below for each volume of EDTA blood extracted. Total final eluate volume is 35µl DNA.

Several extracts from this experiment were profiled. These experiments were predominantly performed to optimise the DNA extraction method to obtain maximum DNA yield.

Table 20. Total DNA yields obtained by the Automated DNA IQ 2-magnet casework extraction method with the final 65ºC incubation offline.

Staff	Volume	Mean	Std Dev	Total DNA (ng)
GRT/LST	1µl	0.1746	0.092	6.11
GRT/LST	5µl	0.9163	0.4727	32.07
GRT/LST	10µl	1.1151	0.8422	39.03

Experiment 4C. Manual Extraction with Both Incubations Performed Offline.

Aim

The aim of this experiment is to ensure optimal DNA yields from samples by performing the both the 56ºC and 65ºC incubation stages offline in an incubator. This experiment was performed manually by staff utilising the robotic DPC shaker.

Method

The initial starting swab was cut off the shaft and cut into 6-8 pieces and placed into a microfuge tube. Samples were extracted manually as per the protocol which was installed on the platform by PerkinElmer called DNA IQ 2-Magnet Casework Extraction Method. The robotics platform performed all steps including all pipetting, dispensing, mixing, and shaking. The initial 56ºC incubation was performed in heat blocks and samples were vortexed every 15-20minutes for 10 seconds. The final 65ºC incubation was performed offline in a Binder (Crown Scientific) incubator with gentle agitation performed manually, approximately every 1-2mins for 5secs.

The following samples were extracted using the DNA IQ 2 Magnet Casework Extraction method manually by staff with all incubation stage performed offline in an incubator.

Table 21. Sample Storage codes for samples extracted using the DNA IQ 2-Magnet Casework Extraction method manually by staff with both incubations performed offline.

Staff	DNA Storage Codes
GRT	V106A4 – V106B9
GRT	V101J5 – V102A10

Results

The following samples have been extracted using the DNA IQ 2 Magnet Casework Extraction method on the MultiPROBE II with all incubation steps performed offline.

Table 22. Total DNA yields obtained by the manual DNA IQ 2-magnet casework extraction method with all incubation offline.

Staff	Volume	Mean	Std Dev	Total DNA (ng)
GRT/LST	1µl	0.1734	0.1008	6.07
GRT/LST	5µl	0.39947	0.26047	13.98
GRT/LST	10µl	0.57967	0.4741	20.29

Experiment 4D. Automated Extraction with Both Incubations Performed Offline.

Aim

The aim of this experiment is to ensure optimal DNA yields from samples by performing the both the 56ºC and 65ºC incubation stages offline. This experiment was an automated extraction utilising the MultiPROBE II system.

Method

The initial starting swab was cut off the shaft and cut into 6-8 pieces and placed into a microfuge tube. Samples were extracted on the MultiPROBE II as per the protocol that was

installed on the platform by PerkinElmer called DNA IQ 2-Magnet Casework Extraction Method.

All pipetting steps (aspirating and dispensing) were performed online. The shaking steps were performed online using the DPC shaker. The initial 56ºC incubation was performed in heat blocks and samples were vortexed every 15-20minutes for 10 seconds. The final 65ºC incubation was performed offline in a Binder (Crown Scientific) incubator with gentle agitation performed manually, approximately every 1-2mins for 5secs.

The following samples have been extracted using the automated DNA IQ 2 Magnet Casework Extraction method on the MultiPROBE II with all incubations stages performed offline.

Table 23. Storage Codes for DNA extracts with all incubations offline.

Staff	DNA Storage Codes
LST	V106E4 – V106F9

Results

Table 24. Total DNA yields obtained by the Automated DNA IQ 2-magnet casework extraction method with all incubation offline.

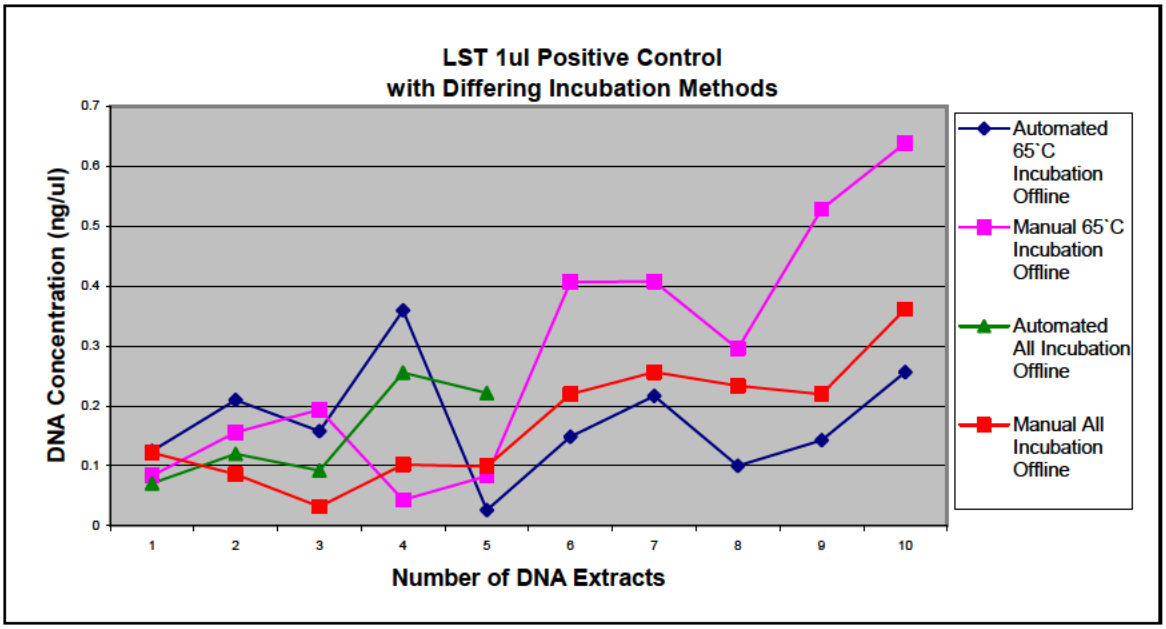
Staff	Volume	Mean	Std Dev	Total DNA (ng)
LST	1µl	0.1522	0.082	5.33
LST	5µl	0.3938	0.10952	13.78
LST	10µl	0.57474	0.3617	20.12

RESULTS for All Experiments (2A-B and 4A–D).

Table 25. Comparison of results for all Experiments using the MultiPROBE II method.

Experiment	Method	LST		
		1µl	5µl	10µl
Experiment 2A	2-magnet CW extn – manual.	2.6	5.5	6.6
Experiment 2B	2-magnet CW extn – automated.	6.0	12.0	26.6
Experiment 4A	Manual – 65°C offline	9.94	28.17	18.31
Experiment 4B	Automated - 65°C offline	6.11	32.07	39.03
Experiment 4C	Manual – all incubations offline	6.07	13.98	20.29
Experiment 4D	Automated - all incubations offline.	5.33	13.78	20.12
	Expected results with DNA IQ.	6-10	30-50	60-100

Graph 2. Graphical representation of 1ul of Positive Control with Differing Incubation Methods.



Conclusion

From the above results there is minimal difference between all incubation performed offline and those performed with incubation online. There is still a large discrepancy in the results obtained and the expected results from the kits. In communication with PerkinElmer it was suggested that the lysis buffer from the DNA IQ Kit be used in place of the Extraction Buffer from the 2-magnet casework method installed by PE.

This was performed with several variations made to the method involving incubation times and temperature. They are listed below in Experiments 5A-C.

Experiment 5 Differing Lysis Solutions.

Aim

The Promega DNA IQ system kit is giving comparable DNA yields to the current extraction method in Forensic Biology using 5% Chelex. Similar DNA yields are desirable for samples on the robotic platform.

The aim of these experiments is to increase the automated DNA yields by replacing the extraction buffer with the lysis buffer in the DNA IQ kit as recommended by Tanja Albers from PerkinElmer.

Method

The extraction buffer utilised in the 2-magnet casework DNA extraction method was replaced with lysis buffer from the Promega DNA IQ systems. The Promega method incubates the lysis buffer at 95ºC for 30mins where the PerkinElmer Robotics Systems incubates the Extraction buffer at 56ºC for 2 hours. In this experiment both temperatures (95ºC and 65ºC) and time (30mins and 2 hours) variations were trialled with the Promega lysis buffer replacing the extraction buffer of the 2-magnet casework extraction method. Volumes remained unchanged (300µl).

Experiment 5A 65ºC Initial incubation for 30 minutes.

Aim

To establish whether the Promega DNA IQ lysis buffer will give greater DNA yields compared to the Extraction Buffer from the PerkinElmer MultiPROBE II method when incubated at 65ºC for 30minutes.

Method

The extraction method was fully automated on the MultiPROBE II with the newly designed heater tile from PerkinElmer. The Promega DNA IQ lysis buffer replaced the PerkinElmer extraction buffer. The initial starting swab was cut off the shaft into 6-8 pieces and placed into a microfuge tube. The robotic platform performed all steps including pipetting, dispensing, mixing, shaking and incubating. The normal 56ºC incubation in extraction buffer for 2 hours was replaced with the DNA IQ lysis buffer (containing DTT) incubated at 65ºC

for 30 mins on the robotic platform. Volumes used for DNA IQ lysis buffer was 300µl as per kit instructions.

The following samples have been extracted using the automated DNA IQ 2 Magnet Casework Extraction method on the MultiPROBE II at 65ºC for 30 minutes.

Table 26. Storage codes for samples incubated at 65ºC for 30 minutes.

Staff	DNA Storage Codes
LST	V115B6 – V115D1
LST	V115F6 – V115H1
LST	V124D7 – V124F2
LST	V124C1 – V124D6

Results

Table 27. DNA yields for extracts incubated at 65ºC for 30 minutes.

Staff	Volume	Mean	Std Dev	Total DNA (ng)
LST	1µl	0.8398	0.2394	29.39
LST	5µl	2.445	1.08597	85.58
LST	10µl	2.6738	1.0363	93.58

Experiment 5B. 95ºC Initial Incubation for 30 Minutes.

Aim

To establish whether the Promega DNA IQ lysis buffer will give greater DNA yields compared to the Extraction Buffer from the PerkinElmer MultiPROBE II method when incubated at 95ºC for 30minutes.

Method

In this experiment, the extraction method was fully automated on the MultiPROBE II however, all incubations were performed offline as this experiment was performed prior to the arrival of the new heater tile. The Promega DNA IQ lysis buffer replaced the PerkinElmer extraction buffer. The initial starting swab was cut off the shaft into 6-8 pieces and placed into a microfuge tube. The robotic platform performed all pipetting, dispensing, mixing and shaking stages. The normal 56ºC incubation in extraction buffer for 2 hours was replaced with the DNA IQ lysis buffer (containing DTT) incubated at 95ºC for 30 mins. 300µl of

DNA IQ lysis buffer was used as per kit instructions. The 95ºC incubation was performed in heat blocks for 30mins and the 65ºC incubation was performed in a Binder incubator. There was no mixing during the 95ºC incubation. Gentle agitation was performed by hand for the 65ºC incubation in the 96well plate approx every 1-2mins for 5secs. The 96well plate was put back on the robot to complete the final transfer into microfuge tubes.

The following samples have been extracted using the DNA IQ 2 Magnet Casework Extraction method on the MultiPROBE II at 95ºC for 30 minutes.

Table 28. Storage codes for samples incubated at 95ºC for 30 minutes.

Staff	DNA Storage Codes
LST	V116F3 – V116G8
LST	V106J5 – V107A10
LST	V107B1 – V107C6

Results

Table 29. DNA yields for extracts incubated at 95ºC for 30 minutes.

Staff	Volume	Mean	Std Dev	Total DNA (ng)
LST	1µl	1.0637	0.3833	37.23
LST	5µl	4.23867	1.57578	148.35
LST	10µl	5.73267	1.8049	200.64

Experiment 5C 65ºC Initial incubation for 2 hours.

Aim

To establish whether the Promega DNA IQ lysis buffer will give greater DNA yields compared to the Extraction Buffer from the PerkinElmer MultiPROBE II method when incubated at 65ºC for 2 hours.

Method

The extraction method was fully automated on the MultiPROBE II with the newly designed heater tile from PerkinElmer. The Promega DNA IQ lysis buffer replaced the PerkinElmer extraction buffer. The initial starting swab was cut off the shaft into 6-8 pieces and placed

into a microfuge tube. The robotic platform performed all steps including pipetting, dispensing, mixing, shaking and incubating. The normal 56ºC incubation in extraction buffer for 2 hours was replaced with the DNA IQ lysis buffer (containing DTT) incubated at 65ºC for 2 hours in a Binder incubator. Samples were mixed by agitation on the shaker during the incubation period. Volumes used for DNA IQ lysis buffer was 300µl as per kit instructions.

The following samples have been extracted using the automated DNA IQ 2 Magnet Casework Extraction method on the MultiPROBE II at 65ºC for 2 hours.

Table 30. Storage codes for samples incubated at 65ºC for 2 hours.

Staff	DNA Storage Codes
LST	V120C1 – V120D6
LST	V120D7 –V120F2
LST	V124H7 – V124J2

Results

Table 31. DNA yields for extracts incubated at 65ºC for 2 hours.

Staff	Volume	Mean	Std Dev	Total DNA (ng)
LST	1µl	0.4634	0.2073	16.22
LST	5µl	2.05967	0.95969	72.09
LST	10µl	1.73467	0.6129	60.71

Conclusion

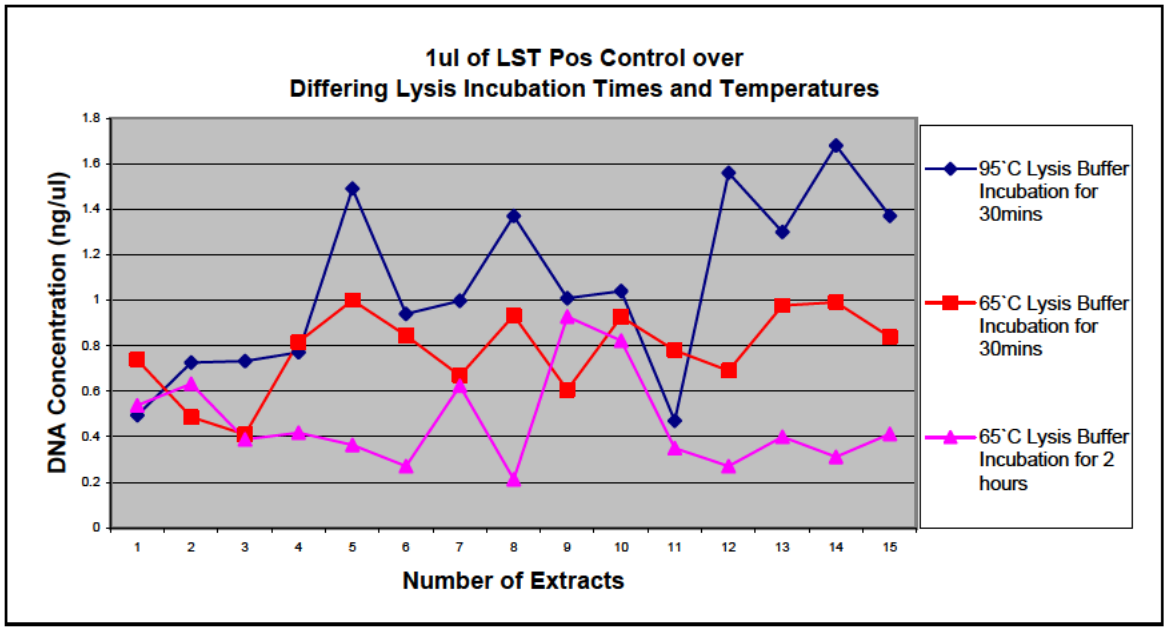
Table 32. Comparison of Differing Lysis Incubation Times and Temperatures.

	Staff	Volume	Mean	Std Dev	Total DNA (ng)
Expected Range		1µl			6-10
		5µl			30-50
		10µl			60-100
95ºC for 30mins	LST	1µl	1.0637	0.3833	37.23
		5µl	4.238	1.5757	148.35
		10µl	5.732	1.80	200.64
65ºC for 30mins	LST	1µl	0.7436	0.197	29.39
		5µl	1.86	0.54	85.58
		10µl	1.9416	0.49	93.58
65ºC for 2 hours	LST	1µl	0.5204	0.233	16.22
		5µl	1.708	0.919	72.09
		10µl	1.539	0.359	60.71

Although the 95ºC 30 min lysis incubation experiment indicates significantly greater DNA yields, profiles obtained are lower in peak height and therefore in the number of reportable alleles when compared to the 65ºC 30min lysis incubation. On average the 95ºC 30min incubation indicated peak heights at approx 200RFU and partial profiles (5/9 profiles were partial). It was possible that the 95ºC incubation degraded the DNA samples.

The 65ºC 30min incubation indicates on average peak heights ranging from 400-1000RFU with the majority of samples being full profiles (2/9 partial profiles).
These profiles are comparable to the profiles obtained from using the 2-magnet casework DNA method supplied with the robotics systems with the added DNA yield.

The 65ºC, 30min incubation with the Promega DNA IQ systems lysis buffer was selected as the method of choice for DNA extraction. This method was chosen due to the increase in DNA yield obtained when comparing lysis buffer to extraction buffer. 65ºC was chosen over 95ºC due to the lower partial profiles obtained. Additionally, 95ºC incubation had the added concern of excess evaporation and increased user handling by incubating on heat blocks and not automated on the robot. The robotic platform currently does not have heater tiles that can incubate at 95ºC. The 30 min incubation was chosen over 2 hours as similar peak heights with greater DNA yields were obtained, therefore the more time efficient method was selected.



Graph 3. Graphical representation of Differing Lysis Incubations Times and Temperatures.

Figure 8. 1µl of LST Positive Control DNA extracted using the MultiPROBE II with the initial 65ºC incubation using Promega DNA IQ Lysis Buffer for 30 minutes.

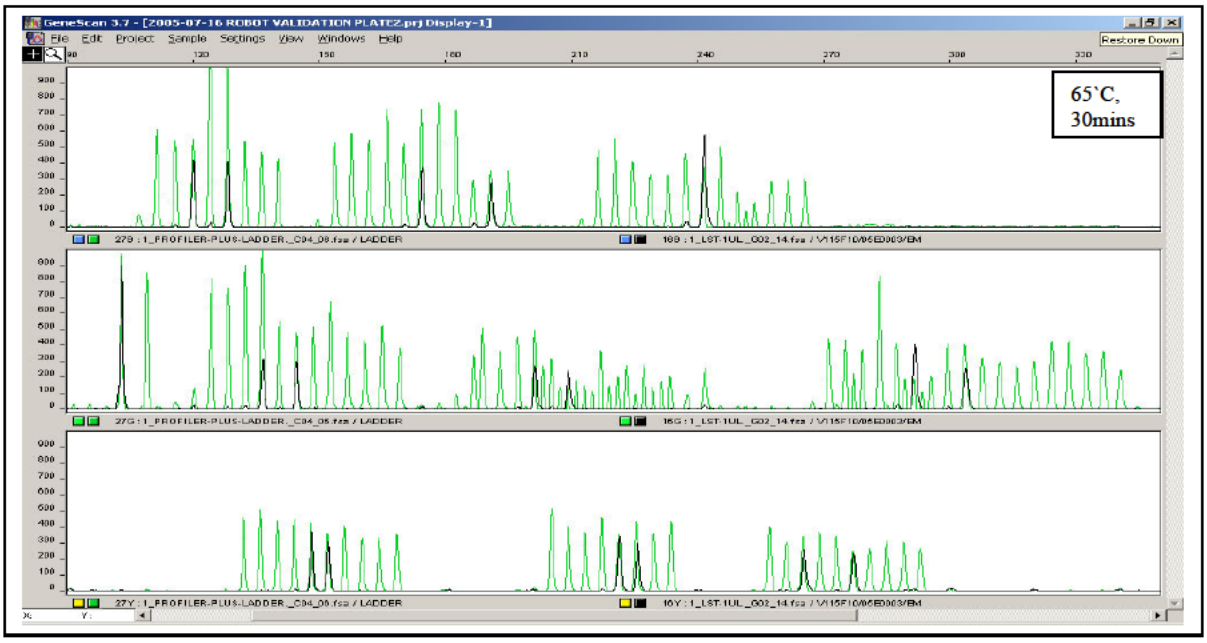


Figure 9. 1µl of LST Positive Control DNA extracted using the MultiPROBE II with the initial 95ºC incubation using Promega DNA IQ Lysis Buffer for 30 minutes.

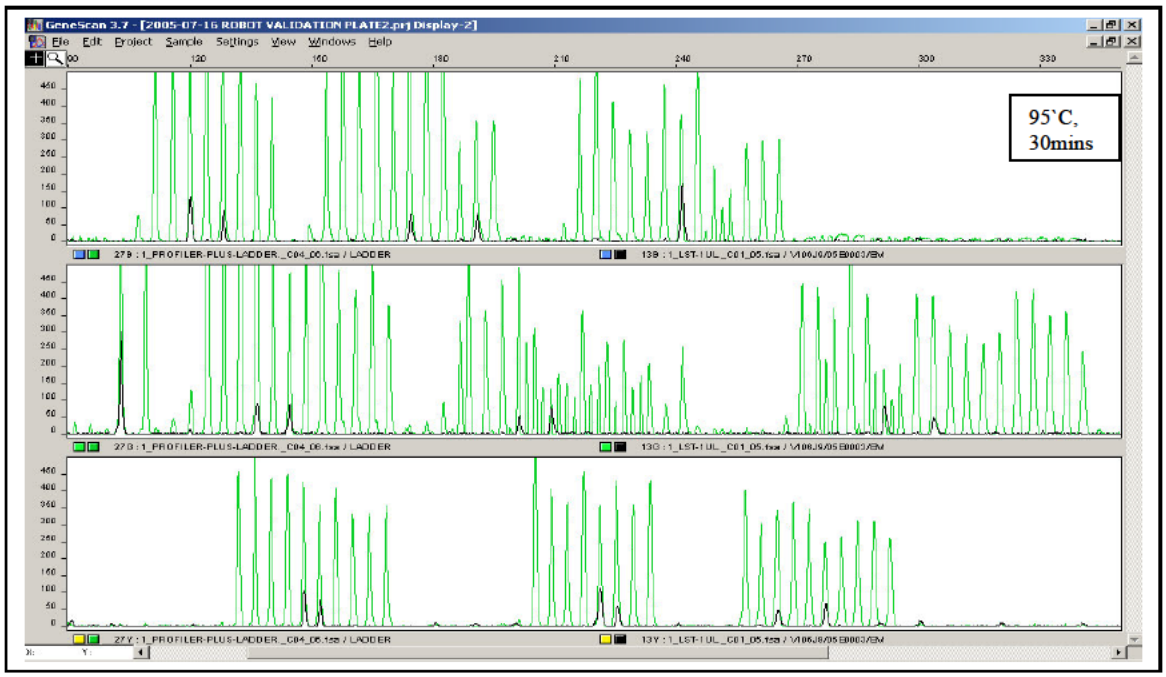
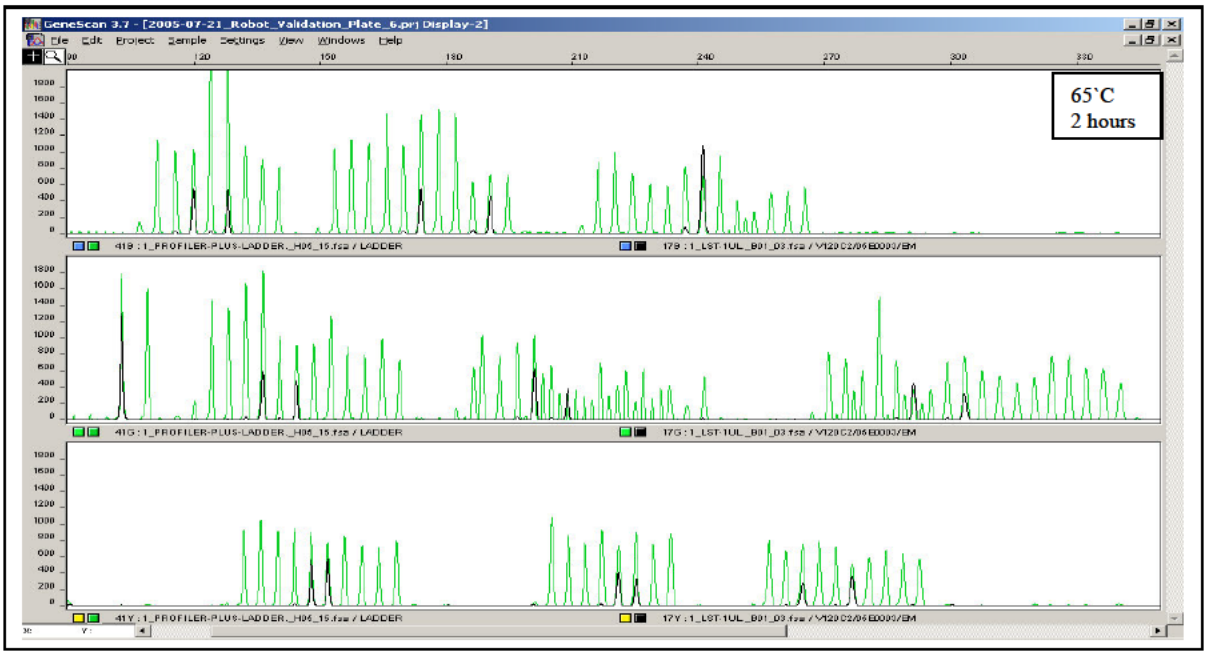


Figure 10. 1µl of LST Positive Control DNA extracted using the MultiPROBE II with the initial 65ºC incubation using Promega DNA IQ Lysis Buffer for 2 hours.



Experiment 6 Sample Tracking.

Aim

In this experiment the aim was to determine if the robotic system transfers samples to the correct microfuge tubes throughout the extraction process. For example: if sample A is placed into microfuge tube 1 will the final eluate be transferred to a sterile microfuge tube located at position 1 at the completion of the extraction.

Method

38 unique staff samples and 2 randomly placed blanks were tested. Staff samples consisted of 10µl of EDTA blood aliquoted onto a sterile Copan Swab. The swab head was dissected into 6-8 pieces and placed into a microfuge tube on the platform. The amended 2-Magnet casework DNA method was utilised (incubation at 65ºC for 30 minutes in DNA IQ kit lysis buffer). This experiment was repeated three times to ensure accuracy of the platform.

All DNA extracts were quantitated using the Applied Biosystems Quantifiler™ Human DNA Quantification Kit (P/N 4343895) on the ABI Prism 7500 according to the current Forensic Biology Method (FBPCRS003).

All samples were then profiled using the Applied Biosystems Profiler Plus Kit (P/N 4303326) on the ABI Prism 3100 Genetic Analyser. Results were interpreted using both Genotyper and GeneScan V3.7.1 software programs and uploaded onto the WADNA Database.

The following samples have been extracted using the automated DNA IQ 2 Magnet Casework Extraction method on the MultiPROBE II at 65ºC for 30 minutes.

Table 33. Storage codes for all DNA extracts performed in Sample Tracking

Sample Extraction Run	Storage Codes
Extraction One	V116J4 – V117D3
Extraction Two	V117H4 – V118B2
Extraction Three	V119B3 –V119F1 (note: one sample lost due to pipetting error)

Results

From the results we can say that 100% accuracy was obtained where all samples were consistent with the expected profile. In conclusion, the known starting profile at the initial step of the extraction process was the same profile obtained at the final elution stage of the extraction method when profiled.

Two out of 114 profiles presented with weak partial profiles that were not reportable. Both of these profiles were consistent with the known profile of the sample starting material.

In concluding, we can be confident that the robot is reliable in all transfer stages of DNA extraction process.

Experiment 7 Contamination

Experiment 7A Checkerboards and Zebra Crossing Checks

Aim

The aim of this experiment is to ensure that the robotic platform does not cross contaminate any sample. These contamination checks consisted of Zebra crossings (both horizontal and vertical) and Checkerboards runs.

Method

The MultiPROBE II was utilised to extract blank swabs and known profiles from staff blood samples. Staff samples consisted of 10µl of EDTA blood aliquoted onto the sterile Copan swab. All blank samples were plain sterile Copan swabs. The swab heads were dissected into 6-8 pieces and extracted using the 2-Magnet Casework Extraction method (65ºC incubation for 30 minutes in DNA IQ kit lysis buffer). This experiment was repeated three times to ensure accuracy and consistency of the platform.

All DNA extracts were quantitated using the Applied Biosystems Quantifiler™ Human DNA Quantification Kit (P/N 4343895) on the ABI Prism 7500 according to the current Forensic Biology Method (FBPCRS003).

All DNA extracts were then profiled using the Applied Biosystems Profiler Plus Kit (P/N 4303326) on the ABI Prism 3100 Genetic Analyser. All results were interpreted using both Genotyper and GeneScan V3.7.1 software programs and uploaded onto the WADNA Database.

The following give diagrammatic representation of the contamination checks that were performed.

Blank
Sample

Plate One
Zebra Crossing – Horizontal

	1	2	3	4	5	6
A						
B						
C						
D						

Plate Two
Zebra Crossing – Vertical

	1	2	3	4	5	6
A						
B						
C						
D						

Plate Three
Checkerboard A (blank starting at A1)

	1	2	3	4	5	6
A						
B						
C						
D						

Plate Four
Checkerboard B (blank starting at B1)

	1	2	3	4	5	6
A						
B						
C						
D						

Samples

The following samples have been extracted using the automated DNA IQ 2 Magnet Casework Extraction method on the MultiPROBE II for the differing contamination checks.

Horizontal Zebra Crossing

Sample Extraction Run	Storage Codes
Extraction One	V129E9 – V129J6
Extraction Two	V132D7 – V132I4

Vertical Zebra Crossing

Sample Extraction Run	Storage Codes
Extraction One	V131E1 – V131I8
Extraction Two	V131VI9 – V132D6

Checkerboards

Sample Extraction Run	Storage Codes
Extraction One	V129A1 –V129E6
Extraction Two	V130E5 – V130J2
Extraction Three	V151I4 – V152D1
Extraction Four	V152D2 – V152H9

Results

From all of the contamination checks that were performed, it can be seen that only a small number of blank samples have given weak partial profiles. These partial profiles are all under out reporting criteria of 100RFU with the majority of alleles present were on average <50RFU.

As the initial contamination checks were performed with the original 2-Magent casework Extraction method, and this method has since been refined to increase the sensitivity and recovery rate of DNA yields, contamination checks were repeated. Similar results were obtained, where weak partial profiles were present in only a few samples. Of an extraction run of 48 samples (24 blanks and 24 known profiles) only 4 blank samples indicated possible contamination with the presence of partial profiles of 1 or 2 alleles. All of these alleles were below our reporting criteria of 100RFU and on average the majority were less than 50RFU.

Zebra Crossing

Figure 11. An electropherogram representing a typical partial profile present in a blank sample utilising the original 2-Magnent Casework Extraction method. (Pre-refinement).

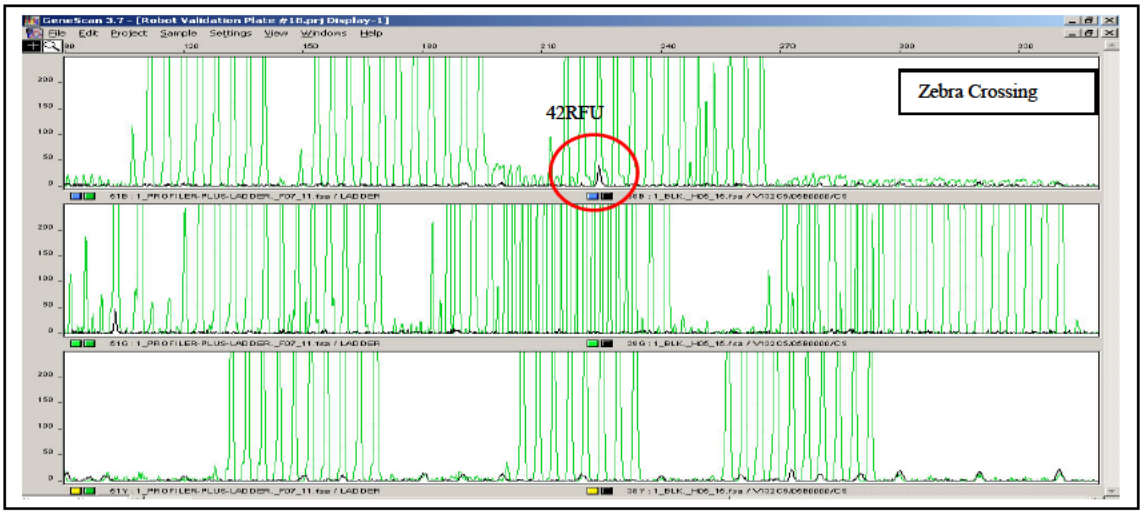
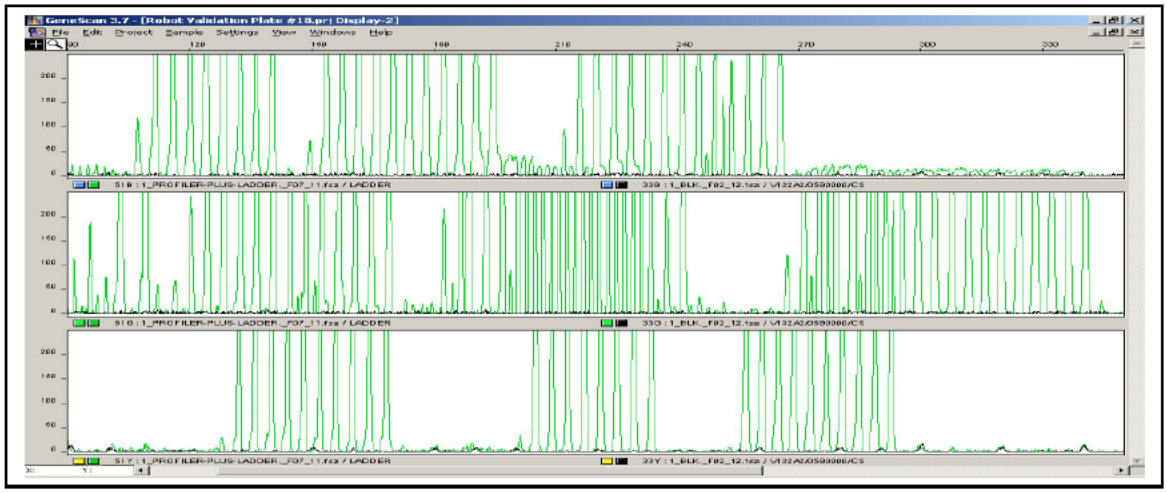


Figure 12. An electropherogram representing a typical partial profile present in a blank sample utilising the amended 2-Magnent Casework Extraction method. (Post-refinement).

Figure 13. A representative example of a Blank sample that shows no indication of any possible contamination.



Checkerboard A

Figure 14. An electropherogram representing a typical example of minimal contamination present in the original 2-Magnet Casework Extraction Method. Maximum peak height is 35RFU.

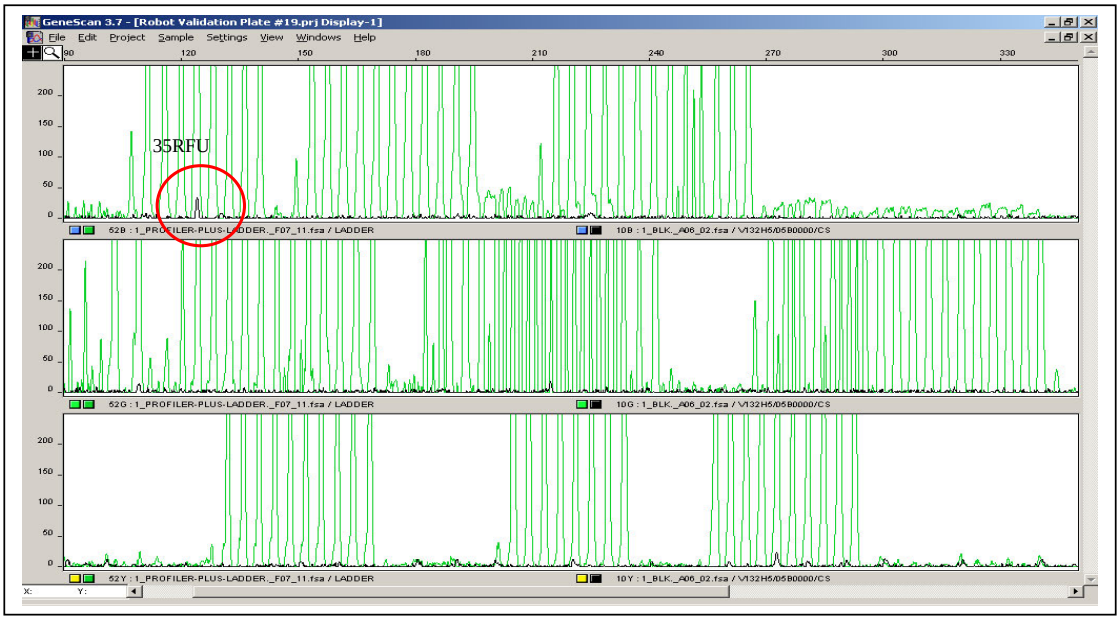
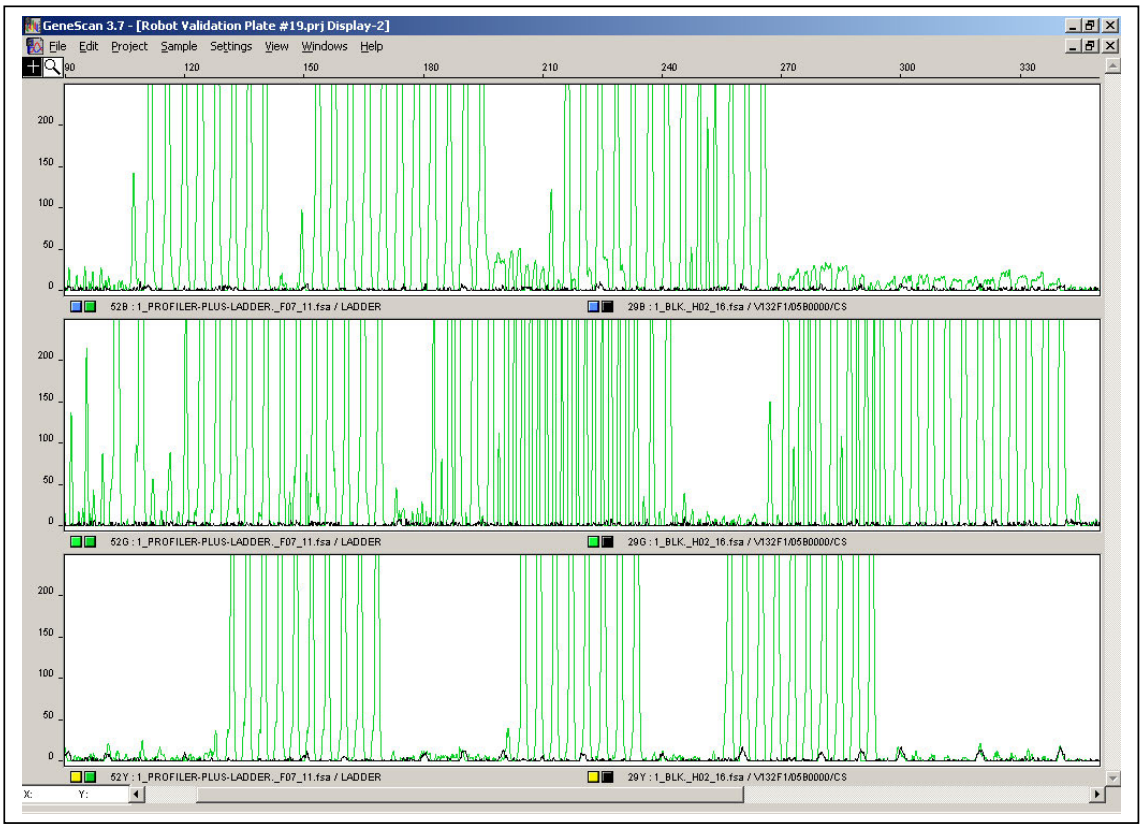


Figure 15. A representative example of a blank sample with no indication of any contamination being present.



Checkerboard B

Figure 16. An electropherogram representing a typical example of minimal contamination present in the original 2-Magnet Casework Extraction Method. Maximum peak height is 25RFU.

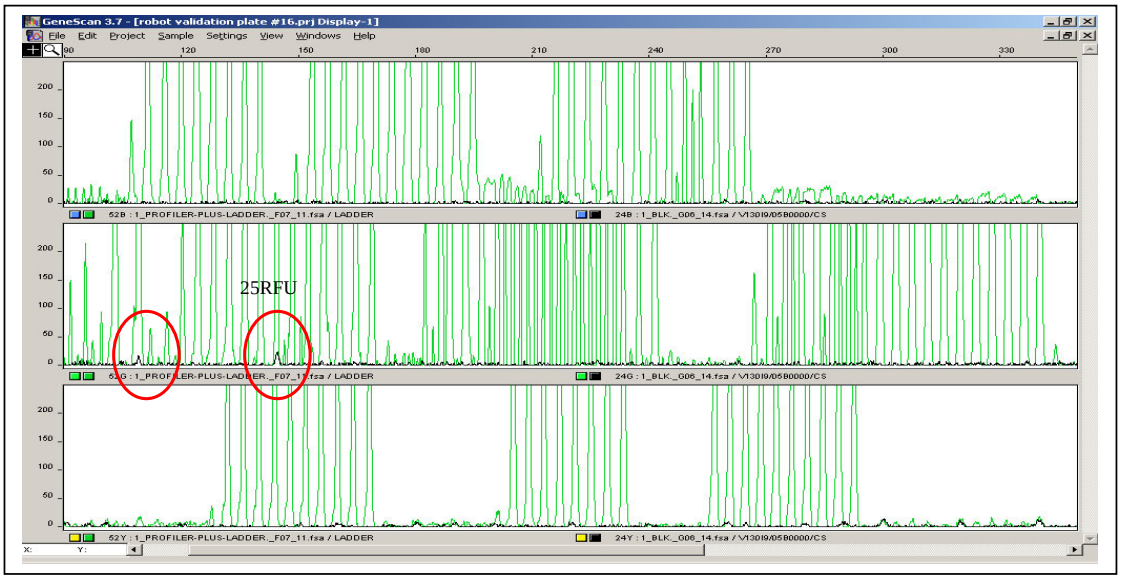
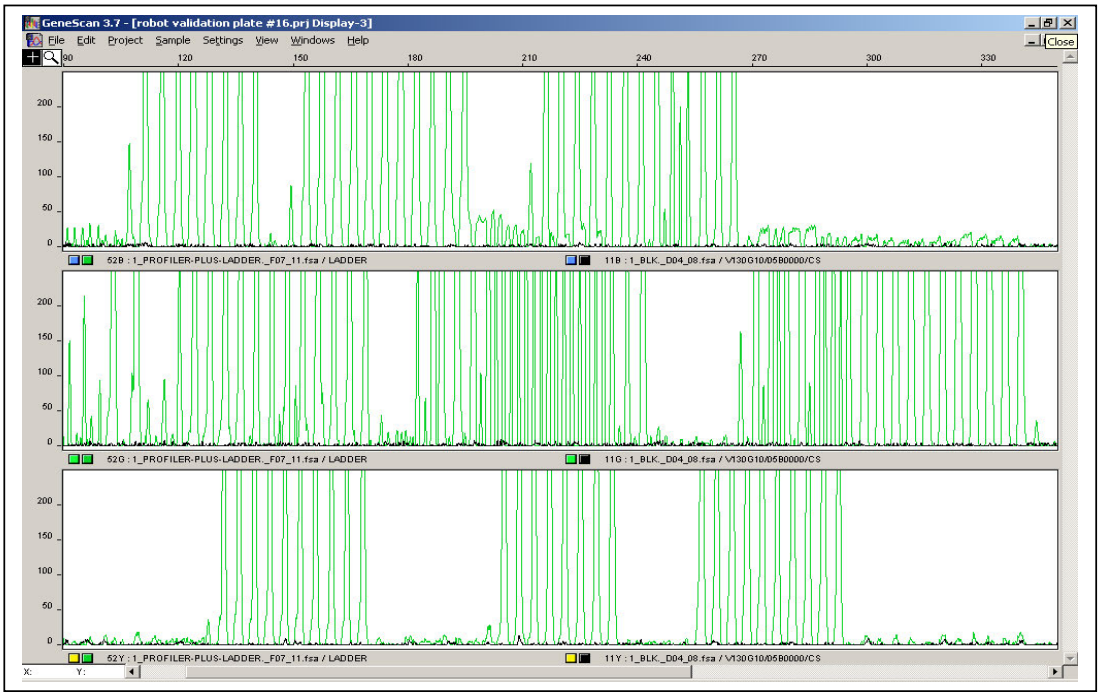


Figure 17. A representative example of a blank sample with no indication of any possible contamination being present.



Experiment 7B Dilution of a DNA Extract containing a Weak Partial Profile.

Aim

The aim of this experiment is to investigate whether a weak partial profile such as contamination would be detected if present a crime scene DNA sample.

Method

The sixteen blank samples that previously indicated a weak partial profile being present were repeated for amplification with Profiler Plus at differing dilutions to mimic the scenario if contamination was present in a quantitated DNA sample. Samples have been amplified neat, 4:11µl and a 1:29µl dilution. These dilutions represented our current dilution range for samples of various DNA concentrations that can be seen in the table below.

Table 33. Quantitation results used for to detect possible contamination in a DNA sample.

DNA Concentration (ng/µl)	Volume of DNA (µl)	Volume of Water (µl)
<0.1	15	0
0.2 - 0.3	4	11
2.0 - 3.0	1	29

Samples

The following samples gave a weak partial profile and were subsequently analysed further.

Extract	Storage Code	Quantitation Result (ng/µl)	Profile Present on Dilutions
1	V152B9	UND	No
2	V152B2	UND	No
3	V151J2	0.00312	No
4	V151I9	UND	No
5	V153H3	UND	No
6	V153H1	UND	No
7	V153G1	UND	No
8	V153G9	UND	No
9	V153I2	UND	No
10	V152F2	UND	Yes*
11	V152E10	0.00581	No
12	V152F4	UND	No
13	V152D6	UND	No
14	V152D2	UND	No
15	V152D4	0.00213	No
16	V152G4	UND	No

* sample excluded from experiment

Results

DNA extract V152F2 was excluded from the experiment as it profiled with a weak partial profile that is not consistent with any known staff members profiles, or profiles that were used in these experiments. This extract has quantitated with an undetectable result and therefore it is questionable whether contamination of the sample occurred post extraction. The profile is reproducible on repeat amplification.

Several blank extracts have given a minimal Quantifiler human DNA results, however no profile is detected.. All of these samples had a quantifiler range from 0.00148ng/µl to 0.00581ng/µl.

For all remaining 15 samples indicating weak partial profiles, it can be seen that on dilution of the DNA extract, no profile is detected at these dilutions. The following electropherograms of DNA extract V151J2 represent this.

Figure 18. Electropherogram representing DNA Extract V151J2 with a weak partial profile amplified neat.

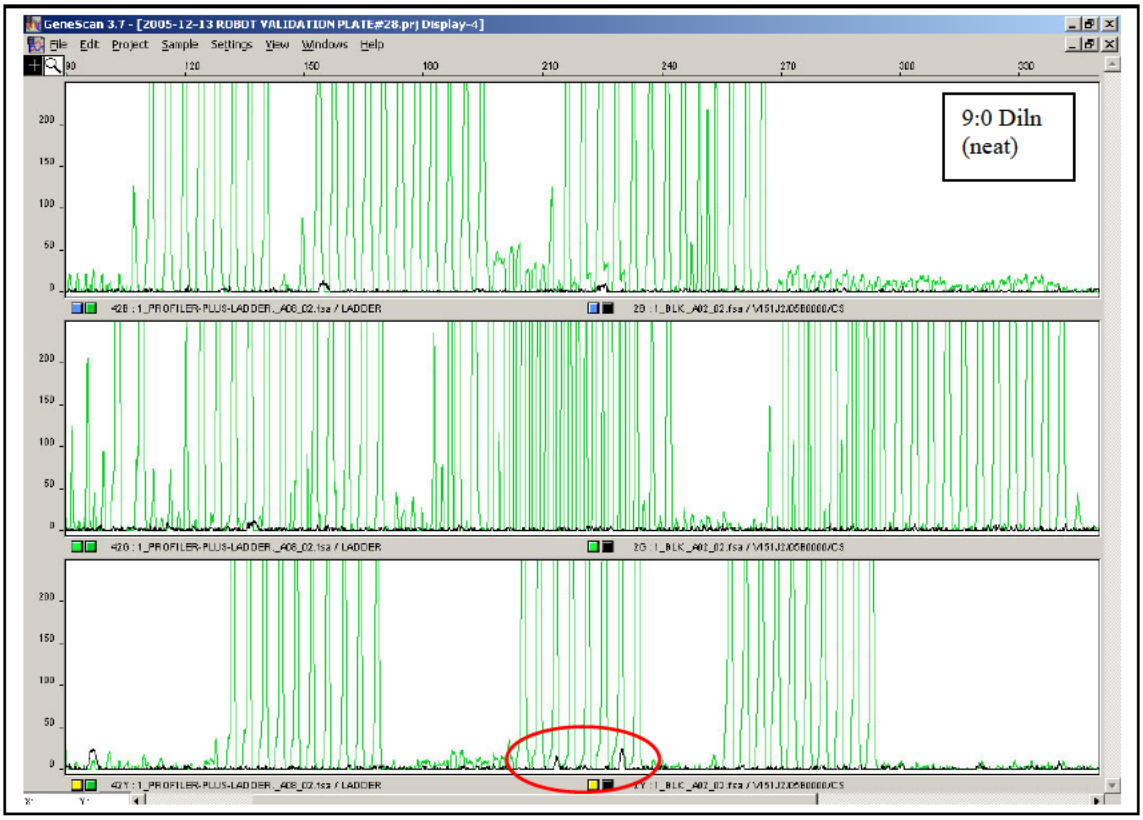


Figure 19. Electropherogram representing DNA Extract V151J2 with a weak partial profile amplified at 4:11µl dilution. No profile is detected.

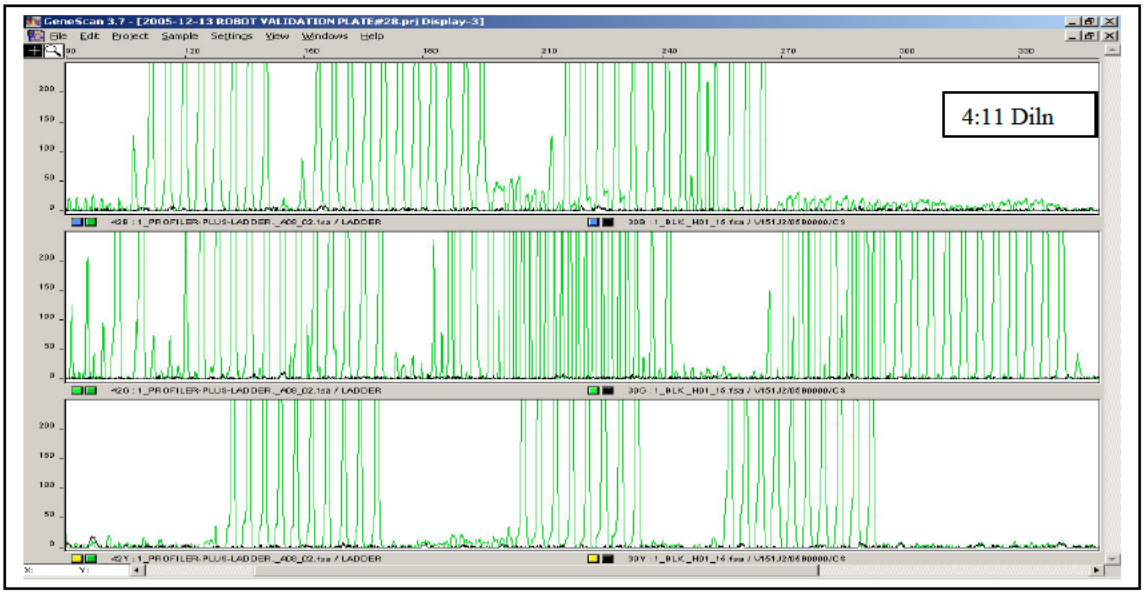
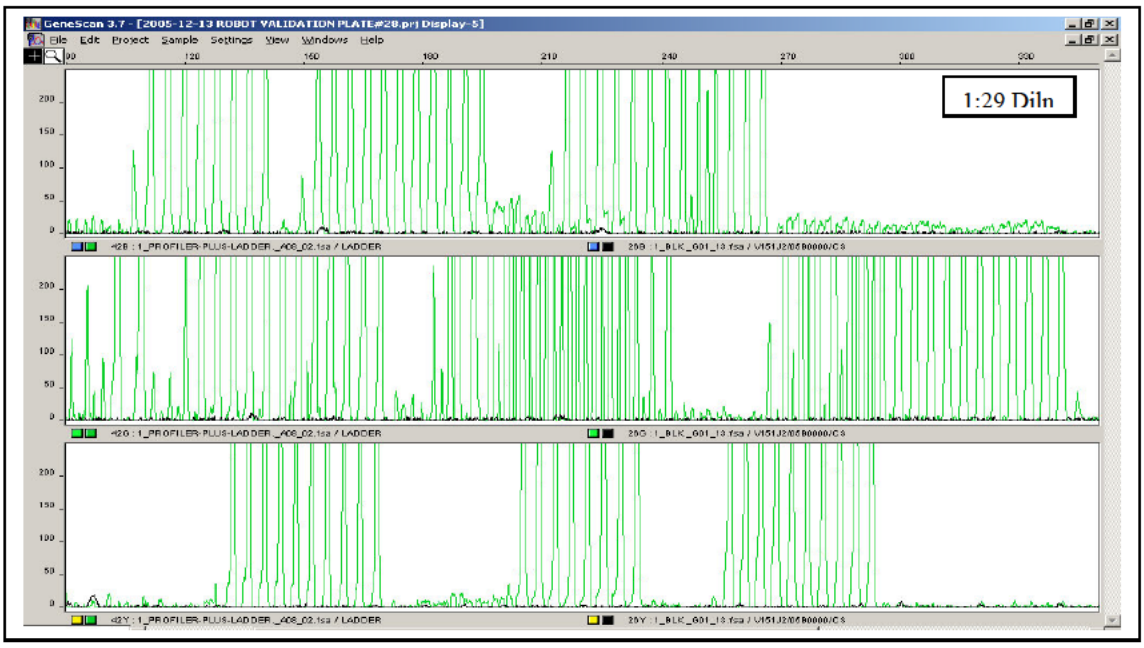


Figure 20. Electropherogram representing DNA Extract V151J2 with a weak partial profile amplified at 4:11µl dilution. No profile is detected.



Conclusion

From the above results we can conclude that if a weak partial profile was present in a sample, then after dilution the minimal peaks would be diluted out of the profile and not inhibit amplification of that sample.

Experiment 7C Reagent Blanks following Heavy Blood Stained Extraction.

Aim

The aim of this experiment is to establish if the robotic platform is entirely decontaminated after each extraction run therefore eliminating any transfer of possible contamination from one extraction run to the next.

Method

Twentyfour saturated blood stained staff swabs containing approximately 70-80µl of EDTA blood were extracted utilising the amended 2-Magnet Casework Extraction method on the MultiPROBE II. At completion of this extraction run, 24 microfuge tubes containing reagent blanks only were extracted using the same method. All DNA extracts were quantitated using the Applied Biosystems Quantifiler™ Human DNA Quantification Kit (P/N 4343895) on the ABI Prism 7500 according to the current Forensic Biology Method (FBPCRS003).

All blank extracts were then analysed using the Applied Biosystems Profiler Plus Kit (P/N 4303326) on the ABI Prism 3100 Genetic Analyser. All results were interpreted using both Genotyper and GeneScan V3.7.1 software programs and uploaded onto the WADNA Database.

The following samples have been extracted using the automated DNA IQ 2 Magnet Casework Extraction method on the MultiPROBE II for contamination checks.

DNA Extracts	Storage Codes
Reagent Blanks	V153F9 –V153I2
Staff Samples	V153D5 – V153F8

Results

All reagent blank samples quantitated with an Undetectable amount of DNA result. Of the 24 reagent blank samples, 4 samples have given a weak partial profile of 1 or 2 alleles. All of these alleles are below our reporting criteria of 100RFU and typically below 50RFU. All of these profiles can be seen in the following electropherograms.

Figure 21. Electropherogram representing a Reagent Blank one extracted following a heavily blood stained extraction run. The sample has a weak partial profile present <50RFU.

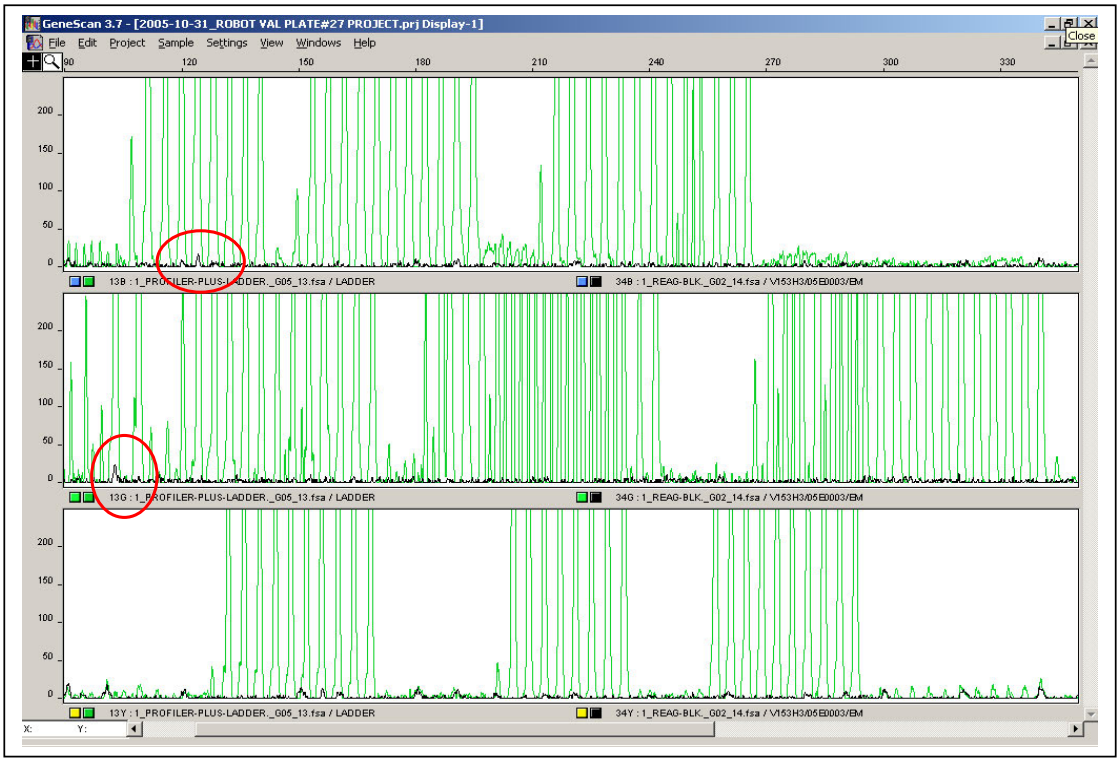


Figure 22. Electropherogram representing a Reagent Blank two extracted following a heavily blood stained extraction run. The sample has a weak partial profile present <50RFU.

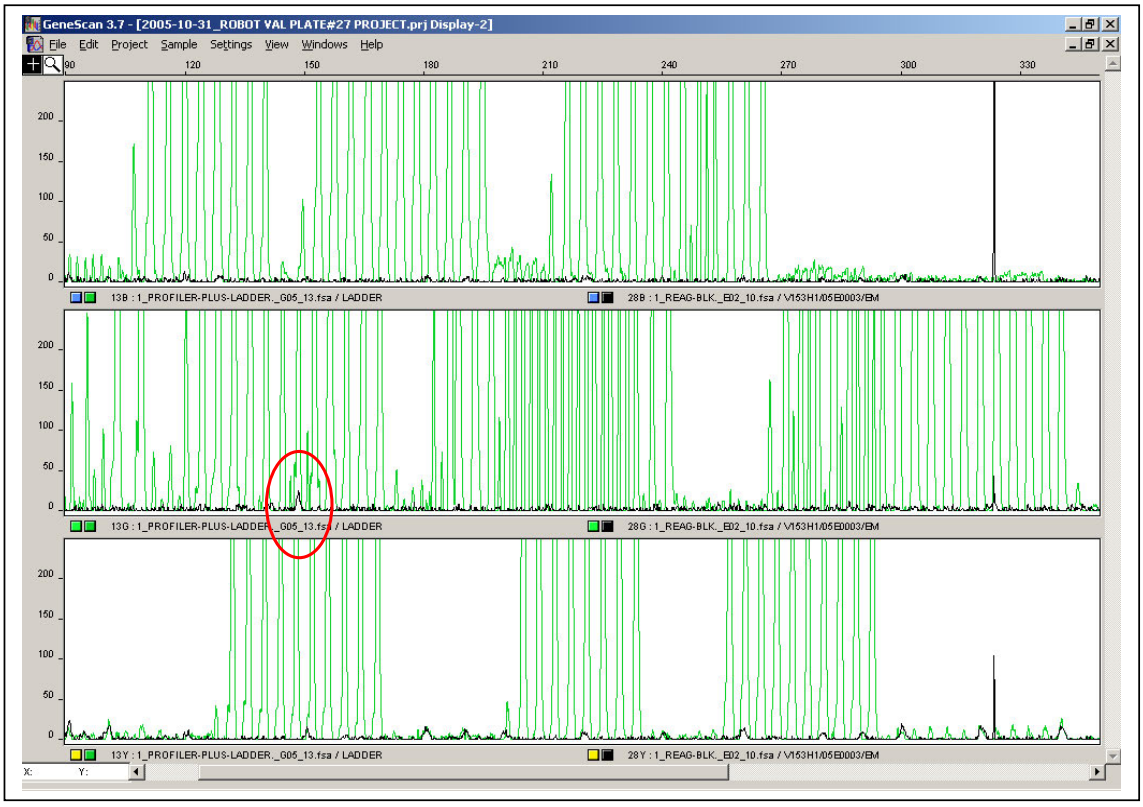


Figure 23. Electropherogram representing a Reagent Blank three extracted following a heavily blood stained extraction run. The sample has a weak partial profile present <50RFU.

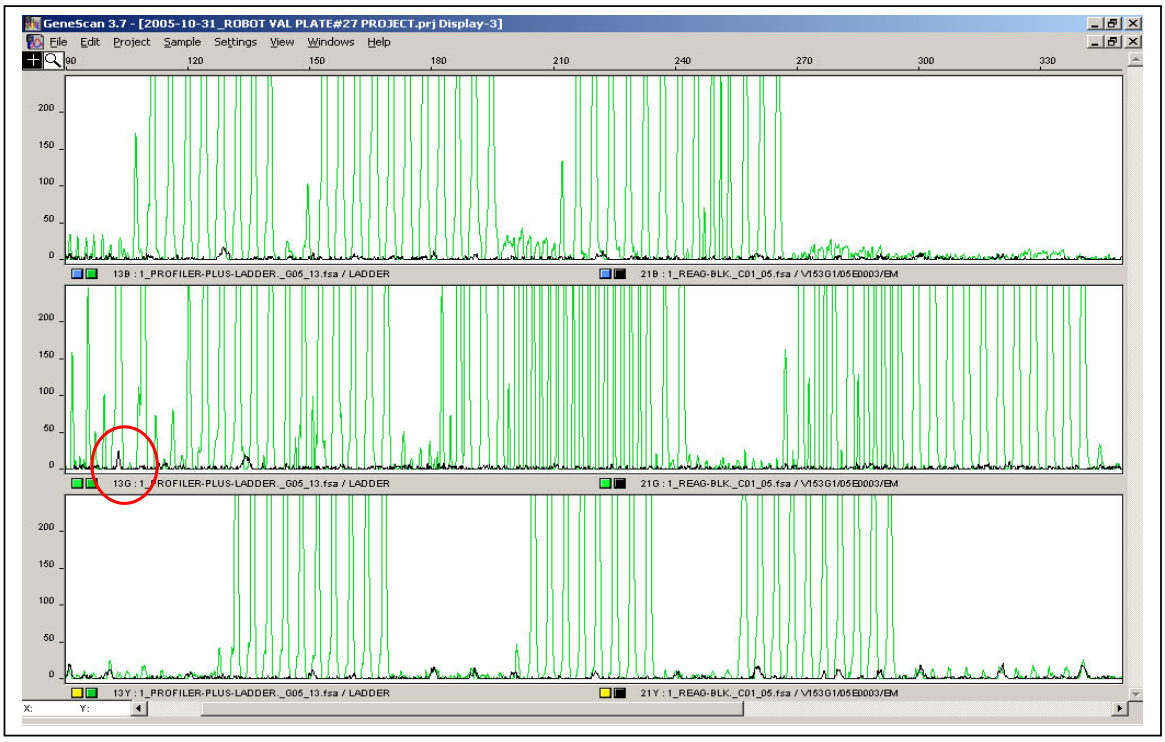


Figure 24. Electropherogram representing a Reagent Blank four extracted following a heavily blood stained extraction run. The sample has a weak partial profile present <50RFU.

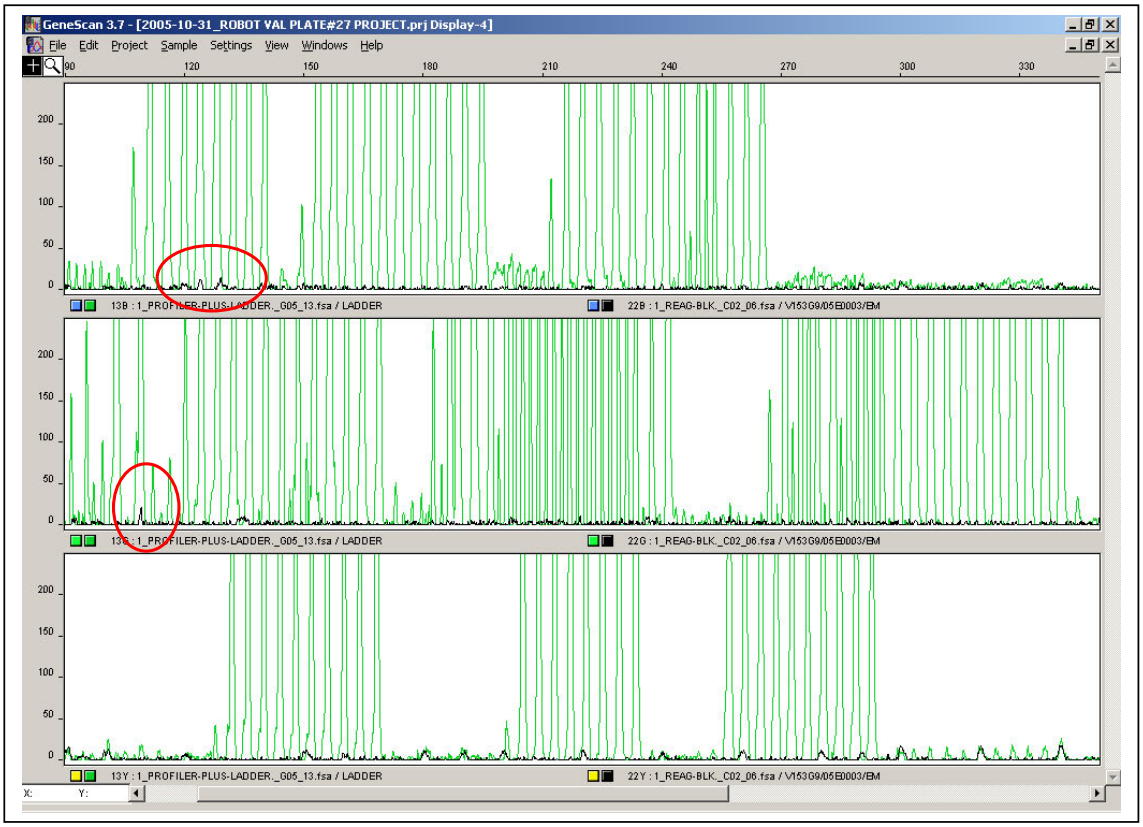
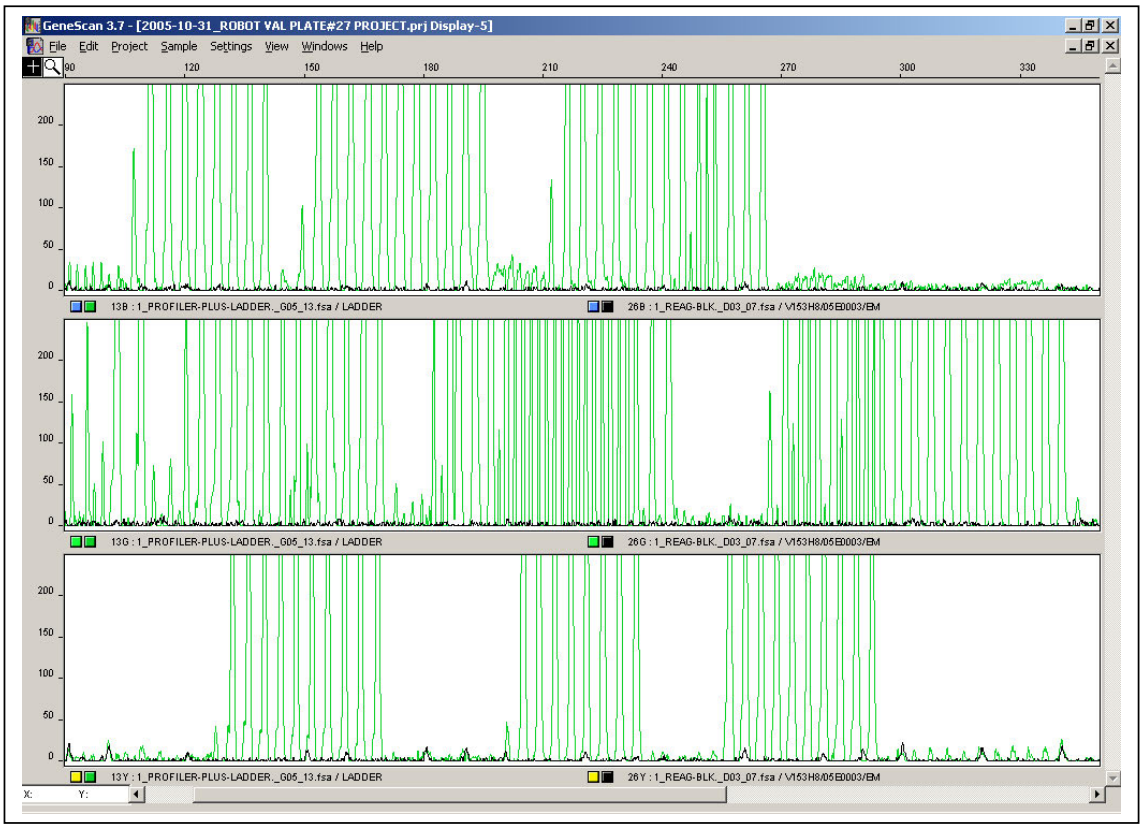


Figure 25. An example of a Reagent Blank extract with no presence of contamination extracted following a heavily blood stained extraction run.



Conclusion

The above results indicate that following extraction of heavily blood stained samples, no reportable DNA contamination is present. Weak partial profiles are present, however they are all under the detectable threshold limits.

Experiment 8 Reproducibility and Yield

Aim

To determine if the same volume of input blood will give similar DNA yields and sample volumes on completion of the DNA extraction.

Method

Various volumes of EDTA blood from a known female staff member were aliquoted onto a sterile Copan swab (1µl, 5µl, and 10µl). The swab heads were dissected into 6-8 pieces and extracted using the 2-Magnet Casework Extraction method (65ºC incubation for 30 minutes in DNA IQ kit lysis buffer). Each extraction run consisted of 47 blood samples of the same volume of blood and one random blank sample to ensure accuracy and consistency of the platform. Volumes of the final eluate were measure for all extracts to ensure final eluate reproducibility.

All DNA extracts were quantitated using the Applied Biosystems Quantifiler™ Human DNA Quantification Kit (P/N 4343895) on the ABI Prism 7500 according to the current Forensic Biology Method (FBPCRS003).

All DNA extracts were then profiled using the Applied Biosystems Profiler Plus Kit (P/N 4303326) on the ABI Prism 3100 Genetic Analyser. All results were interpreted using both Genotyper and GeneScan V3.7.1 software programs and uploaded onto the WADNA Database.

Results

Reproducibility and DNA Yields

1µl EDTA blood

From the 47 blood samples extracted, 43 extracts had full reportable profiles (91%), one had a 9/10 partial profile and 3 were a weak partial profile with only 3 reportable loci (6%). The expected profile was obtained for all samples.

5µl EDTA blood

From the 47 blood samples extracted, 44 extracts had full reportable profiles (94%). The remaining 3 extracts had a 9/10 partial profile. The expected profile was obtained for all samples.

10µl EDTA blood

From the 47 blood samples extracted, 45 extracts had a full reportable profile (96%). The remaining two extracts were partial, (1x 9/10 profile and 1x 6/10 profile). The expected profile was obtained for all samples.

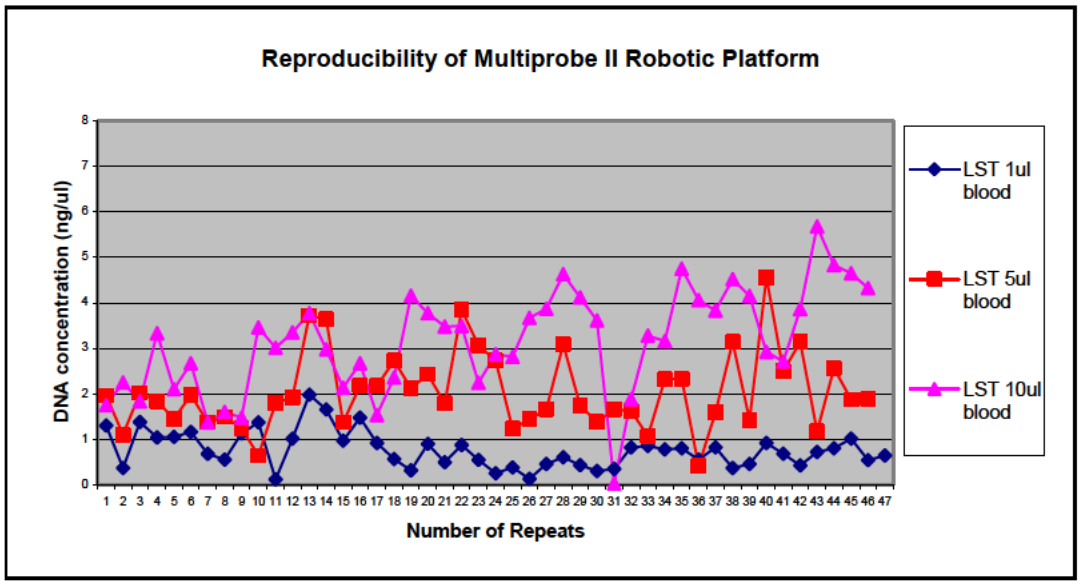
Quantifiler results for the mean and standard deviation of the DNA concentration were calculated and listed in the table below.

Staff	Volume	Mean (ng)	Std Dev (ng)	Total DNA (ng)
Expected Range	1µl			6-10
	5µl			30-50
	10µl			60-100
LST	1µl	0.763	0.402	26.7
	5µl	2.051	0.863	77.1
	10µl	3.151	1.13	110.3

Positions on the 96well plate were plotted for samples that appeared as an outlier (those samples that had significantly differing values) to determine if one particular probe, column or row was performing differently to the other positions within the plate and across plates. No consistencies were found amongst the outliers over several extraction runs.

Possible reasons for these differences in final concentrations may be due to manual pipetting error at several of the stages (aliquoting blood onto the swabs or quantitation pipetting error), poor mixing of the resin beads, poor heat transfer to the 96 well plates by not seating properly on the heater tile due to warping.

Graph 4. Graphical representation of the reproducibility on the MultiPROBE II for one female staff member over various volumes of EDTA blood for 47 extracts.



DNA Extract Volume

On completion of an extraction run the final DNA eluate was measured for every sample to ensure consistency amongst samples. The robotic platform is programmed to add 35µl of Elution buffer. Each sample repeatedly measured 34µl of DNA indicating reproducible and reliable results for final extract volume.

Conclusion

From the above results we can conclude that the MultiPROBE II will consistently and reliably give reproducible final DNA extract volumes of 34µl.

The amount of DNA obtained from the blood sample is reasonably consistent across the extraction run for the various volumes. All DNA concentrations obtained are greater than the expected DNA concentrations for the Promega DNA IQ kit as recommended by PerkinElmer. There were no consistencies were found amongst the outliers.

It must also be considered that the DNA IQ system isolates a maximum of 100ng of DNA from blood. For samples containing less than 100ng of DNA the small casework extraction method is used which is the method being utilised by Forensic Biology. This method isolates DNA at varying concentrations as the amount of resin is added in excess.

The system becomes more efficient with samples containing less than 50ng of DNA, which can be seen in the results above, with 1µl blood samples having the lowest standard deviation results.

For samples containing more than 100ng DNA then the Database protocol should have been used as the resin is saturated and the eluted DNA has a more consistent concentration between the samples. This method was not analysed by Forensic Biology in this validation, which could explain some of the inconsistencies at the higher DNA concentrations.

Experiment 9 Overloaded Samples

Aim

The aim of this experiment is to determine what is the maximum amount of DNA that can be obtained from a blood swab using the Promega DNA IQ kit on the MultiPROBE II.

Method

Various volumes of EDTA blood from known staff members were aliquoted onto a sterile Copan swab (25µl, 50µl and 70-80µl). 70-80µl of blood was the saturation point for the Copan swab and therefore volumes used in this range may vary slightly. The swab heads were dissected into 6-8 pieces and extracted using the 2-Magnet Casework Extraction method (65ºC incubation for 30 minutes in DNA IQ kit lysis buffer).

All DNA extracts were quantitated using the Applied Biosystems Quantifiler™ Human DNA Quantification Kit (P/N 4343895) on the ABI Prism 7500 according to the current Forensic Biology Method (FBPCRS003).

All DNA extracts were then profiled using the Applied Biosystems Profiler Plus Kit (P/N 4303326) on the ABI Prism 3100 Genetic Analyser. All results were interpreted using both Genotyper and GeneScan V3.7.1 software programs and uploaded onto the WADNA Database.

Samples

DNA Extracts	Storage Codes
LGW	V125F3 – V125I2
CC	V126D3 – V126G2
MEB	V126G5 – V126J4

Results

The following table indicate the total DNA concentrations in nanograms extracted from heavily blood stained swabs. From the results below we can see that the system can extract in excess of 100ng of DNA as guaranteed by Promega and on average in excess 300ng of DNA from 50µl of blood. In some instances upto 750ng of DNA was obtained from 50µl blood. For downstream applications such as Profiler Plus amplification, only 0.5 to 1.25ng of DNA is required.

Full reportable profiles were obtained in 83% of all samples. 13% of samples were partial profiles with 8 or greater reportable loci. Less than 1% (3/90 samples) failed to amplify.

Sample	Volume	Mean	Std Dev	Total DNA (ng)
Expected Range	1µl			6-10
	5µl			30-50
	10µl			60-100
LGW	25µl	4.726	2.378	165.4
	50µl	9.462	5.345	331.2
	80µl	6.539	2.261	228.9
MEB	25µl	8.272	1.611	289.5
	50µl	9.707	3.415	339.8
	80µl	7.392	2.466	258.7
CC	25µl	3.589	1.051	125.6
	50µl	9.626	2.74	336.9
	80µl	8.162	3.08	285.7

Figure 26. DNA extract from a male staff sample with 25µl of EDTA blood aliquoted onto a Copan swab. Total DNA concentration is 100ng.

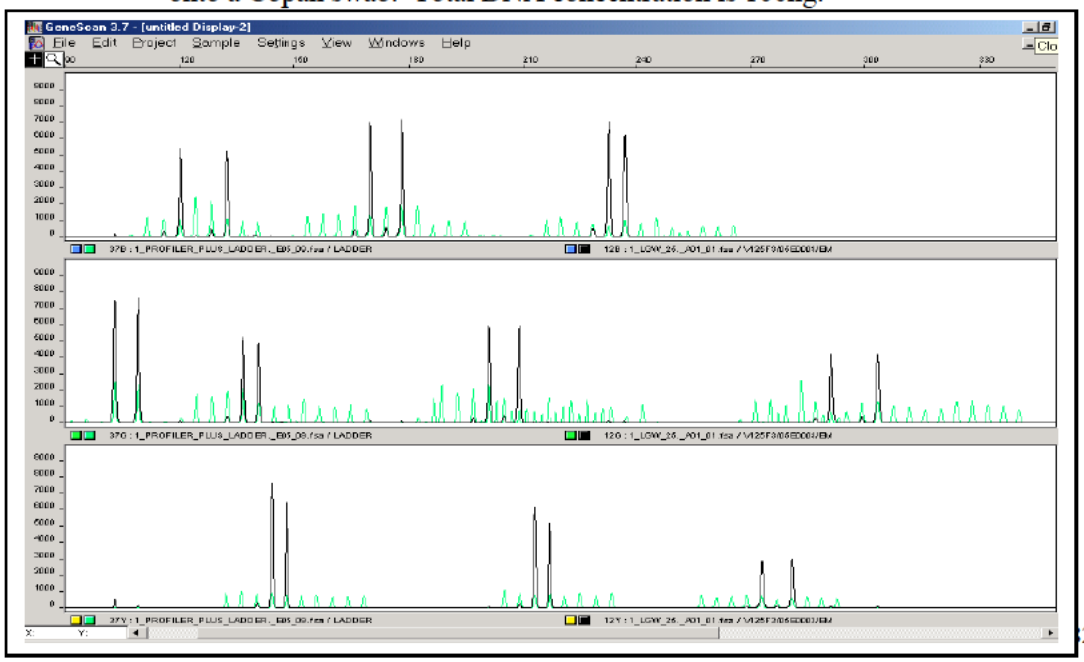


Figure 27. DNA extract from a male staff sample with 50µl of EDTA blood aliquoted onto a Copan swab. Total DNA concentration is 750ng.

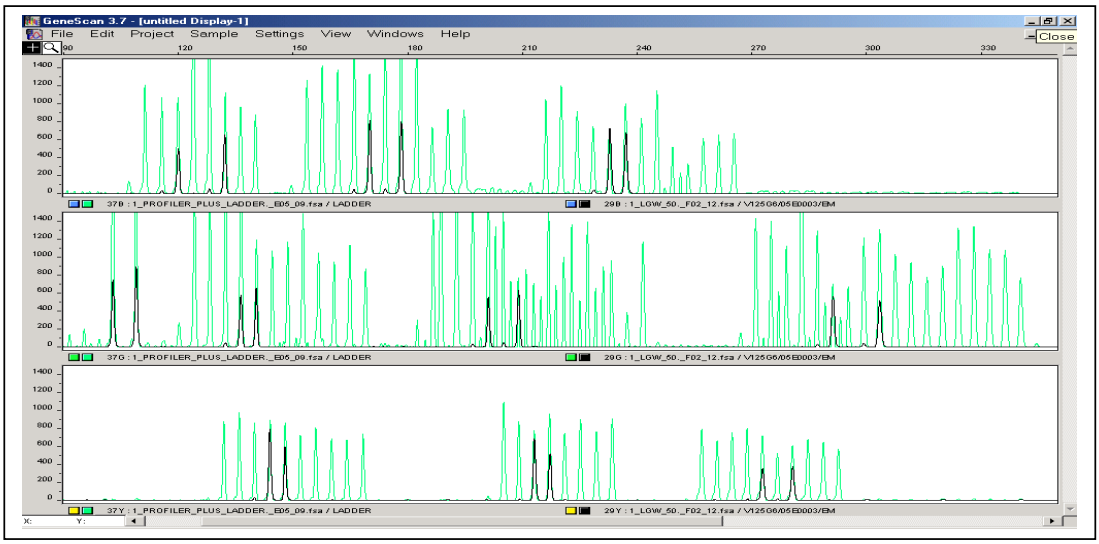
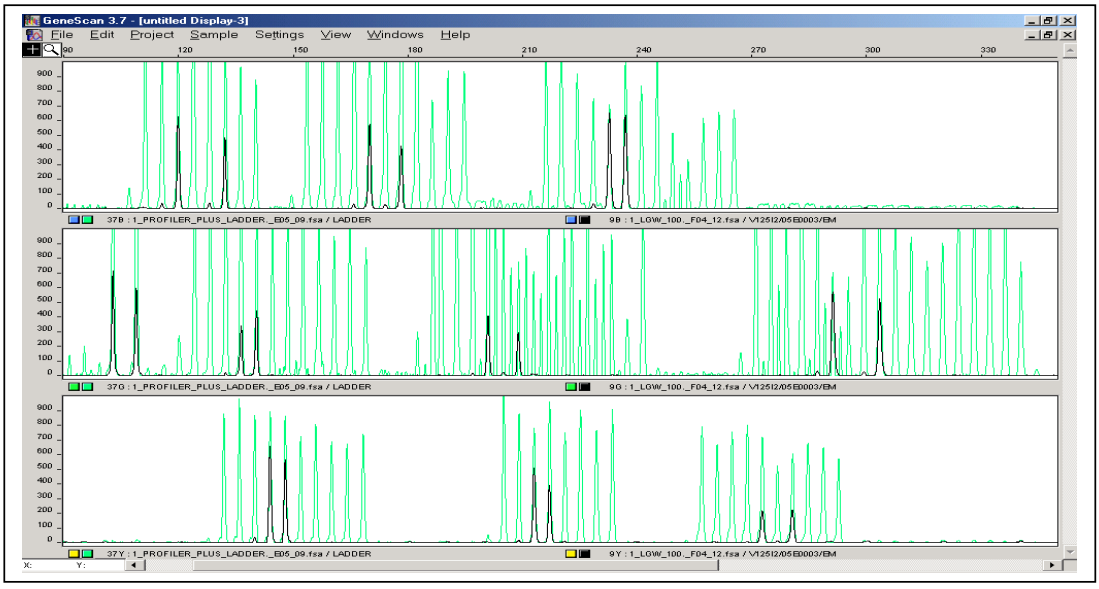


Figure 28. DNA extract from a male staff sample with 100µl of EDTA blood aliquoted onto a Copan swab. Total DNA concentration is 300ng.



Conclusion

The above results indicate that the maximum amount of DNA extracted using the 2-Magnet Casework Extraction on the MultiPROBE II is 750ng DNA with an average of 300ng of DNA from 50µl EDTA blood. In 97% of all samples, profiles with 8 or greater reportable loci were obtained. Less than 1% (3/90 samples) failed to amplify.

Experiment 10 **Minimum Input DNA**

Aim

The aim of this experiment is to determine the minimum amount of DNA that can be detected from a swab.

Method

Serial dilutions were performed on a female staff member's blood. A whole blood count was obtained to determine the starting white cell count (8.6 x10^9/L). The blood was then diluted in 0.85% saline at various volumes and 1µl of the diluted blood was aliquoted onto a sterile Copan swab. The swab head was dissected into 6-8 pieces and placed into a microfuge tube on the robotic platform. The amended 2-Magnet casework DNA method was utilised (incubation at 65ºC for 30 minutes in DNA IQ kit lysis buffer) to extract the DNA.

All DNA samples were quantitated using the Applied Biosystems Quantifiler™ Human DNA Quantification Kit (P/N 4343895) on the ABI Prism 7500 according to the current Forensic Biology Method (FBPCRS003).

All samples were then profiled using the Applied Biosystems Profiler Plus Kit (P/N 4303326) on the ABI Prism 3100 Genetic Analyser. All results were interpreted using both Genotyper and GeneScan V3.7.1 software programs and uploaded onto the WADNA Database.

Results

Amount of Blood Added in Total	Volume of Blood Added to Dilution	Volume of 0.85% Saline Added to Dilution	WCC (x10^3/µl)	Total WCC	Storage Codes
5µl	5	0	43	43000	V167D5 – D10
3µl	3	0	25.8	25800	V167E1 – E9
1µl	10	0	8.6	8600	V167H3 – H5
0.5µl	5	5	4.3	4300	V167H6 – H8
0.25µl	2	6	2.15	2150	V167H9 – I1
0.2µl	1	4	1.72	1720	V167I2 – I4
0.1µl	1	9	0.86	860	V167I5 –I7
0.05µl	1	19	0.43	430	V167I8 – I10
0.02µl	1	49	0.172	172	V167J1 – J3
0.01µl	1	99	0.086	86	V167J4 – J6

Full reportable DNA profiles were obtained for samples diluted down to 0.05µl of DNA (WCC=0.43 x10^9/L). Partial profiles were obtained for samples diluted from 0.02µl down to 0.01µl of EDTA blood. Excluding one sample, full reportable profiles (10/10 profile) were obtained for all dilutions down to 0.05ng of blood. Partial profiles were obtained for 0.02µl blood dilution and non-reportable profiles were obtained for 0.01µl whole blood.

Kastle-Meyer (KM) testing for blood was performed on the 0.05µl of blood multiple times. All 10 KM testings for these samples was positive, with all results coming up instantly.

Figure 29. 1µl of blood (neat) extracted using the MultiPROBE II.

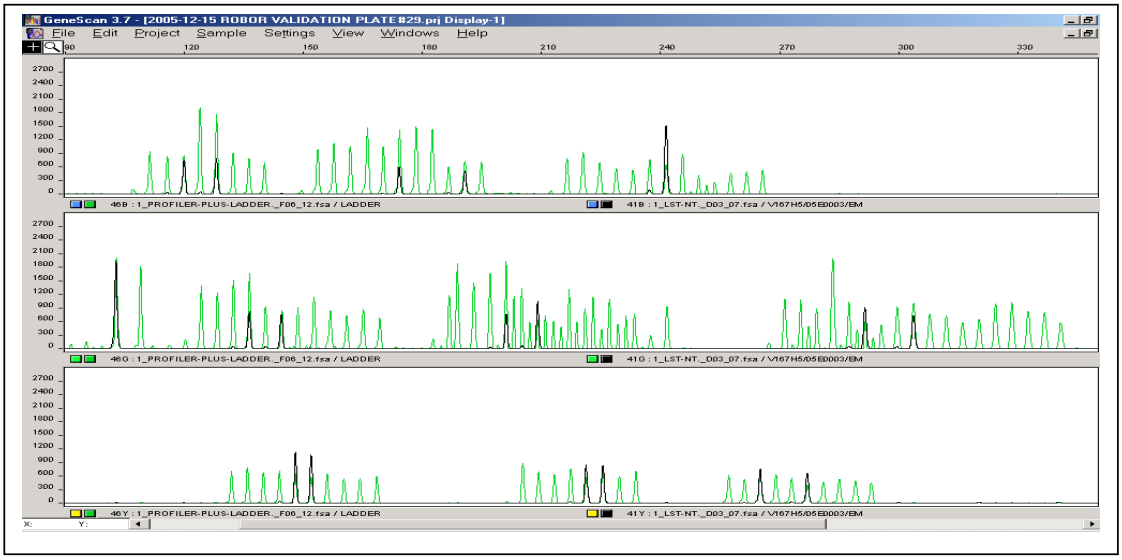


Figure 30. 0.5µl of blood extracted using the MultiPROBE II.

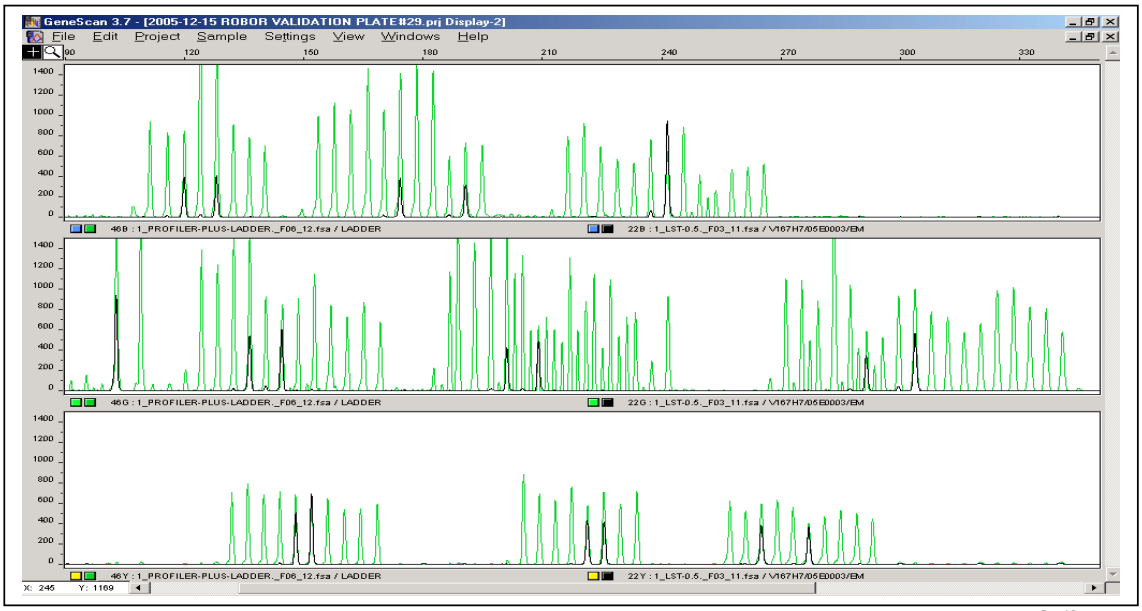


Figure 31. 0.25µl of blood extracted using the MultiPROBE II.

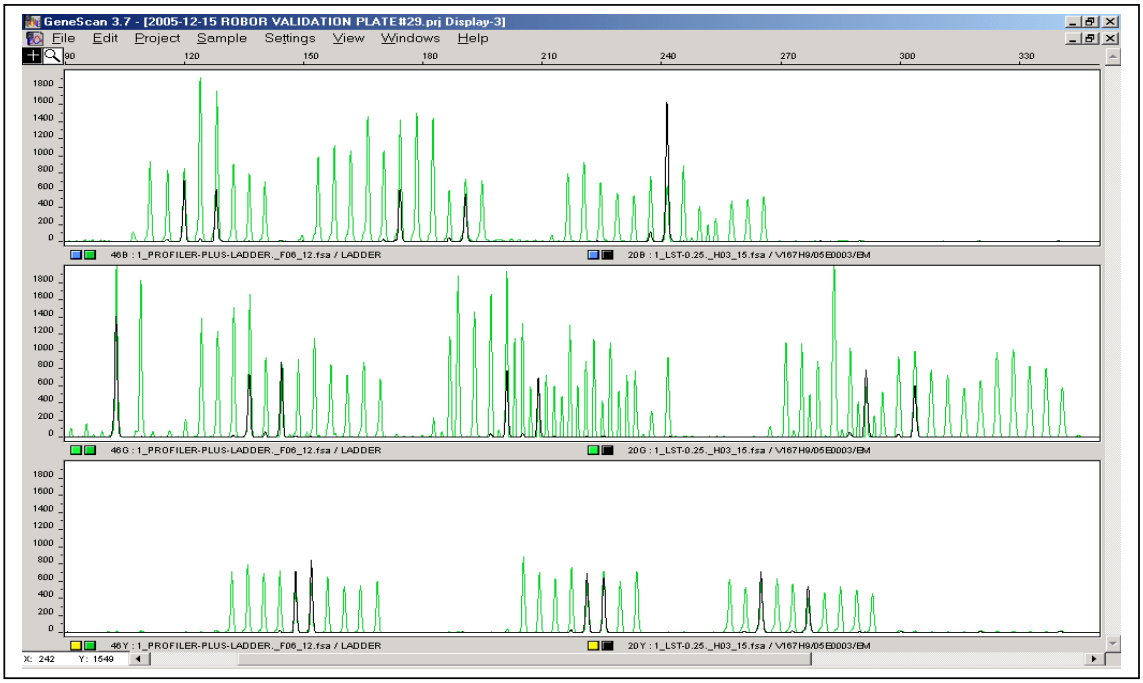


Figure 32. 0.20µl of blood extracted using the MultiPROBE II.

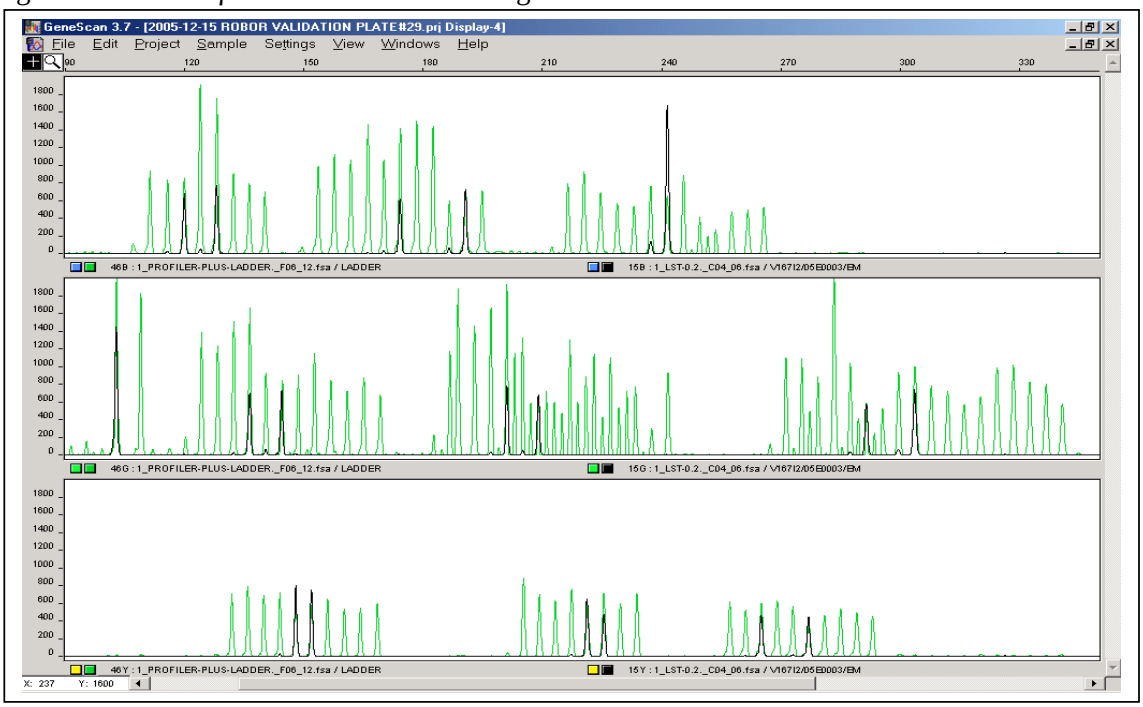


Figure 33. 0.1µl of blood extracted using the MultiPROBE II.

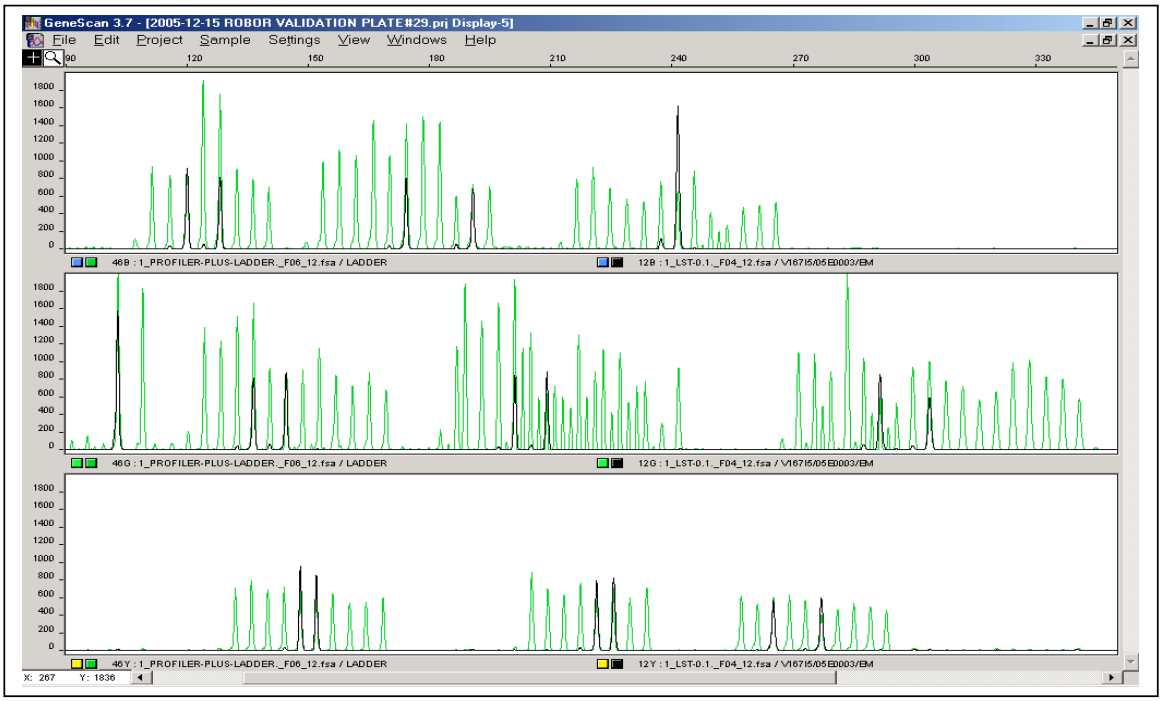


Figure 34. 0.05µl of blood extracted using the MultiPROBE II.

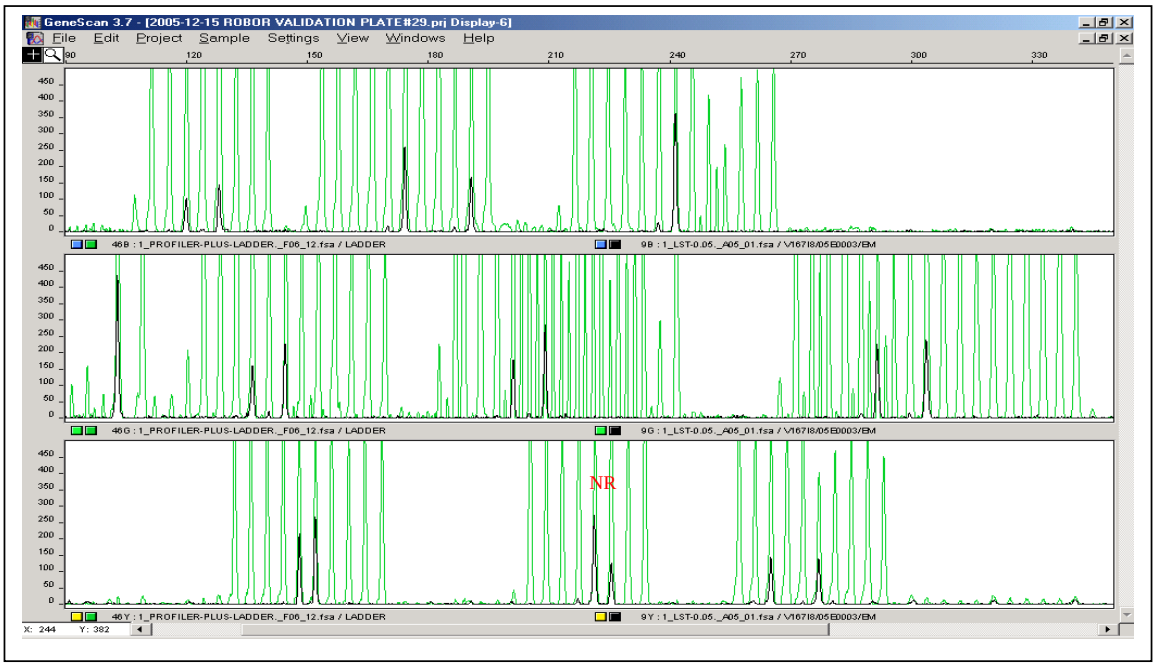


Figure 35. 0.02µl of blood extracted using the MultiPROBE II.

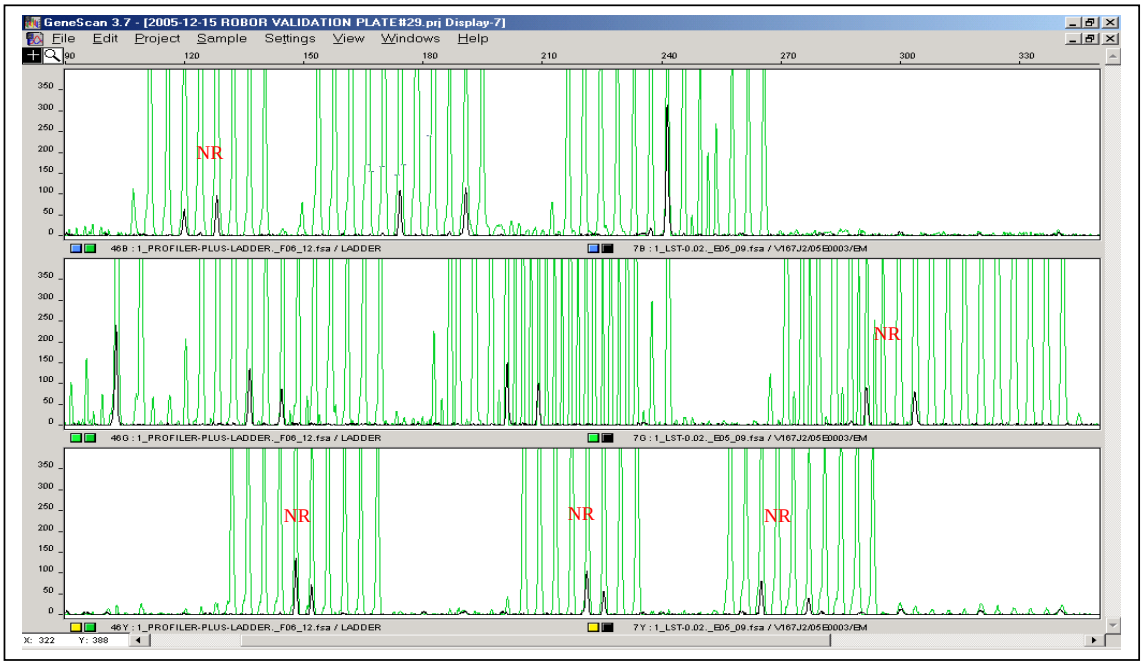
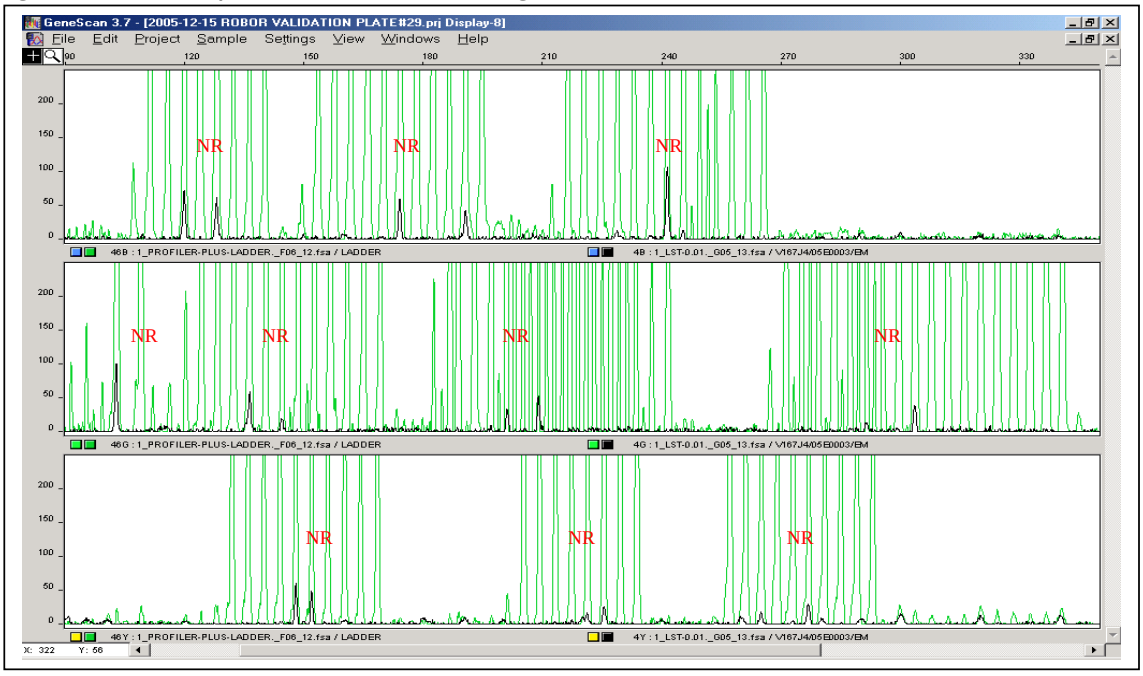


Figure 36. 0.01µl of blood extracted using the MultiPROBE II.



Conclusion

From the above results we can conclude that a full reportable profiles can consistently be obtained from as little as 430 WCC.

Experiment 11 Mixtures

Aim

To investigate whether multiple blood samples can be detected within a mixed sample.

Method

Various ratios of EDTA blood from 2 known staff members were mixed together and aliquoted onto sterile Copan swabs. The mixed blood sample consisted of one male and one female representative. Samples chosen for the experiment were paired with samples sharing similar profiles and those with differing profiles to determine whether any combination of profiles could be detected. A positive control consisting of 1uL of staff blood and a blank control were extracted also. The swab head was dissected into 6-8 pieces and placed into a microfuge tube on the robotic platform. The amended 2-Magnet casework DNA method was utilised (incubation at 65ºC for 30 minutes in DNA IQ kit lysis buffer) to extract the DNA. This experiment was performed for both 5µl and 10µl of blood sample.

All DNA extracts were quantitated using the Applied Biosystems Quantifiler™ Human DNA Quantification Kit (P/N 4343895) on the ABI Prism 7500 according to the current Forensic Biology Method (FBPCRS003).

Profiles were then amplified using the Applied Biosystems Profiler Plus Amplification Kit (P/N 4303326) on the ABI Prism 3100 Genetic Analyser. All results were interpreted using both Genotyper and GeneScan V3.7.1 software programs and uploaded onto the WADNA Database.

DNA Extracts (5µl)	Storage Codes
MB-AMF	V133I4-V133J4
GRT-LST	V133J5-V134A5
SEE-EL	V134A6-V134B6
CDJ-JB	V134B7-V134C7

DNA Extracts (10µl)	Storage Codes
MB-AMF	V133D8-V133E8
GRT-LST	V133E9-V133F9
SEE-EL	V133F10-V133G10
CDJ-JB	V133H1-V133I1

Table 34. Representation of the volumes of EDTA blood mixed from staff sample 1 with staff sample 2.

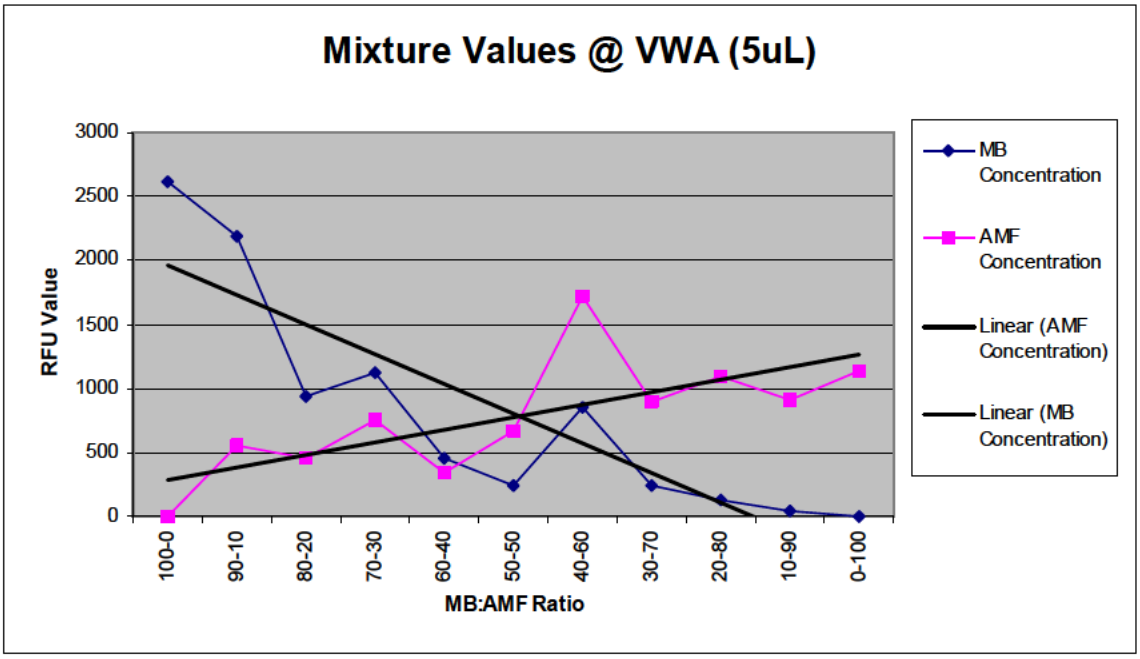
Staff One	100	90	80	70	60	50	40	30	20	10	0
Staff Two	0	10	20	30	40	50	60	70	80	90	100

Table 35. Profile of Staff Members used in Mixtures

Initial	D3S1358	Vwa	FGA	Amel	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820	WCC (x10^9/L)
MB	14/16	18/18	20/22	X/Y	14/16	30.2/32.2	15/17	11/12	11/12	8/11	5.5
AMF	17/18	17/17	21/22	x/x	12/13	30/30	13/20	10/11	12/14	10/10	6.7
GRT	15/16	14/17	21/25	X/Y	13/14	31/31.2	15/18	10/12	10/11	10/10	6.3
LST	14/16	16/20	24/24	X/X	11/13	28/30	14/17	11/12	12/13	8/11	7.4
SEE	18/18	14/15	20/22	X/Y	11/13	29/30	12/17	12/13	11/12	9/12	8.4
EL	14/18	17/18	20/21	X/X	11/13	30/30	14/16	11/11	8/11	12/12	5.0
CDJ	15/15	15/18	22/23	X/Y	14/14	28/33.2	15/18	11/12	11/12	8/11	11.5
JB	15/15	15/17	22/23	X/X	14/15	29/29	16/20	10/12	11/11	11/12	8.4

Results

It is visible in the electropherograms that as the concentration of one person in a mixture increases then the RFU values increase proportionately. As shown in graph xx where both concentrations are seen to be about equal around the 50-50 ratio. The VWA was the locus chosen because both staff have heterozygote alleles.



Graph 5. Graphical representation of two staff members mixed blood samples

Figure 37. Representation of the markings of Staff member one and two for the following electropherograms.

MB
AMF

Figure 38. Electropherogram representing a 0-100µl mix of a male and female staff member.

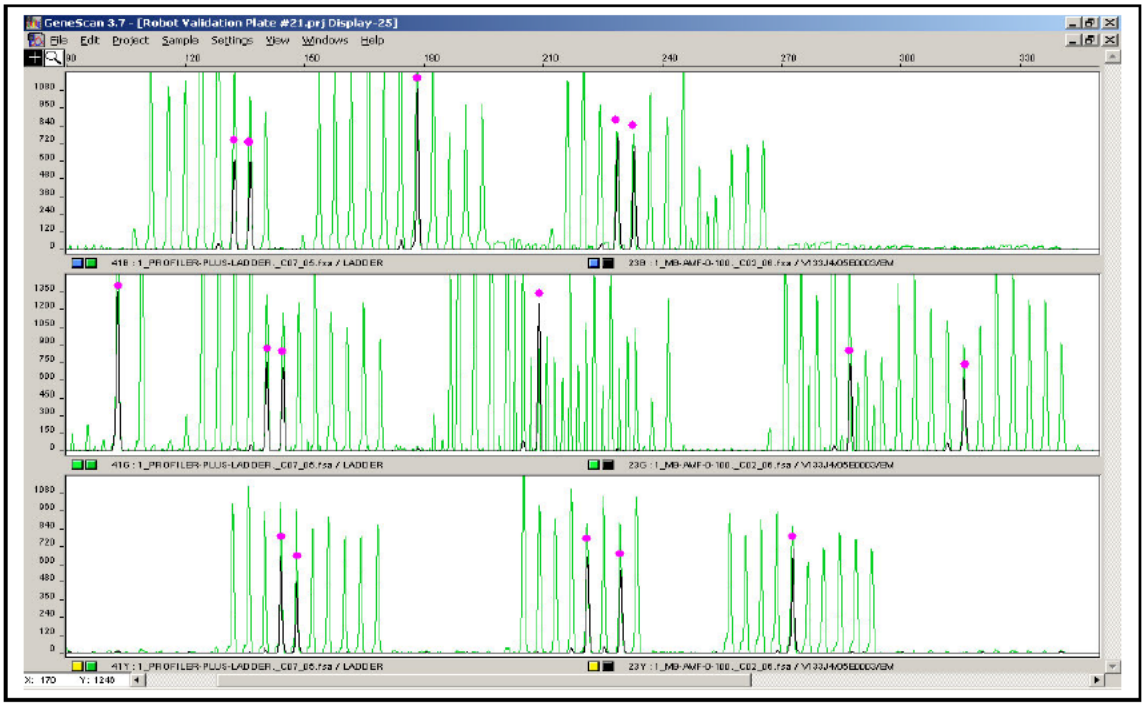


Figure 39. Electropherogram representing a 10-90µl mix of a male and female staff member.

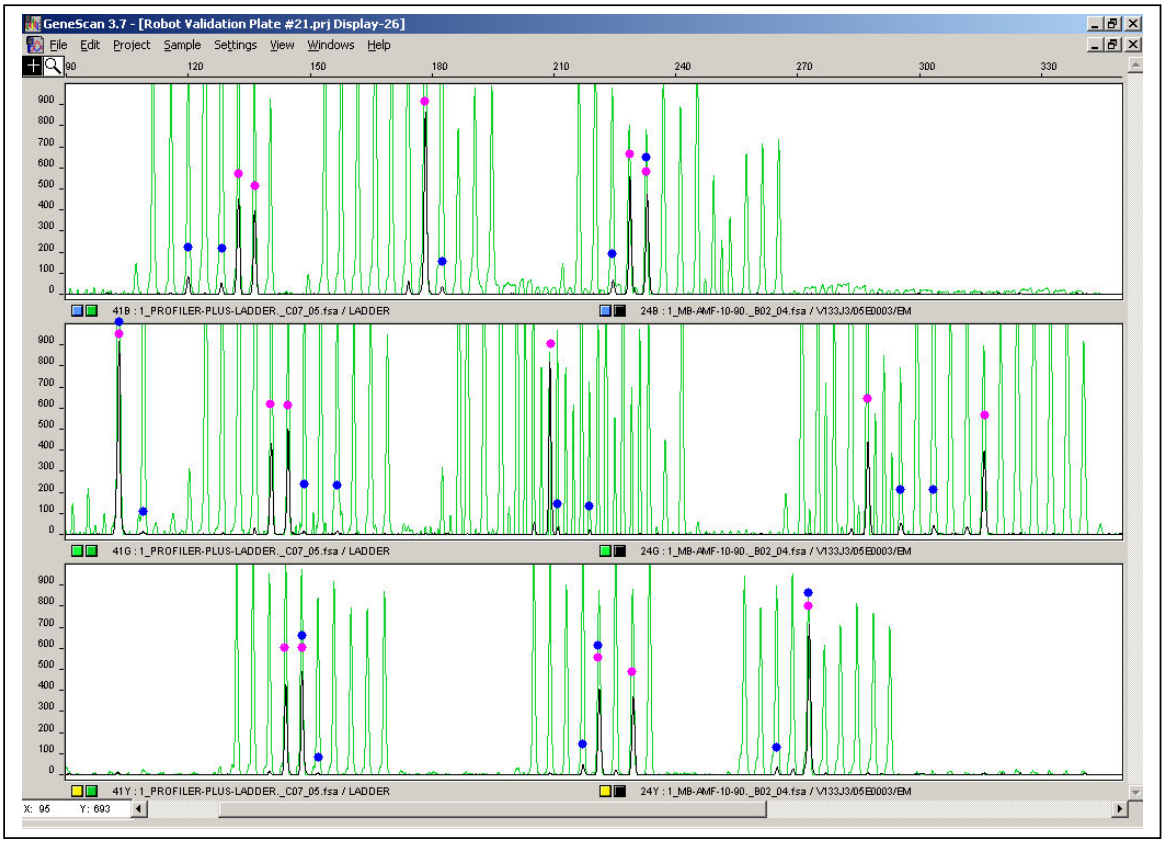


Figure 40. Electropherogram representing a 20-80µl mix of a male and female staff member.

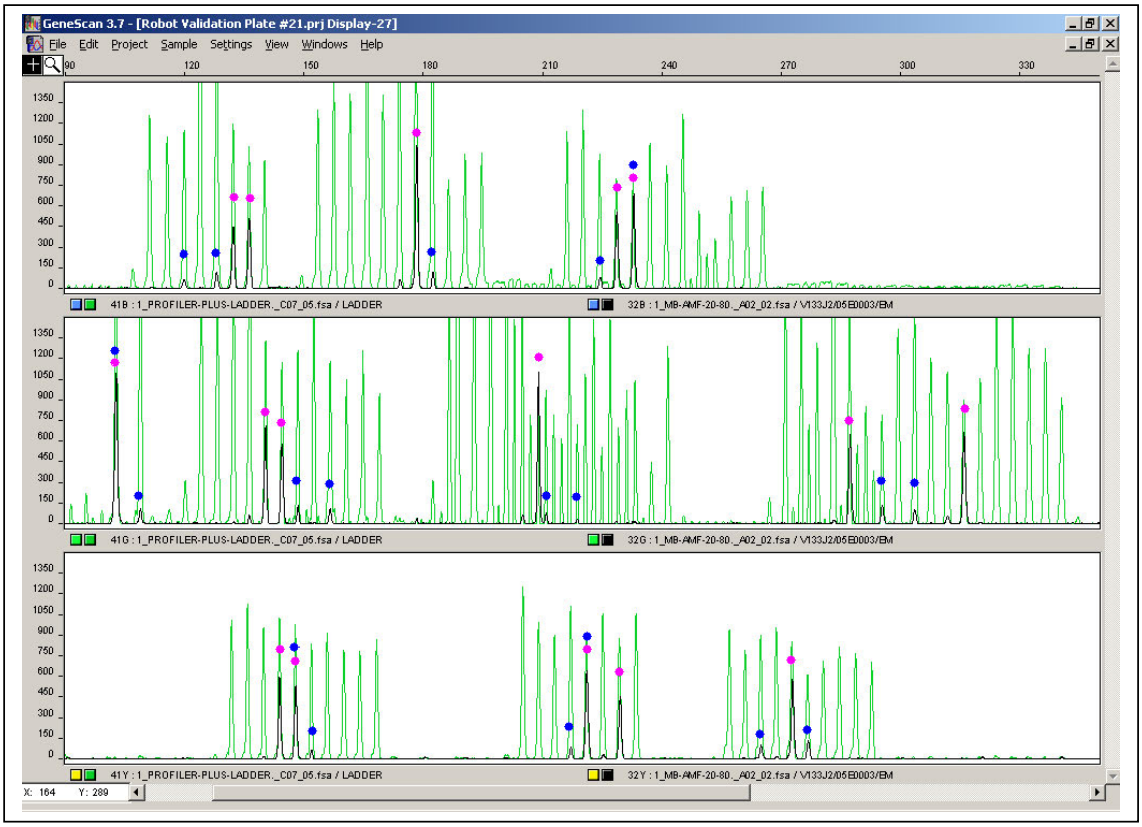


Figure 41. Electropherogram representing a 30-70µl mix of a male and female staff member.

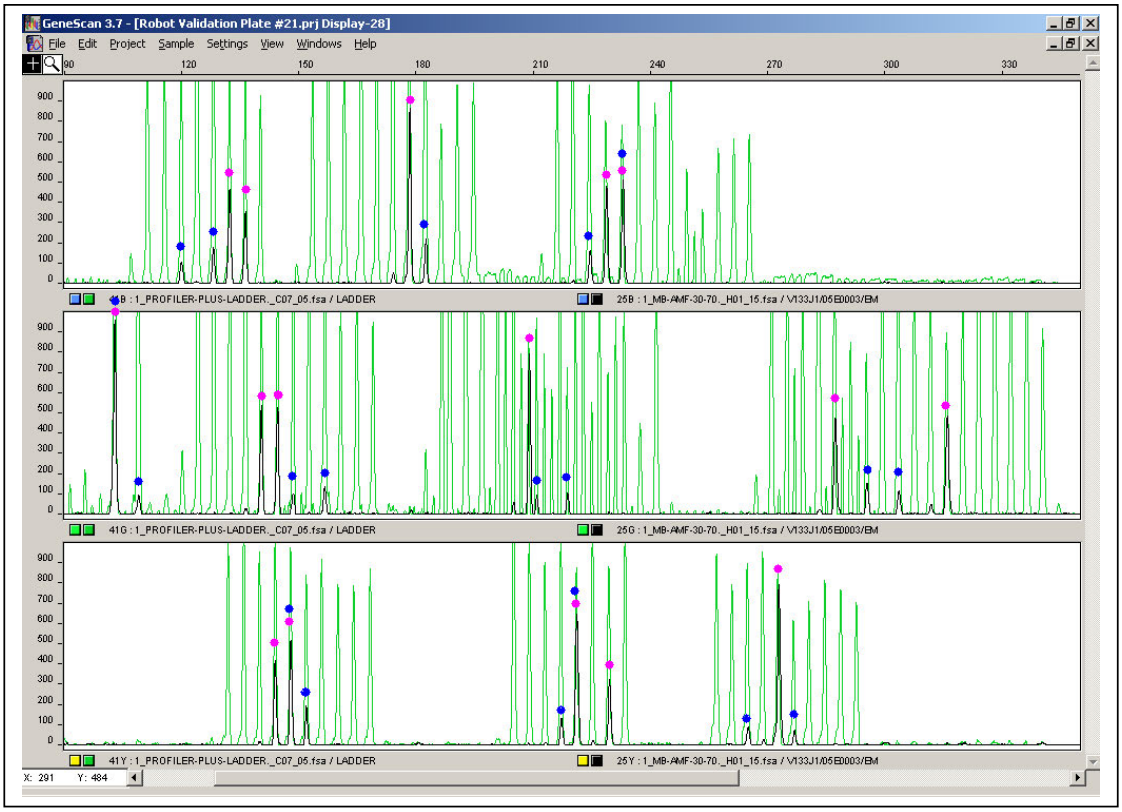


Figure 42. Electropherogram representing a 40-60µl mix of a male and female staff member.

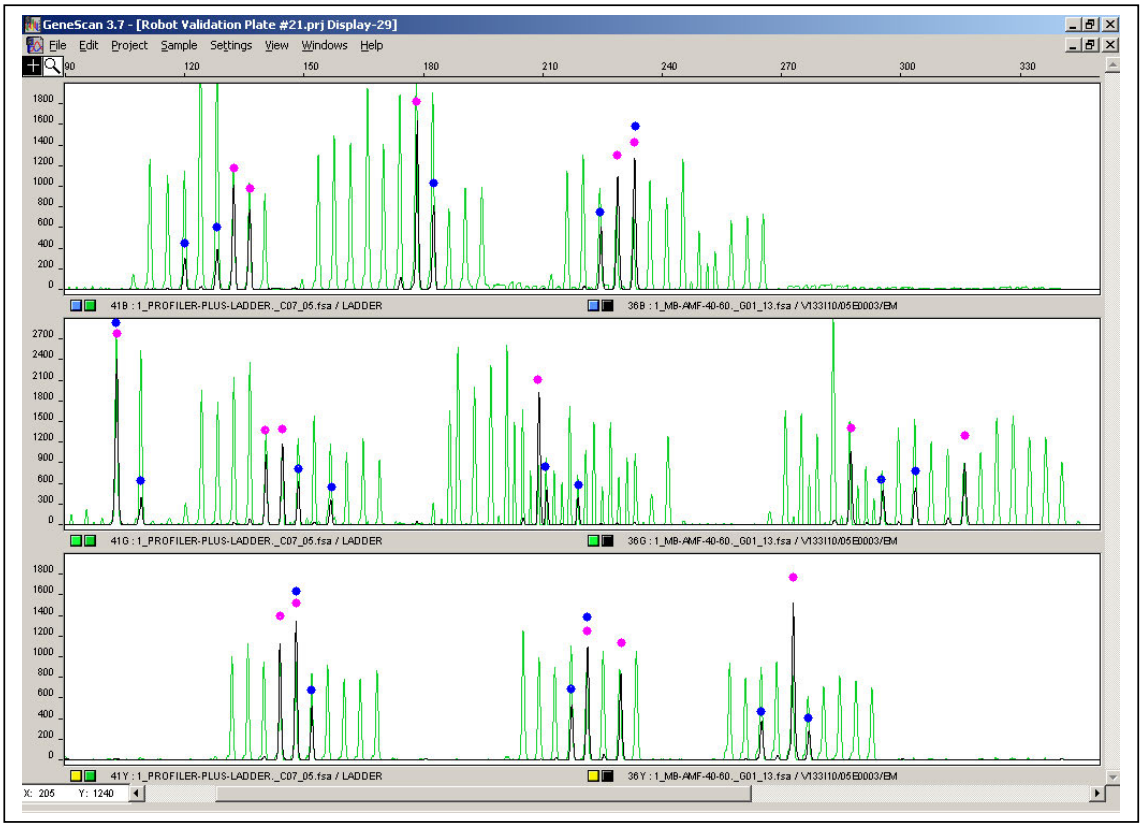


Figure 43. Electropherogram representing a 50-50µl mix of a male and female staff member.

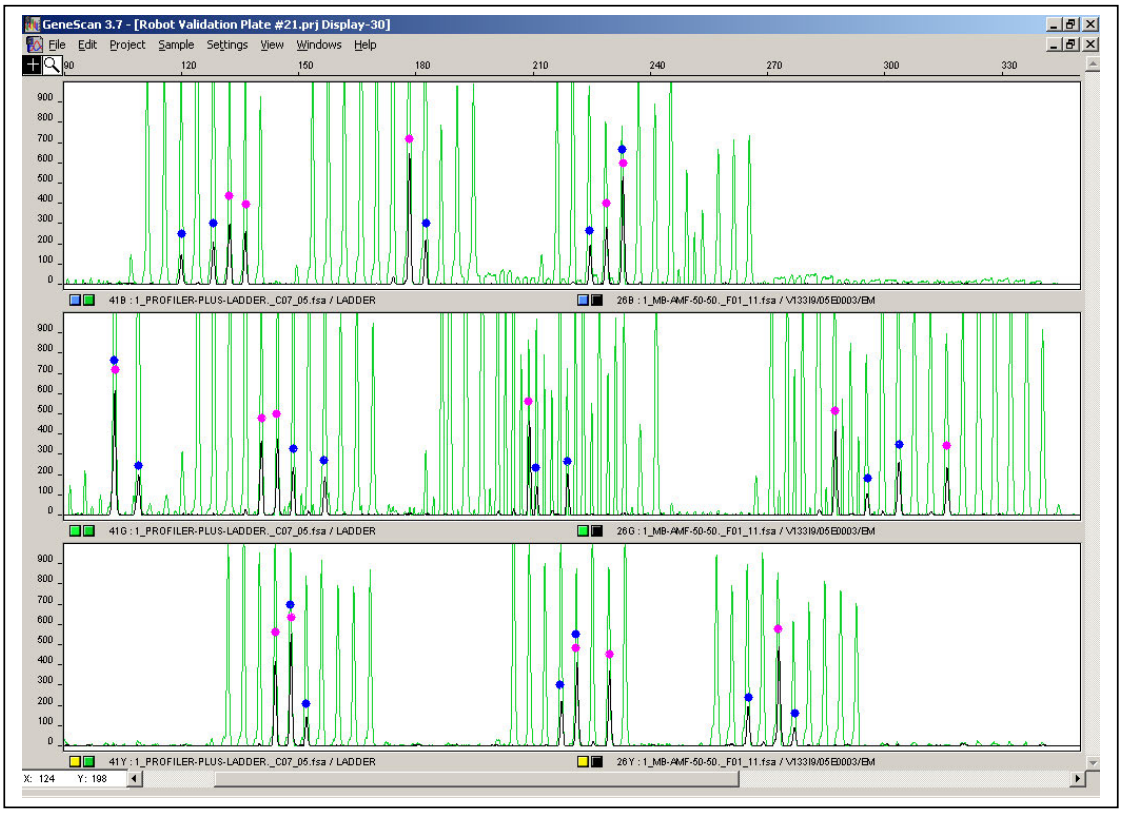


Figure 44. Electropherogram representing a 60-40µl mix of a male and female staff member.

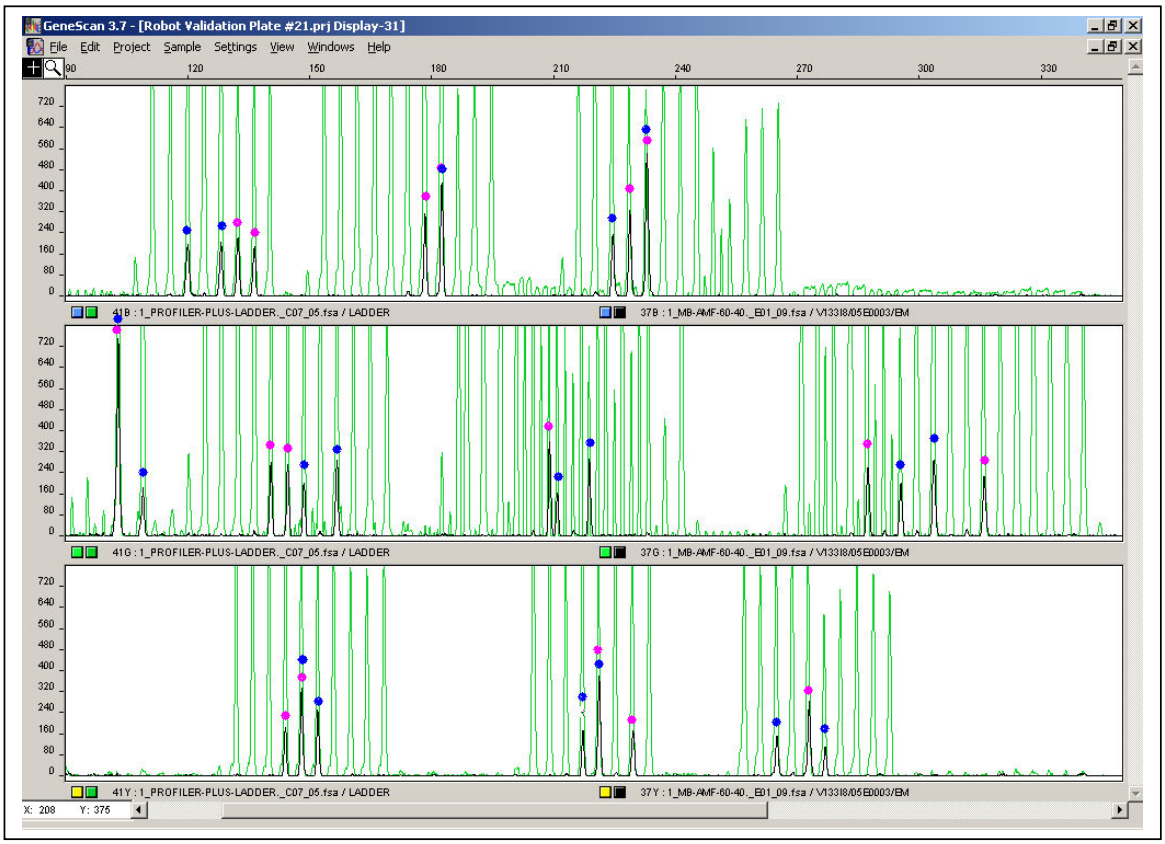


Figure 45. Electropherogram representing a 70-30µl mix of a male and female staff member.

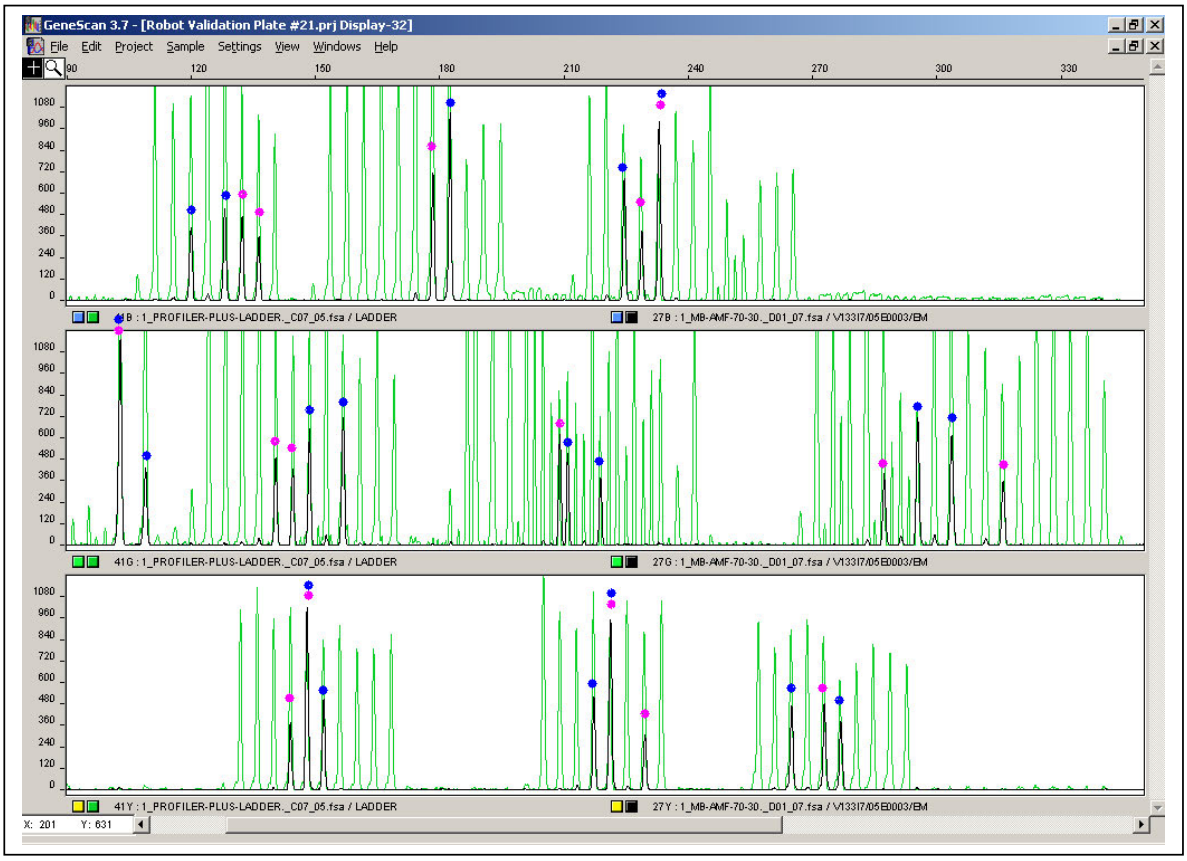


Figure 46. Electropherogram representing a 80-20µl mix of a male and female staff member.

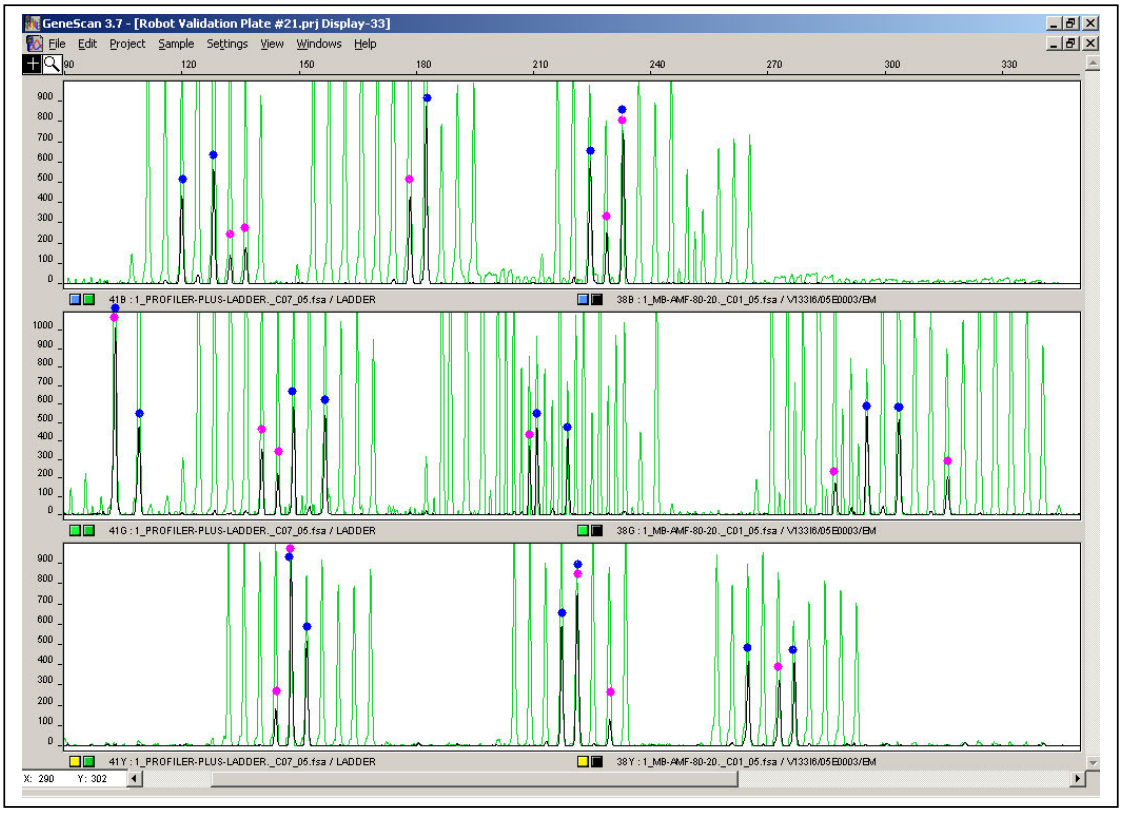


Figure 47. Electropherogram representing a 90-10µl mix of a male and female staff member.

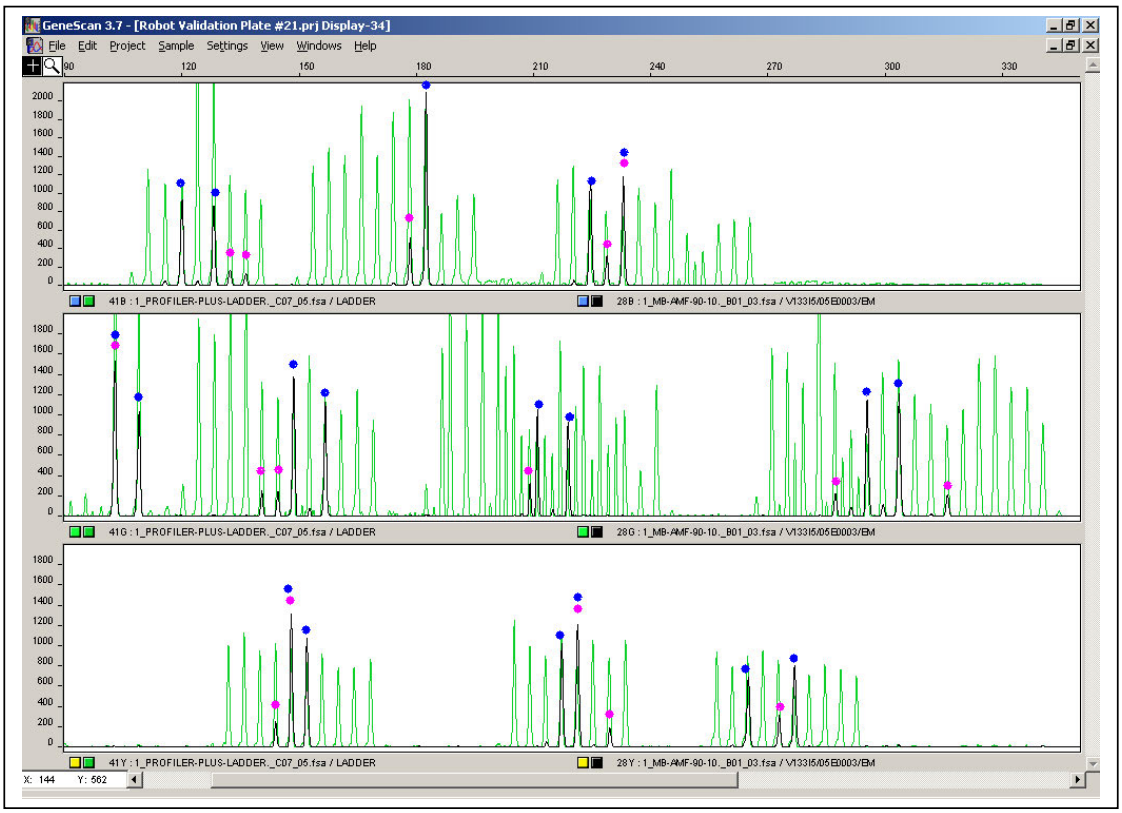
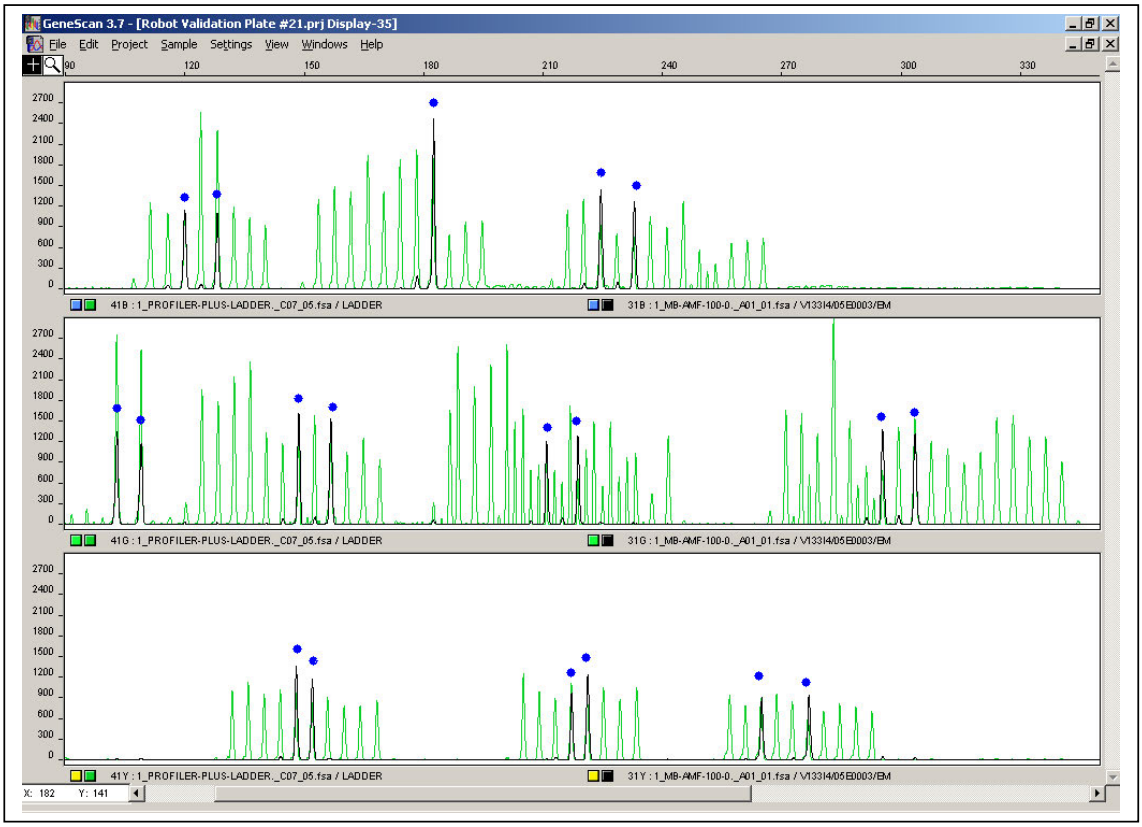


Figure 48. Electropherogram representing a 100-0µl mix of a male and female staff member.



Conclusion

From the above results it can be seen that a mixture at a ratio of 10:90 and 20:80µl of blood, although is below the detectable limits, can visually still be seen in the electropherograms. A mixed blood sample at 30:70µl ratio of blood is within the reportable range and can easily be detected.

Experiment 12 Re-Elution of Magnetic Beads

Aim

To determine if DNA profiles can be obtained from the dried magnetic beads that are left as waste following a completed extraction. Results will establish whether the dried magnetic beads in 96 well microtitre plates will be stored post extraction, or discarded immediately.

Method

Twentyfour heavily blood stained staff swabs containing 70-80µl of EDTA blood were extracted utilising the amended 2-Magnet Casework Extraction method on the MultiPROBE. At completion of this extraction run the 96well microtitre plate was left on the robotic platform overnight and covered with an adhesive plate cover, inplace of discarding it.
The following morning, an additional 35µl of elution buffer was added to the 96 well plate and samples were re-incubated at 65ºC as per steps 49-54 of the PathWest DNA IQ Casework Extraction protocol. The plate was then placed on the DNA IQ magnet and the eluate was separated and transferred into sterile microfuge tubes, producing a 2nd extract from the one extraction run.

All DNA extracts were quantitated using the Applied Biosystems Quantifiler™ Human DNA Quantification Kit (P/N 4343895) on the ABI Prism 7500 according to the current Forensic Biology Method (FBPCRS003).

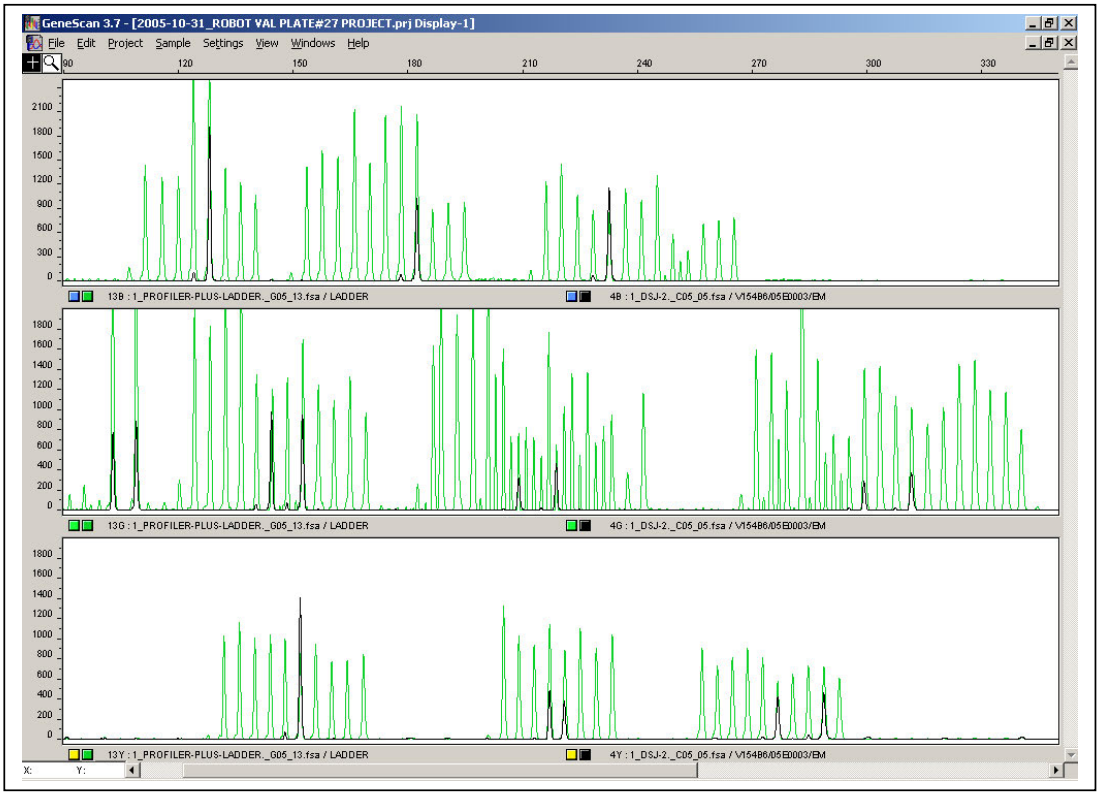
Sample profiles were then analysed using the Applied Biosystems Profiler Plus Kit (P/N 4303326) on the ABI Prism 3100 Genetic Analyser. All results were interpreted using both Genotyper and GeneScan V3.7.1 software programs and uploaded onto the WADNA Database.

DNA Extracts	Storage Codes
Staff Extracts	V153D5 – V153F7
Staff Re-Elution Extracts	V154A7 – V154C10

Results

Full reportable profiles were obtained for all of the profiles analysed from the re-elution of the original extracts.

Figure 49. Electropherogram representing a staff sample re-eluted following completion of an extraction run.



Conclusion

The above results indicate that full reportable profiles can be obtained after the completion of an extraction run. Therefore storage of a 96well plate is recommended and will allow re-elution of an extract or extraction run in the event of post extraction problem such as post extraction contamination.

Experiment 13 Performing Overnight Extractions

Aim

The aim of this experiment is to establish whether the MultiPROBE II may be used to perform DNA extraction runs overnight, therefore maximising the number of extraction performed per day.

Method

Sixteen heavily blood stained staff swabs containing both 3µl and 5µl of EDTA blood were extracted utilising the amended 2-Magnet Casework Extraction method on the MultiPROBE II. The extraction run was started at 3:19pm and left unattended overnight until 7am the following morning.

All DNA extracts were quantitated using the Applied Biosystems Quantifiler™ Human DNA Quantification Kit (P/N 4343895) on the ABI Prism 7500 according to the current Forensic Biology Method (FBPCRS003).

Samples were then analysed using the Applied Biosystems Profiler Plus Kit (P/N 4303326) on the ABI Prism 3100 Genetic Analyser. All results were interpreted using both Genotyper and GeneScan V3.7.1 software programs and uploaded onto the WADNA Database.

Samples

DNA Extracts	Storage Codes
Staff Extracts	V167D5 – V167E10

Results

It was observed that for every samples, the entire 35µl of DNA extract had evaporated throughout the night. An additional 35µl of elution buffer was added to the microfuge tube and vortexed to rehydrate the DNA sample. The results indicate that 33% of samples tested have failed to amplify. The 67% of samples that have amplified have given a full 10/10 reportable profile.

Figure 50. An electropherogram indicating a sample containing 5µl of blood that has been amplified after complete evaporation and re-elution has occurred. A complete profile was obtained.

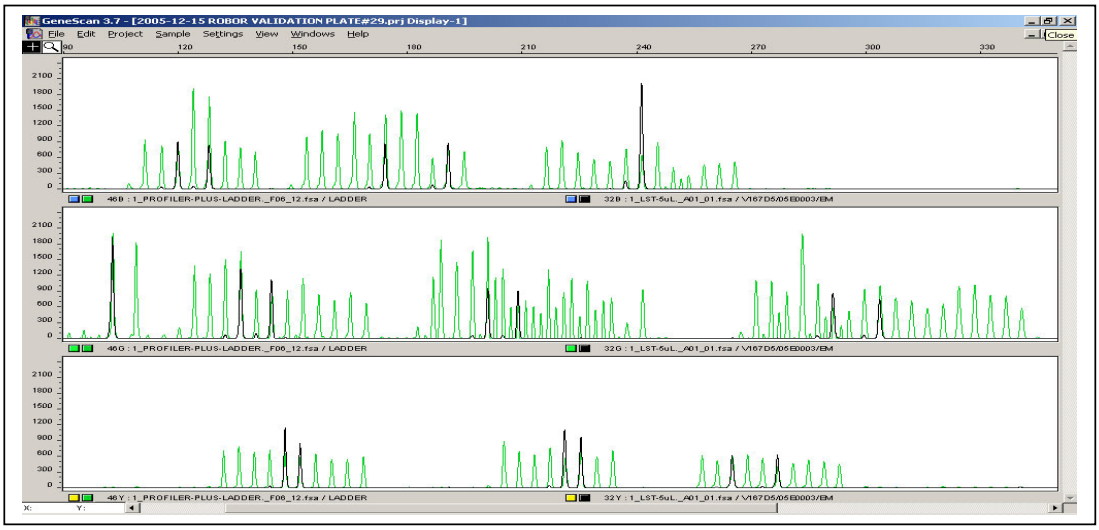
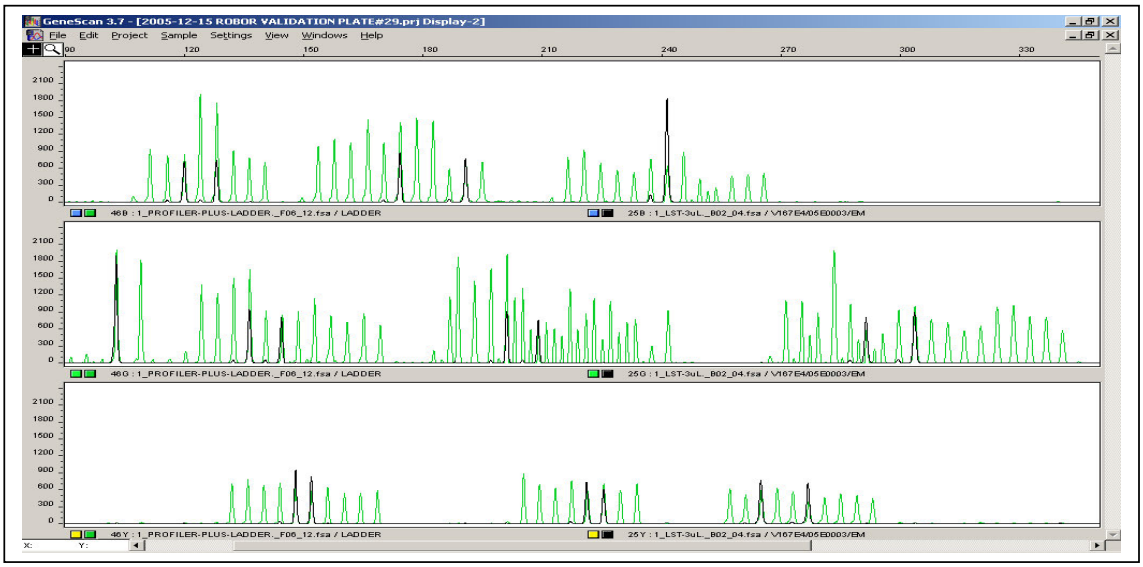


Figure 51. An electropherogram indicating a sample containing 3µl of blood that has been amplified after complete evaporation and re-elution has occurred. A complete profile was obtained.



Conclusion

It is not desirable to perform DNA extraction runs overnight as the heat generated in the dust cover caused complete evaporation of the DNA extracts. If for any reason sample extracts evaporate, then two thirds of the samples can be recovered with the addition of elution buffer. No contamination was seen in this experiment.

Completed
23rd December 2005.

Louise Taylor
Forensic Scientist
Forensic Biology
PathWest

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DNA IQ™ Method of Extracting DNA from Casework and Reference Samples

1 PURPOSE AND SCOPE

This method describes the routine method of DNA extraction using the PerkinElmer MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms and Promega DNA IQ™ kit. The manual method has been included as a back-up method should it be required.

This method applies to all DNA Analysis staff that are required to extract DNA from samples.

Reference samples and Casework samples must be extracted separately. If Casework and Reference samples are to be extracted on the same instrument, the instrument must be decontaminated between operations.

2 DEFINITIONS

Samples	Samples awaiting DNA extraction
Lysates	Samples that have had the Lysis step performed, but have not yet completed the entire extraction process
DNA Extracts	Samples that have had a DNA extraction processes performed
DNA IQ™ Resin	Magnetic Resin Beads used to bind DNA
MP II	MultiPROBE® II PLUS HT EX Platform
DTT	1,4 Dithiothreitol
Pro K	Proteinase K
SDS	Sodium Dodecyl Sulphate
TNE	Tris, NaCl and EDTA Buffer
EDTA	Ethylenediaminetetraacetate
EP-A	Extraction Platform A
EP-B	Extraction Platform B

3 PRINCIPLE

Sample Pre-lysis

The Extraction Buffer used in the pre-lysis of cellular material contains Proteinase K (Pro K), TNE (Tris, NaCl, EDTA) and SDS. TNE acts as a basic buffer with EDTA chelating ions in solution. SDS is a detergent that lyses open cell membranes. Proteinase K is added to digest protein and cellular material that interferes with the DNA binding capacity of the resin. It is also added to rapidly inactivate enzymatic activity that could potentially degrade DNA (e.g. nucleases).

Proteinase K (also known as Endoproteinase K) is a fungal enzyme from *Engyodontium album* (formerly *Tritirachium album*). It is a stable S8 family serine alkaline protease that has broad specificity and is inactivated by diisopropylfluorophosphate, phenyl-methane sulfonyl fluoride and Hg^{2+} ions. As a highly active endopeptidase, it cleaves peptide bonds adjacent/next to the carboxyl group of N-substituted (blocked alpha amino) hydrophobic, aliphatic and aromatic amino acids. Therefore, it actively hydrolyses proteins, glycoproteins, peptides and esters of amino acids. The smallest peptide that can be hydrolysed with Proteinase K is a tetrapeptide.

DNA IQ™ Kit

The DNA IQ ™ kit is designed to purify DNA from a number of different substrates and has been optimised for trace DNA samples. An in house validation was performed using a modified version of the PerkinElmer automated protocol. The protocol has been modified to incorporate a number of work practices used in DNA Analysis FSS. These are:

- The use of the Slicprep™ 96 device (Promega) for removing substrate from lysate.

- The increase of Extraction Buffer volume to 500µL for use with the Slicprep™ 96 device.
- The use of tubes and spin-baskets for the off-deck lysis of samples prior to extraction on MPII. Use of a 96-deepwell plate for completion of extraction on MPII.
- The provision of initial incubation with TNE Buffer and retention of a portion thereof for further testing (retained supernatant testing).
- The increase of Lysis Buffer volume to 957µL proportional to the increase of Extraction Buffer volume, according to the manufacturer's instructions.
- Double Elution step, with an Elution Buffer volume of 60µL for a final volume of 100µL.
- The use of NUNC Bank-It tubes for storage of final extracts.

Cell lysis is performed with Promega Lysis Buffer containing Dithiothreitol (DTT). 1,4 Dithiothreitol is a reducing agent used in extractions to break disulfide bonds of proteins. The Lysis Buffer used is a proprietary buffer containing chaotropic salts required for binding of DNA to the magnetic beads. According to the MSDS, the Lysis Buffer contains 50-75% guanidinium thiocyanate, < 2.5% EDTA, < 2% 3-[(3-Choalamidopropryl)dimethylammonio] propanesulfonic and < 2% polyethylene glycol tert-octylphenyl ether.

The basis of the DNA IQ™ kit is a magnetic bead resin which contains novel paramagnetic particles covered with silica. The magnetic bead resin usually has a DNA binding capacity of 100ng but the addition of Pro K will increase the binding capacity. Samples with small amounts of DNA are more efficient than samples with large amounts of DNA, and as a result a small sample size is critical to ensure efficient recovery of DNA.

The magnetic beads with silica have a negative charge at basic and near neutral pH. The Lysis Buffer changes the pH and salt concentration of the solution and the silica on the magnetic beads becomes positively charged which is then able to bind the DNA.

Several washing steps are employed in the protocol to remove inhibitors. The first washing step is performed with Lysis Buffer. This wash ensures the DNA is bound to the magnetic bead resin and washes out inhibitors. The next three washing procedures are with a Wash Buffer. This buffer contains an alcohol/aqueous mixture which ensures the DNA is not eluted during washing by keeping the DNA dehydrated, and aqueous phase washes out the inhibitor.

Elution Buffer removes the DNA from the magnetic bead resin. The Elution Buffer changes the salt content. Heating the complex to 65°C allows the DNA to be released from the magnetic beads. The Elution Buffer is a low ionic strength buffer that reduces the affinity of the DNA for the silica that covers the magnetic beads, by re-hydration of the phosphate backbone.

The DNA IQ™ kit isolates DNA greater than 80bp, smaller DNA is removed selectively to prevent PCR inhibition.

MultiPROBE® II HT EX PLUS with Gripper™ Integration Platform

Within DNA Analysis, routine DNA extractions are performed using either one of two MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms (Extraction Platform A, EP-A) and (Extraction Platform B, EP-B) perform casework or reference samples.
Each platform uses a computer – controlled Cartesian X-Y-Z liquid handling system designed for the efficient automation of sample preparation. Liquid transfer is performed by an 8-tip System with VersaTip® and VariSpan™ options. The VersaTip® option allows the use of fixed and/or disposable tips (both clear and conductive). The VariSpan™ option permits variable probe spacing between each of the individual probes so that a wide range of labware such as micro plates, tubes, vials and reagent troughs can be accessed. Each

sample probe is capable of independent motion in the Z direction due to independent Z drives.

The 8-tip System is also capable of multichannel liquid-level sensing by utilising Accusense™ technology. This technology works by each probe detecting a change in capacitance within the liquid. This capacitive mode of detection is also possible when using conductive disposable tips. Accusense™ also permits the detection of low or non-ionic polar solutions and solvents. Pipetting on the platforms is driven by 8 individually controlled syringes via the positive displacement of the system liquid (nanopure water) when transferring liquid.

The Gripper™ Integration on all the platforms (except for the Post – PCR MPII) allows for automated identification of labware via the scanning of barcodes and also allows for automated transfer of plates from one position on the deck to another. To increase deck space and capacity, all platforms include a left deck extension.

In this program a platemap is utilised to provide the necessary information for correct volumes and locations for pipetting. It also contains information regarding positions where pipetting must take place at various locations, the number and IDs of samples. Without a platemap the program will fail to work.

4 REAGENTS AND EQUIPMENT

4.1 Reagents

1. DNA IQ™ System Kit – 400 sample Kit
    - Resin
    - Lysis Buffer (LB)
    - 2x Wash Buffer (2xWB)
    - Elution Buffer (EB)
2. Tris/Sodium chloride/EDTA Buffer (TNE)
3. Proteinase K (Pro K) 20mg/mL
4. Dithiothreitol (DTT) 1M
5. 5% TriGene
6. 70% Ethanol
7. 1% Amphyl
8. 0.2% Amphyl
9. Isopropyl alcohol
10. AnalR 100 %Ethanol
11. 20% SDS
12. Decon® 90 solution
13. Nanopure H_2O

Table 1. Reagent storage locations.

Reagent	Device	Storage Location
Pro K	Freezer	Room 6120
DTT	Freezer	Room 6120
20% SDS	Shelf	Room 6122
Isopropyl alcohol	Shelf	Room 6122
TNE pH 8 Buffer	Shelf	Room 6122
DNA IQ™ Kit	Shelf	Room 6122
Amphyl (1% and 0.2%)	Shelf	Room 6127
Nanopure Water	Shelf	Room 6127
5% TriGene	Shelf	Room 6127
AnalR 100 %Ethanol	Shelf	Room 6127

Please see Table 2 for the volume of reagents for a full plate or half plate. Refer to "*Receipt, Storage and Preparation of Chemicals, Reagents and Kits*" (QIS 17165) for

preparation of the TNE Buffer. All reagents can be made on the bench in Room 6122, except for the Lysis Buffer with DTT which needs to be made in a fume hood. DNA IQ reagents are prepared by staff performing the DNA IQ method.

Table 2. Table of reagent volumes.

Reagent (volume per sample)	Constituent (volume per sample)	Volume req'd for 96 Samples (mL)	Volume req'd for 48 Samples (mL)
Extraction Buffer (500 µL/sample	TNE Buffer 462.5µL	54	27
	Prot K (20 mg/mL)25.0 µL	2.9	1.5
	SDS (20 %) 12.5µL	1.5	0.7
Lysis Buffer (with DTT) (1.127mL/sample)	Lysis Buffer (no DTT)	130	66
	DTT (add to Lysis Buffer)	1.3	0.66
Lysis Buffer (with DTT) Reagent Trough	As above	125	63
DNA IQ RESIN Solution (50µL/sample)	Lysis Buffer (with DTT) (from above) 43µL	6	3
	DNA IQ RESIN 7µL	1	0.5
DNA IQ 1X Wash Buffer (300µl/sample)	See Below for preparation	35	18
DNA IQ Elution Buffer (120µl/sample)	Use directly from Kit	14	8

NOTE: For batches not equal to either 96 or 48 samples samples, refer to Appendix 1 "*18.1 Reagents Calculation Tables*" Table 7 for batches of <48 samples, and Table 8 for <96 (but >48)

Extraction Buffer

Prepare the buffer just before extraction in a 100mL sterile glass bottle according to Table 2. Use one aliquot of 20mg/mL Proteinase K (1.5mL) for a 48 sample run and two aliquots for a 96 sample run. Remove aliquot/s of Proteinase K from the freezer, defrost, vortex and centrifuge before use. Ensure that the 20% (v/v) SDS is completely dissolved (clear) in the stock solution before making the Extraction Buffer. If not dissolved, invert the container a few times and leave longer at room temperature.

Lysis Buffer with DTT

Lysis Buffer is supplied with the kit. Lysis Buffer with DTT is prepared at the start of each run. Remove the DTT from the freezer, defrost, vortex and centrifuge before use. Into a sterilised glass bottle, add 1.3mL of DTT to 130ml of Lysis Buffer for 96 samples. If 48 samples are to be run, use 660µl of DTT to 66ml of Lysis Buffer, again, made up in a sterile glass bottle. Make up the Lysis Buffer with DTT within the Laminar Flow and ensure that full PPE is worn, including face shield. Warning: Lysis Buffer and DTT are toxic.

DNA IQ™ Resin

DNA IQ™ Resin is supplied with the kit. The resin is prepared at the start of each run in a 10mL sterile tube. Ensure the resin is properly mixed by vortexing before pipetting. Look for calculations in Table 2 for the correct volumes of resin and Lysis Buffer (with DTT). In the 10mL tube, mix by inversion before adding to column 4 in the 12-channel reagent plate.

1X Wash Buffer

2X Wash Buffer is supplied with the kit. Once a new kit has been opened, add 35mL of ***AnalR*** Ethanol and 35mL of Isopropyl alcohol to the 2X Wash Buffer. Once the reagents have been added, label the lid and side of the bottle with "1X Wash Buffer," initial and date.

4.2 Equipment

The following equipment (Table 3) and consumables (Table 4) are required for the DNA IQ extraction.

Table 3. Equipment used and location.

Equipment	Asset No.	Location
STORstar (B system)	10238493	6122
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (Ext A Platform)	10076438	6127
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (Ext B Platform)	10076437	6127
DPC Shaker (Ext A Platform)	N/A	6127
DPC Shaker (Ext B Platform)	N/A	6127
Automated Temperature Controller, Heat Block tiles and heat block adapters (Ext A Platform)	N/A	6127
Automated Temperature Controller, Heat Block tiles and heat block adapters (Ext B Platform)	N/A	6127
Eppendorf 5804 centrifuge	10238416	6127
Vortex	30087015	6127
Fridge	30433424	6127
Micro centrifuge	None	6127
Decapper	None	6127

Table 4. Consumables used for extraction

Consumables	Location
175uL Clear Non-Conductive Filter Tips – Pre-sterilized	6127
MβP Pure 1000uL Tips – Pre-Sterilized	6127
SlicPrep™ 96 device plate	6122
ABgene 96-deepwell plate	6120
Axygen 2mL Deep Well storage plate	6127
1.5ml or 2ml Eppendorf tubes with Spin baskets	6120
12 Channel plate	6127
Nunc tubes	6120
Nunc Caps	6127
Sterile 50mL Falcon tubes	6122
Sterile 10mL tubes	6122
Autoclaved 100mL glass bottles	6122
Autoclaved 250mL glass bottles	6122
Aluminium sealing film	6127
1000uL disposable tips	6120

5 SAFETY

As per the procedures in the QIS document "*Operational Practices in the DNA Dedicated Laboratories*" (QIS 17120), PPE is to be worn by all staff when performing this procedure.

The automated platforms, labware and associated equipment should be wiped with 5% TriGene™ followed by 70% ethanol before and after use.

While the MPII is running a procedure, refrain from placing any part of the body inside the cabinet. Either pause the program or use the emergency STOP button located at the front of the instrument before touching anything within the cabinet or on the deck surface.

Warning: Tris base, EDTA, SDS and Lysis Buffer are irritants. DTT is a reducing agent that destroys disulfide bonds. Chaotropic reagents such as guanidinium thiocyanate (GuSCN) are toxic. Do not breathe alcohol fumes. Handle carefully and wear appropriate PPE.

Lysis Buffer contains guanidinium thiocyanate which can be harmful if inhaled, swallowed or comes in contact with skin. Any left over Lysis Buffer with DTT is to be disposed of in a brown Winchester bottle in the fume hood. Never dispose down the sink. If spillage occurs, absorb with liquid-binding material (such as sand, kitty litter, etc) and dispose in a biohazard bin. Again, handle carefully and wear appropriate PPE, including face shield.

6 SAMPLING AND SAMPLE PREPARATION

Samples waiting to be extracted are stored in freezers as described in Table 5.

Table 5. Sample storage locations.

Sample type	Storage Device	Storage Location
Urgent/High/Medium Priority Samples	Freezer	6117-2
Medium Priority Samples	Walk in Freezer or Freezer in 6117	6109 or 6117-5
Low Priority Samples	Walk in Freezer or Freezer in 6117	6109 or 6117-5
Lysates in 1.5ml tubes	Fridge	6120
96 deep well plate containing lysates	Fridge	6127

QC samples

All extraction batches require two controls to be registered. These controls, UR numbers and positions are listed in Table 6.

Table 6. Extraction Quality Controls

QC Name	UR Number	Description
Negative Control	FBOT277	Negative Extraction control – Empty well
Positive Control	FBOT279	Positive Extraction control – Known Donor dried blood swab

Registration of QC

1. Log into the **AUSLAB Main Menu**.
2. Select **1. Request Registration**.
3. Select **2. Full Reception Entry**.
4. Scan in barcode of control.
5. Enter the UR number as per Table 6 and press **[Enter]**.
6. Enter the appropriate Specimen type (e.g. **Blood** for blood control).
7. Request a **9PLEX** test, when prompted to enter the processing comment, enter **EXTP** (Positive extraction control) or **EXTN** (Negative extraction control). Do no assign a priority.
8. Press **[F7]** ***Save*** to save the Billing details.
9. Enter **LAB** in the Billing code field and **t** in the date field and **FBQC** in the Loc/Client field.
10. Press **[F4]** ***Save twice*** to save the registration details.

Note 1: Quality controls should not have a DNA priority allocated at time of registration to ensure they are included in the top positions of a batch

Note 2: for DNA IQ Lysis batches with more than 46 samples (excluding controls) two sets of controls should be registered

Create the DNA IQ Lysis or Retain Supernatant Batch (as required)

1. Log into the **AUSLAB Main Menu**.
2. Select **5. Workflow management**.
3. Select **1. DNA workflow table**.
4. Highlight the appropriate batch type and press **[F5]** ***Batch Allocation***.
5. Press **[F6]** ***Create batch***.
6. Press **[F8]** ***Print menu***.
7. Press **[F7]** ***Print Sample Label***. (print 3 sets)
8. Press **[F8]** ***Print Worksheet***. (print 2 copies)
9. Press **[SF5]** ***Main menu***.
10. Press **[SF11]** ***Print***.

11. Press **[SF6]** ***Accept batch***.
12. Press **[Pause/Break]** to exit to the **Main Menu**.
13. Obtain worksheets **(FBLASER3)** and labels **(FBLABEL13-16)** from the Analytical Section printing bench **(Room 6117)**.

Locating Samples

To locate samples refer to "*Analytical Sample Storage*" (QIS 24255).

7 OFF-DECK LYSIS PROCEDURE (NO RETAINED SUPERNATANT)

1. Print or obtain a copy of Appendix 2. "*18.2 Reagent & Batch details recording tables (DNA IQ™ Lysis Batch & Extraction Batch)*".

2. Separate the batch into two smaller batches of 48 samples, including one set of controls. (If only a single operator the second batch can be started during step 11)

Note: Positions 1-4 will be the two sets of controls, positions 5-50 will be the first 46 samples and 51-96 will be the second set of 46 samples

3. Remove samples from 5mL storage tubes and label side with barcodes if necessary. If substrates are in a 0.5mL tube, transfer to a labelled 1.5mL tube. Label lid of each sample with position number within the lysis batch. Retain the original 5mL storage tube for substrate storage.

4. Prepare / assemble spin basket assembly or 1.5mL (or 2.0mL) tube and label side with a barcode and lid with position number as required.

Note: substrates from each sample need to be retained

a. Samples requiring a spin basket include swabs, fabric, paper and cigarette butts.
b. Samples requiring a 1.5mL include tapelifts, chewing gum, straws and toothbrush bristles.

5. Label the side of sterile 1.0mL Nunc Bank-It tubes with barcode.

6. Have a second operator sequence check all tubes and complete the sequence check in AUSLAB.

7. Prepare Extraction Buffer (store at 4ºC when not in use).

8. Using a multi-stepper pipette add 500µL of Extraction Buffer and vortex briefly.

9. Incubate in a hotblock at 37°C for 45minutes (note temperature on worksheet).

10. Remove samples from hot block and vortex briefly then return to rack.

11. Increase temperature on hotblock to 65°C (preparation for second incubation step).

12. Transfer substrates to spin baskets if required using twirling sticks (if unable to remove with twirling sticks, use forceps. Forceps must be cleaned between each sample by rinsing in bleach followed by ethanol and flaming).

13. For samples not requiring spin baskets, transfer the lysate to the newly labeled 1.5mL tube. Then store original 1.5mL containing substrate in the original 5mL tube.

14. Centrifuge spin baskets at maximum speed (14000rpm) for 2 minutes.

15. Transfer flow through from spin baskets back to original lysis tube, retain spin basket in 5mL tube.

16. Vortex Lysates briefly, then incubate in hotblock at 65°C for 10 minutes (note temperature on worksheet)

17. Enter reagent details, temperatures etc. into AUSLAB.

18. Complete batch in AUSLAB.

19. Store lysates at 4°C (fridge in 6120).

20. Enter into completed batch in AUSLAB and determine the DNA IQ extraction batch ID the DNA lysates have progressed to.

21. Print the appropriate worksheets and 3 sets of batch labels for the DNA IQ extraction batch and note the lysis batch the samples have progressed from on the worksheets. Stamp as "Urgent" if necessary.

8 OFF-DECK LYSIS PROCEDURE (RETAINED SUPERNATANT)

1. Print or obtain a copy of Appendix 2. "*18.2 Reagent & Batch details recording tables (DNA IQ™ Lysis Batch & Extraction Batch)*".

2. Remove samples from 5mL storage tubes and label side with barcodes if necessary. If substrates are in a 0.5mL tube, transfer to a labelled 1.5mL tube. Label lid of each sample with position number. Retain 5mL tube for substrate storage.

3. Label the side of 1.5mL tubes with barcodes for retaining supernatant. Also label lid of 1.5mL tube indicating it contains supernatant.

4. Prepare spin basket assembly or a 1.5mL (or 2.0mL) tube and label side with a barcode and lid with position number as required. **Note:**
   a. Samples requiring a spin basket include swabs, fabric, paper and cigarette butts.
   b. Samples requiring a 1.5mL include tapelifts, chewing gum, straws and toothbrush bristles.

5. Label the side of sterile 1.0mL Nunc Bank-It tubes with barcode.

6. Have a second operator sequence check all tubes and complete the sequence check in AUSLAB.

7. Using a pipette add 650µL of TNE Buffer and vortex briefly.

8. Incubate at room temperature for 30 minutes.

9. During 30 minute incubation prepare Proteinase K and SDS solutions.

10. Vortex, then centrifuge for 3 minutes at maximum speed (14000rpm).

11. Remove 150µL of supernatant and place into labelled 1.5ml tube (for further testing).

12. Add 25µL of 20ng/µL (mg/mL) Proteinase K and 12.5µL 20% (w/v) SDS to each original sample tube containing TNE Buffer. Vortex briefly.

13. Incubate in hotblock at 37°C for 45 minutes (note temperature on worksheet).

14. Remove samples from hotblock, vortex briefly and return to rack.

15. Change settings on hotblock to temperature of 65°C (preparation for second incubation step).

16. Transfer substrates to spin baskets if required using twirling sticks (if unable to remove with twirling sticks, use forceps. Forceps must be cleaned between each sample by rinsing in bleach followed by ethanol and flaming).

17. For samples not requiring spin baskets, transfer the lysate to the newly labeled 1.5mL tube. Then store original 1.5mL containing substrate in the original 5mL tube.

18. Centrifuge spin baskets at maximum speed (14000rpm) for 2 minutes.

19. Transfer flow through from spin baskets back to original lysis tube, retain spin basket in 5mL.

20. Vortex Lysates briefly, then incubate in hotblock at 65°C for 10minutes (note temperature on worksheet).

21. Enter reagent details, temperatures etc. into AUSLAB.

22. Complete batch in AUSLAB.

23. Store supernatants in Freezer 6117-2 (-20°C).

24. Store lysates at 4°C (Fridge in 6120).

25. Enter into completed batch in AUSLAB and determine the DNA IQ extraction batch ID the DNA lysates have progressed to.

26. Print the appropriate worksheets and 3 sets of batch labels for the DNA IQ extraction batch and note the retained supernatant batch the samples have progressed from on the worksheets. Stamp as "Urgent" if necessary.

9 MPII EXTRACTION PROCEDURE

Automated setup of DNA IQ extractions in 96-deep well format is performed using the MultiPROBE® II PLUS HT EX EP-A and EP- B platforms located in Room 6127.

Refer to "*Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform*" (QIS 23939) for instructions on the use and maintenance of the MultiPROBE® II PLUS HT EX platforms.

Summary of DNA IQ EXTRACTION Version 2 ODL

1. ***Binding of paramagnetic resin to DNA and further Lysis:*** add Resin solution (50µL) and Lysis Buffer (957µL). Automated mixing and shaking at room temperature for 5

minutes. (this occurs at steps 10-15 of the protocol)

2. ***Removing lysis reagents:*** Slicprep plate is moved to the PKI Magnet to separate beads. Removing of supernatant (1600µL) without disturbing resin, dispense this solution in the storage plate. (this occurs at steps 16-18 of the protocol)

3. ***Washing of the resin-DNA complex:*** To remove any inhibitors in solution. The first wash is with Lysis Buffer (125µL), shaking at room temperature for 1 minute. The plate is moved to the PKI Magnet and the supernatant is removed into the storage plate. The next three washes are with 1X Wash Buffer (100µL), shaking at room temperature for 1 minute. During each wash cycle, the plate is moved to the PKI Magnet and the supernatant is discarded. (this occurs at steps 21-59 of the protocol)

4. ***Removing any excess of 1X Wash Buffer:*** air dry at room temperature for 5 minutes. (this occurs at step 60 of the protocol)

5. ***Elution of DNA from the Resin-DNA complex:*** Add Elution Buffer (60µL) and incubate at 65 °C for 6 minutes (3 minutes no shaking and 3 minutes shaking). The plate is moved to the PKI Magnet. The eluted solution (supernatant) is removed to the Nunc tubes. Elution is repeated twice. (this occurs at steps 63-83 of the protocol)

6. ***Flushing of capillaries:*** The capillaries are washed with Amphyl and nanopure water.

Preparation of Reagents prior to extraction

1. Refer to table 2 for reagent volumes to make up the required amount of Lysis Buffer (with DTT) and Resin solution. Also measure the required amount of 1X Wash Buffer.

2. Record the Lot numbers of all the reagents used onto the AUSLAB worksheet.

Sequence Check the Nunc Bank-It™ tubes and Sample Lysates

To sequence check storage tubes and transfer DNA lysates to ABgene 96-deep well plates, please refer to method "*Procedure for the Use of the STORstar unit for automated sequence checking*" (QIS 24256).

ENSURE the Nunc tube rack is labelled with the AUSLAB Batch ID and barcode on the front of the plate.

Setting up the EP-A or EP-B MPIIs

These steps are to be carried out in the Automated extraction Room (Room 6127)

3. Turn on the instrument PC.

4. Log onto the network using the **Robotics** login.

5. Double click the WinPrep® icon on the computer desktop (Figure 1).

Figure 1 The WinPrep® icon.

6. Log onto the WinPrep® software by entering your username and password, then press **"Enter"**.

7. Ensure the **System Liquid Bottle is FULL** before every run and perform a Flush/Wash.

8. Ensure that the daily/weekly start-up has been performed before running any program. If WinPrep® has been closed or been idle for a long period of time initialise the MP II platform as described in QIS 23939.

9. Open the Extraction setup MP II test file in WinPrep® by selecting:
   - **File**
   - **Open**, navigate to **C:\PACKARD\MULTIPROBE\BIN\QHSS PROTOCOLS**
   - Select "**DNA IQ Extraction_Ver 2_ODL.mpt**"
   - Click the "**Open**" button

10. Check the whole program there are no bold fonts (e.g. plates may lose gripability and the program will not run). See the line manager.

11. Open the required plate map from the network **I:\EXTRACTION**. Check that the plate map is complete. If there are blanks (samples that have been removed), make all volumes to 0. If it is not a full batch, delete the rows of blank samples at the end of the platemap, then save the changes. Copy the plate map to the following file path: **C:\PACKARD\EXT PLATE MAPS**

12. Decontaminate the required labware by wiping with 5% TriGene followed by 70% ethanol before placing the labware on to the designated grid positions as outlined in the virtual deck view within WinPrep® (Figure 2).

    - The white WALLAC Isoplates (catalogue #1450-514) that are used to support the ABgene 96-deep well plate must be placed into positions **D16** and **C19**.

    - Ensure that the PKI Magnet at **F16** is stable in its insert. This is critical so that beads and DNA are not lost during pipetting.

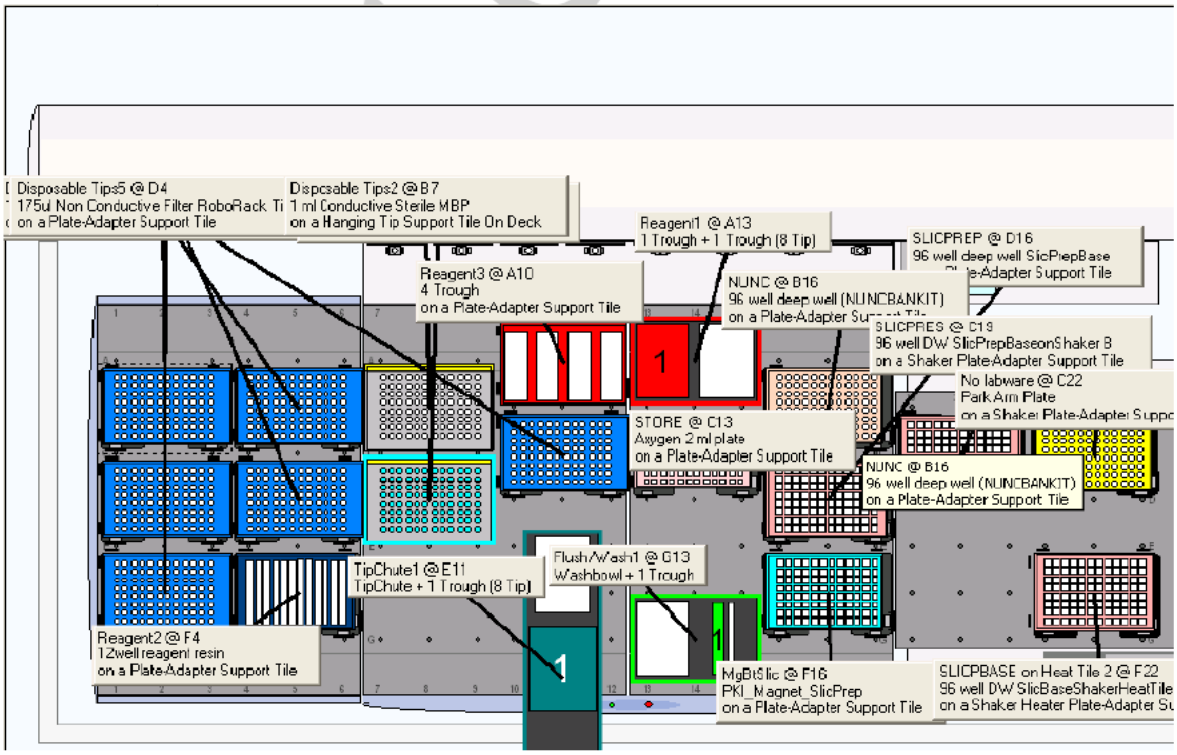

Figure 2. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

13. Ensure that the DPC Shaker and Heater Controller Box are switched on.
    For EP-A: Tile 1 at F22 (85^0C).
    For EP-B: Tile 2 at F22 (85^0C).
    Note: Press the start/stop button twice at the front of the DPC Shaker to ensure that it displays zero on the screen.

14. To the Amphyl wash station at **A10**, add fresh 1% Amphyl to the trough on the far left hand side, 0.2% diluted Amphyl to the middle left and place an empty reagent tough in the middle right position of the station. The nanopure water goes to position **G13** into a 160mL trough in the Flush-Wash station.

15. **Nunc tube rack**: Check that is the same Auslab batch number as the platemap to be run. Ensure that the barcode of the first (A1) and last tube (H12) are the respective ones. Check that the batch label and batch barcode labels are attached to front side of rack. Add B1-Lite generated "**NUNC**" barcode to the right side of the Nunc tube rack. Then place nunc rack into position **B16**.

16. On an Axygen 2ml deep well Storage plate, label the left side of the plate with both the Batch ID and barcode. Label the right side of the plate with a B1-Lite generated "**STORE**" barcode. Then place in position **C13**.

17. Pour the required amount of Lysis Buffer into the labelled 150mL reagent trough. Place Lysis Buffer on the left hand side of the 2 trough holder located in position **A13**.
    Note: Ensure that full PPE is worn, including face shield when handling these reagents.

18. **ABgene 96-deep well plate containing lysates**: Centrifuge plate for 2 minutes at 3021rpm before gently removing adhesive seal and place into position **D16** ensuring the plate is oriented such that the long side of the plate with the words "Front" written on at time of STORstar processing is visible from the front. (This should correspond with the cut corner at H1 being visible to the front of the operator)

19. After ensuring that all the necessary labware has been positioned in the correct grid position as displayed within WinPrep®, click the "**EXECUTE TEST**" button. While the test is loading, record all run information in the Run Log book.

20. Select the correct platemap by browsing to **C:\PACKARD\EXT PLATE MAPS**. Ensure that the platemap selected matches the batch ID affixed to the ABgene 96-deep well plate containing Lysates located in position **D16.** Once this has been done, click "**Start**", to continue.

21. Message will appear (Figure 3 below):

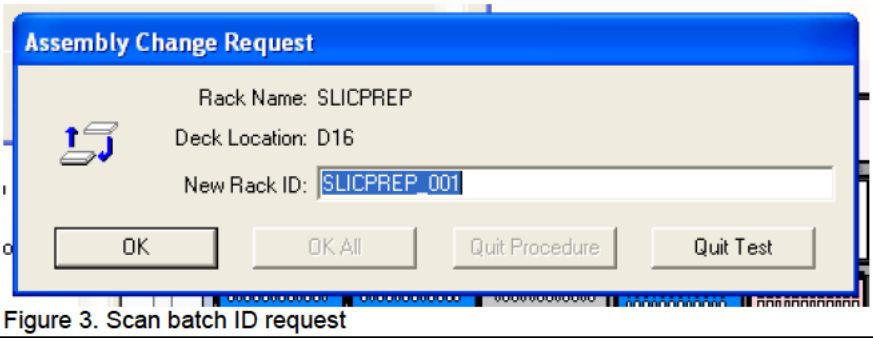

Figure 3. Scan batch ID request

Into "New Rack ID:" Scan barcode of ABgene 96-deep well plate (matches batch ID) and press "OK"

22. Click "**Reset Tip Boxes**" and ensure that the number of tips displayed in this window match those as available on the deck platform. Fill new tips if necessary. Click "**Close**" to accept the tip count, followed by clicking "**Next**"

23. After the barcodes have been read, a user prompt will appear as a reminder to:
"**Ensure**
1. Shaker and heat box are on.
2. Deck has been populated correctly.
3. The Lysis Buffer is on the left side at A13."
Click "**OK**" to continue.

24. Add the Resin to the 12 Channel plate. Pipette mix thoroughly. Then dispense 50uL of resin into each well of the ABgene 96-deep well plate containing Lysates.

25. Place the 12 channel plate into position **F4** then add the Elution Buffer to the plate by splitting the amount of Elution Buffer in half between channels 11 and 12.

26. Place the Wash Buffer in the far right hand side of the 4 well reagent trough holder (Amphyl wash station at **A10)**

27. The next User prompt will appear with the following directions:
"**Ensure Wash Buffer has been added. Manually add 50uL of Resin and place the ABgene plate in position D16. Ensure Elution Buffer has been added.**" Press **"OK"** when steps 24-26 have been performed.

28. Once the lysis and wash steps have been completed, a message will appear waiting for the heating tile to reach 85^0C (real temp 65^0C). **DO NOT PRESS CONTINUE** it will continue automatically when temperature has reached 85^0C.

29. After the first elution step when the plate is moved to the PKI Magnet, a User message will appear requesting:
"**Push down the plate on the PKI Magnet, Check Nunc tubes are uncapped at position B16, then press OK.**"
Note: Allow the plate to cool slightly before pushing down on all four corners of the plate to lock it into place on the PKI Magnet.

30. After the second elution step, the above prompt will appear again. **Note:** Allow the plate to cool slightly before pushing down on all four corners of the plate to lock it into place on the PKI Magnet

31. Once the program is completed, a final User Message prompt appears asking to:
"**Remove plates and cover them with aluminium sealing film. Remove Nunc rack and recap Nunc tubes.**"
Once all plates are removed from the deck and sealed, place into small clipseal plastic bags. Click "**OK**" to proceed to the Amphyl wash step to decontaminate the system tubing.

Finalising the MP II run

32. Remove the resin-Lysis-DTT solution from the 12 channel plate in the glass Lysis-DTT bottle used. Discard the plate in the biohazard waste bin.

33. Remove Lysis Buffer with DTT (wear face shield) and dispose of left over reagent into the glass bottle used previously. Bring this bottle to the room 6122 and dispose in the brown Winchester bottle located in the fume hood.

34. Empty the contents of the Amphyl wash station into the sink and rinse the troughs with nanopure H_2O upon completion of the run.

35. Remove all labware from the deck and clean with 5% TriGene™ followed by 70% ethanol, and setup for the next run if necessary.

36. Remove the tip chute and funnel, rinse with warm tap water to help loosen and remove any residue inside the chute before cleaning with 5% TriGene and 70% ethanol. Hang the chute to dry on the rack beside the sink. If running another extraction straight after, replace the tip chute with another spare, clean chute.

37. Move the platemap to **C:\PACKARD\EXT PLATE MAPS** to the "**Completed Extractions**" folder.

Recording Reagent Details and other information in AUSLAB

38. To record reagent lot numbers, log into the **AUSLAB Main Menu.**
39. Select **5.Workflow Management**.
40. Select **2. DNA Batch Details**.
41. Scan in the Extraction Batch ID.
42. Press **[F6]** ***Reagents***.
43. Press **[SF8]** ***Audit***.
44. Press **[F5]** ***Insert Audit Entry***, enter the lot number details, operator name and Extraction platform the batch was run on and press **[Enter]**.

Importing the MP II log file into AUSLAB

45. To extract the MP II log file, click on the Microsoft Access icon in the WinPrep® main menu to open the MultiPROBE log database.
46. Click on the relevant run Test ID in the Test Selection box. In the Report/Query/Action Selection dropdown menu, select "Report: Test Summary (Sorted by Destination Rack ID)"
47. In the Output Selection dropdown menu, select "File". Save the output file as *.csv format to **C:\PACKARD\EXT PLATE MAPS\EXT LOGS** with the same name as the AUSLAB batch ID and click "**Apply**". (refer to Figure 4. below)

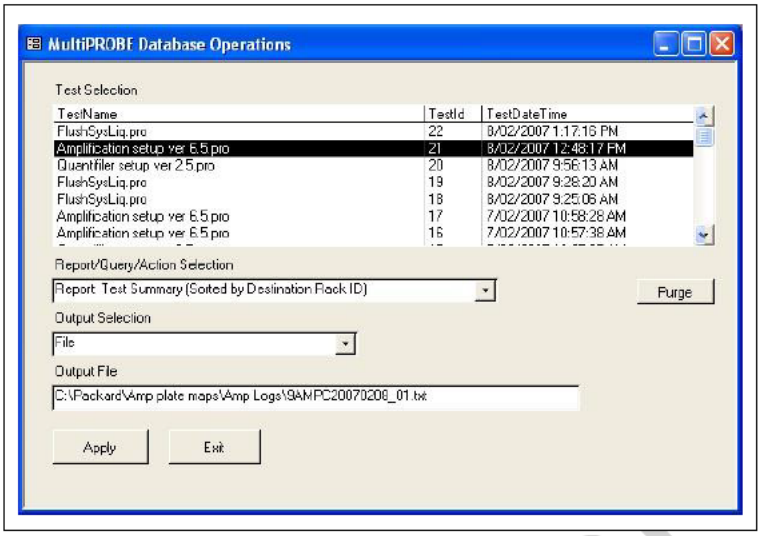

Figure 4. The MultiPROBE log database for collecting MP II run information

48. Open the log file and check for any errors that may have arisen during the Extraction process. Compare the listed errors to any that were encountered during the run. Report any critical errors to the line manager.
49. Copy the log file to **I:\EXTRACTION\EXT A MPII\LOGS or I:\ EXTRACTION\EXT B MPII\LOGS** for uploading to AUSLAB.
50. Log into the **AUSLAB Main Menu**.
51. Select **5.Workflow Management**.
52. Select **2. DNA Batch Details**.
53. Scan in the Extraction Batch ID barcode.
54. Press **[SF6]** ***Files***.
55. Press **[SF6]** ***Import Files***.
56. AUSLAB prompts "***Enter filename***"; enter the filename and extension and press **[Enter]**. (e.g. I:\EXTRACTION\EXT A MPII\Logs\CWIQEXT20071115_01.csv)
57. AUSLAB prompts "***Is this a result file Y/N?***" enter ***N*** and press **[Enter]**.
58. Press **[Esc]**.

Importing Extraction "Results" into AUSLAB

59. Log into the **AUSLAB Main Menu**.
60. Select **5. Workflow Management**.
61. Select **2. DNA Batch Details**.
62. Scan the Extraction batch ID barcode located on the worksheet.
63. Press **[SF6]** ***Files***.
64. Press **[SF6]** ***Import Files***.
65. AUSLAB prompts "***Enter filename***"; enter batch name and extension and press **[Enter]**.(e.g. CWIQEXT20071115_01.txt)
66. AUSLAB prompts "***Is this a results file y/n?***" enter "**y**" and press **[Enter]**.
67. The file will be imported into AUSLAB and appear in the DNA file table.
68. Highlight entry and press **[Enter]**, for access to the DNA results table.
69. Page down through the table and check that all sample results have been imported.
70. Press **[SF8]** ***Table Sort Order***, this sorts the table so any samples that have failed Autovalidation are sorted to the top of the table.
71. For all samples that have failed check the **Processing Comments,** by entering into the sample.
72. **a)** If processing comments state sample is to be sent to another batch type **other** than quant. Return the sample to the correct next batch type – e.g. Microcon, NucleoSpin and pooling

b) Press **[Esc]** to exit back to the DNA results table.
c) Do not toggle accept.

73. **a)** If processing comment does not state next step for sample the sample will be processed as normal.
    b) Press **[Esc]** to exit back to the DNA results table.
    c) Highlight any entries to be changed and press **[SF7]** ***Toggle Accept***
74. Press **[F7]** ***Complete Batch***, all of the samples with a ***Yes*** in the Accept column will be transferred to the outstanding Quant Casework or Quant Reference lists.
75. File the Extraction worksheet into the relevant folder in Room 6117.

10 SAMPLE STORAGE

Please refer to "*Analytical Sample Storage*" (QIS 24255) for how to store the old original 5 mL sample tubes, the DNA extract NUNC tubes, ABgene 96-deep well and Axygen store plates.

11 TROUBLESHOOTING

1. If the barcode reader is not reading the barcodes of the Nunc tube rack, or the Slicprep Plate or the Store plate, manually scan the appropriate barcodes.
2. When reading the Nunc tube rack barcode, if the Gripper is not picking up or placing the Nunc tube rack properly on the deck, just manually place the rack properly on the plate adapter support tile.
3. When reading the Store plate barcode, if the Gripper is not picking up or placing the Store plate properly on the deck, just manually place the plate properly on the plate adapter support tile.
4. When reading the Slicprep plate barcode if the Gripper is not picking up the plate properly :
    a. if the plate was not properly placed on the plate adapter support tile with the Wallac Isoplate, just manually place the plate properly.
    b. if the plate was properly placed on the plate adapter support tile with the Wallac Isoplate on it: it means that the gripper needs to be initialised. Abort the run, Initialise the instrument and restart the run. If problem persists, shutdown the MPII and PC, restart and then initialise the whole instrument. Otherwise, contact your line manager.
    c. Calibrate relevant labware using the SlicPrep Calibration plate. This has preset standardised positions that need to be the same on all labware where the Slicprep plate is being moved. The same plate is used on both extraction platforms A and B.
    d. Check the calibrations against the run program DNAIQGripperTest.pro. This program moves the Slicprep across all the labware the gripper moves across. Start with the Slicprep at D16.

5. In steps 18 or 26, if a message is stating that the instrument is having a motor problem when picking up 1 mL tips and the only option is to Abort, abort, initialise and open program version **1.3a** (if the problem is in step 18) or version **1.3b** (if the problem is in step 26).

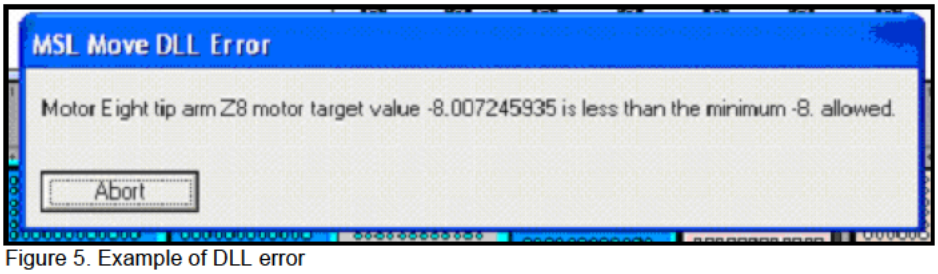

Figure 5. Example of DLL error

As the program will start the gripper will pick up the plates, it is not necessary that the Nunc tube rack is in position (B16), only ensure that it is reading the correct barcode. It is **important not** to place the Slicprep in the original position (E13) as the Slicprep plate has the Spin basket part removed (ie keep at D16), ensure it will scan the correct batch barcode. The Store plate remains in the original position. If the problem persists even after restarting, replace the rack of disposable 1 mL conductive sterile MBP tips for a new one.

6. If the program has already started step 18 and the previous message is appearing, you need to abort. Initialise the instrument and open program version **1.3a.**
   Go to the plate map in C:\PACKARD\ EXT PLATE MAPS and change volume to 0 for all the samples that the Lysis Buffer have been dispensed (Column 6), ensure that the number of samples where the Lysis Buffer was added is the same as the ones where the volume needs to be changed.
   Save the changes and use this plate map for the restart of the program. Please read troubleshooting 5 for barcode reading of plates.
   If the problem persists even after restarting, replace the rack of disposable 1 mL conductive sterile MBP tips for a new one.

7. If the program has already started step 26 and the previous message is appearing, you need to abort. Initialise the instrument and open program version **1.3b**.
   Go to the plate map in C:\PACKARD\ EXT PLATE MAPS and change volume to 0 in all the samples that the Lysis Buffer and Extraction Buffer have been removed (Column 9), ensure that the number of samples where the solution was removed is the same that the ones the volume need to be changed.
   Save the changes and use this plate map for the restart of the program. Please read troubleshooting 5 for barcode reading of plates.
   If the problem persists even after restarting, replace the rack of disposable 1 mL conductive sterile MBP tips for a new one.

8. If a disposable tip gets stuck on the 8 tip arm during disposal of tips a user message will appear. Remove and press retry and then continue.

9. If the message Figure 6 below has appeared:

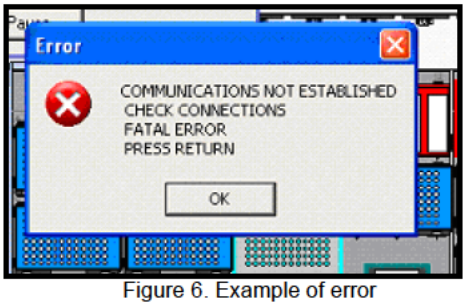

Figure 6. Example of error

press OK and the program will be aborted automatically. Check that all the connections to the instrument (shaker, heater and computer) are properly plugged in. If everything is OK, you need to close WinPrep, shut down the instrument, shaker, heater and PC. After 2 min restart everything. Once Winprep has been opened, reinitialise the instrument and start the program (check version number according to which step the message has came up). Please read troubleshooting 5 for barcode reading of plates.

12 VALIDATION

- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 9. Automation: Report on the Evaluation of DNA Extraction Chemistries." June 2007.
- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 11: Report on the Validation of the Automated Extraction Chemistry Kit using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Casework Platform." 2007.
- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 13: Report on the Verification of the Automated Extraction Chemistry Kit using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Casework Platform." 2007.
- Muharam I., Hlinka V., Gallagher, B., Lundie, G., Iannuzzi, C., Nurthen T., McNevin A., Ientile V. "Project 21: A Modified DNA IQ™ Method Consisting of Off-Deck Lysis to Allow Supernatant Retention for Presumptive Identification of α-Amylase" 2008
- Muharam I., Hlinka V., Gallagher, B., Lundie, G., Iannuzzi, C., Nurthen T., McNevin A., Ientile V. "Project 22: A Modified DNA IQ™ Method for Off-Deck Lysis Prior to Performing Automated DNA Extraction" 2008

13 QUALITY ASSURANCE/ACCEPTANCE CRITERIA

- A negative control (also known as the reagent blank) is included with each batch of extractions. This reagent blank is processed as a normal sample through to completion. If any results are obtained from this sample, either at the quantitation step or the Genescan analysis step, then the possible source of the contamination is investigated. The samples extracted with this control are thoroughly checked and repeated if possible.
- Positive and negative controls are included in each extraction batch as per table 4.

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15. Promega, DNA IQ™ System-Database Protocol. Promega Technical Bulletin #TB297, 2006. Rev 4/06: p. 1-14.
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18. Vandenberg, N., van Oorchot., R. A. H., & Mitchell, R. J., An evaluation of selected DNA extraction strategies for short tandem repeat typing. Electrophoresis, 1997. 18: p. 1624-1626.

15 STORAGE OF DOCUMENTS

All worksheets are stored in the Analytical area (Room 6117).

16 ASSOCIATED DOCUMENTS

QIS 17120 Operational Practices in the DNA Dedicated Laboratories
QIS 17171 Method for Chelex Extraction
QIS 17165 Receipt, Storage and Preparation of Chemicals, Reagents and Test Kits
QIS 23939 Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform
QIS 24255 Analytical Sample Storage
QIS 24256 Sequence Checking with the STORstar Instrument
QIS 24469 Batch functionality in AUSLAB

17 AMENDMENT HISTORY

Revision	Date	Author/s	Amendments
0	23 Oct 2007	B. Gallagher, T. Nurthen, C. Iannuzzi, V. Hlinka,	First Issue

		G. Lundie, I Muharam.	
1	12 Dec 2007	M Harvey, C Iannuzzi, A McNevin	Reviewed and updated after initial training
2	19 March 2008	M Harvey, B Andersen, C Iannuzzi, A McNevin	Addition of Off-deck Lysis procedure, Retention of fully automated method as Appendix, addition of reagent record tables into Appendix

18 APPENDIX

18.1 Reagents Calculation Tables

Table 7. **Less than 48 samples** (note difference is in DNA IQ RESIN Solution)

Lysis-DTT Buffer		Volume (in mL)
Lysis Buffer	(Nx1.35)+0.75	
DTT (1M)	Lysis Buffer/100	
Extraction Buffer		
TNE Buffer	Nx0.56	
Prot K (20 mg/L)	Nx0.03	
SDS (20 %)	Nx0.015	
DNA IQ RESIN Solution		
LYSIS Buffer	0.054x(N+8)	
DNA IQ RESIN	0.009x(N+8)	
DNA IQ 1X Wash Buffer	Nx0.36	
DNA IQ Elution Buffer	Nx0.144	

N = number of samples on batch including positive and negative controls, round up to nearest multiple of 8)

Table 8. **Greater than 48 samples** (note difference is in DNA IQ RESIN Solution)

Lysis-DTT Buffer		Volume (in mL)
Lysis Buffer	(Nx1.35)+0.75	
DTT (1M)	Lysis Buffer/100	
Extraction Buffer		
TNE Buffer	Nx0.56	
Prot K (20 mg/L)	Nx0.03	
SDS (20 %)	Nx0.015	
DNA IQ RESIN Solution		
LYSIS Buffer	0.054x(N+16)	
DNA IQ RESIN	0.009x(N+16)	
DNA IQ 1X Wash Buffer	Nx0.36	
DNA IQ Elution Buffer	Nx0.144	

N = number of samples on batch including positive and negative controls, round up to nearest multiple of 8)

18.2 Reagent & Batch details recording tables (DNA IQ™ Lysis Batch & Extraction Batch)

Off Deck Lysis Batch ID:	DNA IQ Extraction Batch ID:

Lysis batch:

Samples located by:	
For samples 1-48	**For samples 49-96**
Operator:	Operator:
Sequence check performed by:	Sequence check performed by:
Transfer tubes sequence checked:	Transfer tubes sequence checked:

Extraction Buffer made by:	TNE Buffer Lot#:
20% SDS Lot#:	Proteinase K Lot#:
Comments:	

Extraction batch:

Plate Lot#:	Lysate/STORstar Operators:
Nunc tube/STORstar Operators:	Lysate Logfile uploaded:
Nunc Logfile uploaded:	
Comments:	

MultiPROBE Platform:	Operator:
Date and Start time:	

Kit Lot#:	1xWash Buffer Lot#:
Lysis Buffer Lot#:	DTT Lot#:
Resin Lot#:	Elution Buffer Lot#:
MP II Logfile uploaded:	Results file uploaded:
Comments:	

18.3 Fully automated method for extraction using DNA IQ™

18.3.1 Sampling and Sample Preparation

FTA® Samples waiting for extraction will have been punched into a Slicprep™ 96 device according to "*FTA® Processing*" SOP (QIS document 24823) and stored in the Fridge located in room 6127.

18.3.2 Procedure

Preparation of Reagents prior to extraction

1. Defrost Prot K and DTT

2. Refer to table 2 for reagent volumes to make up the required amount of Extraction Buffer, Lysis Buffer (with DTT) and Resin solution. Also measure the required amount of 1X Wash Buffer.

3. Record the Lot numbers of all the reagents used onto the AUSLAB worksheet.

Setting up the EP-A or EP-B MPIIs

These steps are to be carried out in the Automated extraction Room (Room 6127)

4. Turn on the instrument PC.

5. Log onto the network using the **Robotics** login.

6. Double click the WinPrep® icon on the computer desktop (Figure 7).

Figure 7 The WinPrep® icon.

7. Log onto the WinPrep® software by entering your username and password, then press **"Enter"**.

8. Ensure the **System Liquid Bottle is FULL** before every run and perform a Flush/Wash.

9. Ensure that the daily/weekly start-up has been performed before running any program. If WinPrep® has been closed or been idle for a long period of time initialise the MP II platform as described in QIS 23939.

10. Open the Extraction setup MP II test file in WinPrep® by selecting:
    - **File**
    - **Open**, navigate to **C:\PACKARD\MULTIPROBE\BIN\QHSS PROTOCOLS**
    - Select "**DNA IQ Extraction_Ver1.3.mpt.**"
    - Click the "**Open**" button

11. Check the whole program there are no bold fonts (e.g. plates may lose gripability and the program will not run). See the line manager.

12. Decontaminate the required labware by wiping with 5% TriGene followed by 70% ethanol before placing the labware on to the designated grid positions as outlined in the virtual deck view within WinPrep® (Figure 2).

    - The white WALLAC Isoplates (catalogue #1450-514) that are used to support the SlicPrep™ 96 device plate must be placed into positions **E13**, **D16** and **C19**.

- Ensure that the PKI Magnet at **F16** is stable in its insert. This is critical so that beads and DNA are not lost during pipetting.

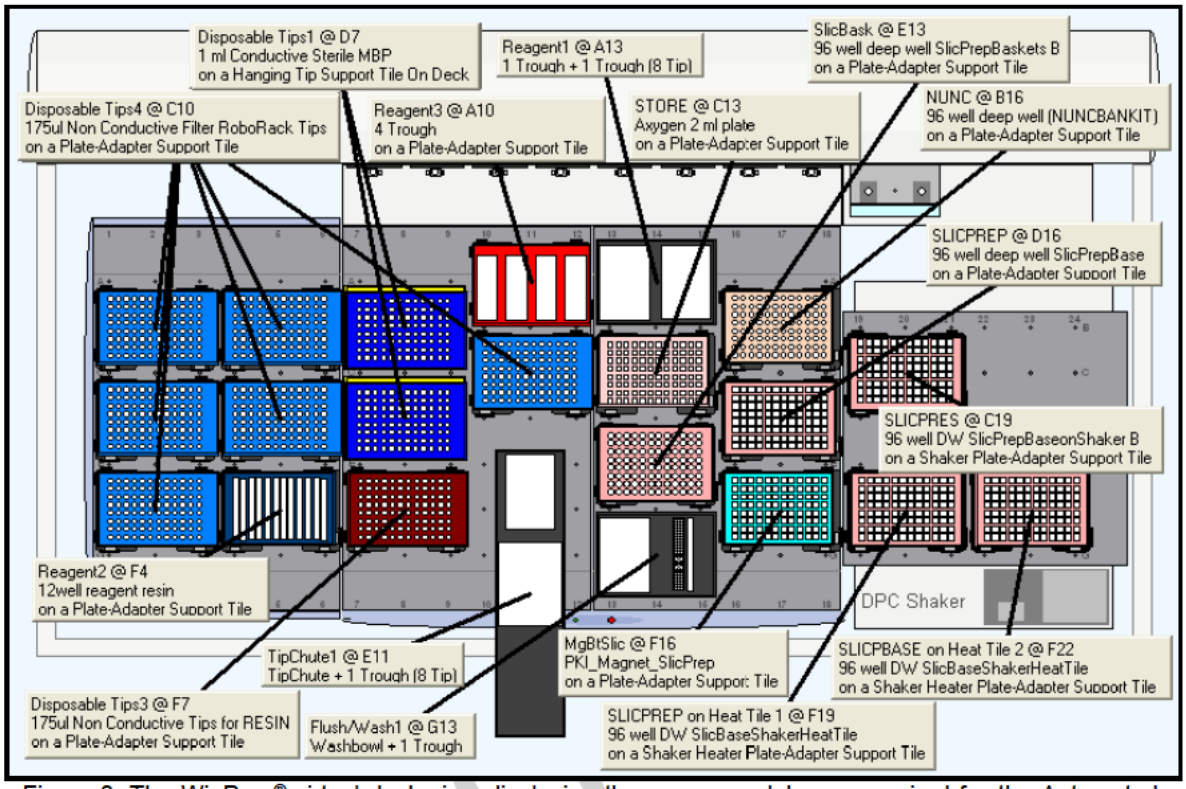

Figure 8. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

13. Ensure that the DPC Shaker and Heater Controller Box are switched on.
    For EP-A: Tile 3 should be at F19 (50^{0}C), Tile 1 at F22 (85^{0}C).
    For EP-B: Tile 1 should be at F19 (50^{0}C), Tile 2 at F22 (85^{0}C).
    Note: Press the start/stop button twice at the front of the DPC Shaker to ensure that it displays zero on the screen.

14. To the Amphyl wash station at **A10**, add fresh 1% Amphyl to the trough on the far left hand side, 0.2% diluted Amphyl to the middle left and place an empty reagent tough in the middle right position of the station. The nanopure water goes to position **G13** into a 160mL trough in the Flush-Wash station.

15. Pour the required amounts of Extraction Buffer and Lysis Buffer into the labelled 150mL reagent troughs. Place Lysis Buffer on the left hand side and the Extraction Buffer on the right hand side of the 2 trough holder located in position **A13**.
    Note: Ensure that full PPE is worn, including face shield when handling these reagents

16. **Nunc tube rack**: Check that is the same Auslab batch number as the platemap to be run. Ensure that the barcode of the first (A1) and last tube (H12) are the respective ones. Attach batch label and batch barcode label to front side of rack. Add B1-Lite generated "**NUNC**" barcode to the right side of the nunc tube rack. Then place nunc rack into position **B16**

17. On an Axygen 2ml deep well Storage plate, label the left side of the plate with both the Batch ID and barcode. Label the right side of the plate with a B1-Lite generated "**STORE**" barcode. Then place in position **C13**.

18. **Slicprep™ 96 device**: Gently remove septa mat from Slicprep™ 96 device and check that substrates are at the bottom of the Spin baskets, if not push them down with a sterile disposable tip and place the Slicprep™ 96 device into position **E13**.

19. In I drive from Extraction folder open the required plate map. Check that the plate map is complete. If there are blanks (samples that have been removed), make all volumes to 0. If it is not a full batch, delete the rows of blank samples at the end of the platemap, then save the changes. Copy the plate map to the following file path: **C:\PACKARD\EXT PLATE MAPS**

20. After ensuring that all the necessary labware has been positioned in the correct grid position as displayed within WinPrep®, click the "**EXECUTE TEST"** button. While the test is loading, record all run information in the Run Log book.

21. Click "**Reset Tip Boxes**" and ensure that the number of tips displayed in this window match those as available on the deck platform. Fill new tips if necessary. Click "**Close**" to accept the tip count, followed by clicking "**Next**"

22. Select the correct platemap by browsing to **C:\PACKARD\EXT PLATE MAPS**. Ensure that the platemap selected matches the batch ID affixed to the 96-well Slicprep™ 96 device in position **D16.** Once this has been done, click "**Start**", to continue.

23. After the barcodes have been read, a user prompt will appear as a reminder to:
    "**Ensure**
    1. Shaker and heat box are on.
    2. Deck has been populated correctly.
    3. The Lysis Buffer is on the left side and Extraction Buffer is on the right at A13."
    Click "**OK"** to continue.

24. Once the Extraction Buffer has been added to the plate, a message will appear waiting for the heating tile to reach 50^0C (real temp 37^0C). When current temperature reaches 50^0C click "**Continue**".

25. The next prompt that appears will request the following:
    "Cover Slicprep with the Aluminium sealing film, then place in position F19. Press "OK."

26. After shaking, a User Prompt will appear with the following directions:
    "**Remove plate, add white plastic collar and centrifuge 5mins at 3021rpm, then in the cabinet, remove the spin basket part and place it in the empty 1 ml tip container**."
    Place the Slicprep™ 96 device into the plate centrifuge and ensure the correct balance plate is used. Once the plate has been centrifuged, carry the plate to the hood and remove the basket of the Slicprep™ 96 device, storing the basket in an empty 1mL tip box, discard the Collar. Complete the step by clicking "**OK**".

27. Add the Resin to the 12 Channel plate. Pipette mix thoroughly. Then dispense 50uL of resin into each well of the Slicprep™ 96 device.

28. Place the 12 channel plate into position **F4** then add the Elution Buffer to the plate by splitting the amount of Elution Buffer in half between channels 11 and 12.

29. Place the Wash Buffer in the far right hand side of the 4 well reagent trough holder (Amphyl wash station at **A10)**

30. The next User prompt will appear with the following directions:
"**Ensure Wash Buffer has been added to trough 4 at A10.**
Manually add 50uL resin to each well of the SlicPrep plate
Place the plate in position D16.
Add the Elution Buffer to the 12 channel plate.
THEN
Press OK when ready." Press **"OK"** when steps 27-29 have been performed.

31. Once the lysis and wash steps have been completed, a message will appear waiting for the heating tile to reach 85^0C (real temp 65^0C). **DO NOT PRESS CONTINUE** it will continue automatically when temperature has reached 85^0C.

32. After the first elution step when the plate is moved to the PKI Magnet, a User message will appear requesting:
"**Check Nunc tubes are uncapped at position B16**
Push down the Slicprep on the PKI Magnet then press OK."
Note: Allow the plate to cool slightly before pushing down on all four corners of the plate to lock it into place on the PKI Magnet.

33. After the second elution step, the above prompt will appear again. **Note:** Allow the plate to cool slightly before pushing down on all four corners of the plate to lock it into place on the PKI Magnet

34. Once the program is completed, a final User Message prompt appears asking to:
"**Remove all the plates starting with the Slicprep plate, place the Spin Basket into the Slicprep plate.**
Cover the Storage plate with the aluminium sealing film."
Recap the NUNC tubes
Once all plates are removed from the deck and sealed, place into small clipseal plastic bags. Click "**OK**" to proceed to the Amphyl wash step to decontaminate the system tubing.

Finalising the MP II run

35. Discard the 12-channel plate used for Resin and Elution Buffer in the biohazard waste bin.
36. Remove Lysis Buffer with DTT (wear face shield) and dispose of left over reagent into the glass bottle used previously. Bring this bottle to the room 6122 and dispose in the brown Winchester bottle located in the fume hood.
37. Empty the contents of the Amphyl wash station into the sink and rinse the troughs with nanopure H_2O upon completion of the run.
38. Remove all labware from the deck and clean with 5% TriGene™ followed by 70% ethanol, and setup for the next run if necessary.
39. Remove the tip chute and funnel, rinse with warm tap water to help loosen and remove any residue inside the chute before cleaning with 5% TriGene and 70% ethanol. Hang the chute to dry on the rack beside the sink. If running another extraction straight after, replace the tip chute with another spare, clean chute.
40. Move the platemap to **C:\PACKARD\EXT PLATE MAPS** to the "**Completed Extractions**" folder.

Recording Reagent Details and other information in AUSLAB

41. To record reagent lot numbers, log into the **AUSLAB Main Menu.**
42. Select **5.Workflow Management**.
43. Select **2. DNA Batch Details**.
44. Scan in the Extraction Batch ID.
45. Press **[F6]** ***Reagents***.
46. Press **[SF8]** ***Audit***.
47. Press **[F5]** ***Insert Audit Entry***, enter the lot number details, operator name and Extraction platform the batch was run on and press **[Enter]**.

Importing the MP II log file into AUSLAB

48. To extract the MP II log file, click on the Microsoft Access icon in the WinPrep® main menu to open the MultiPROBE log database.
49. Click on the relevant run Test ID in the Test Selection box. In the Report/Query/Action Selection dropdown menu, select "Report: Test Summary (Sorted by Destination Rack ID)"
50. In the Output Selection dropdown menu, select "File". Save the output file as *.csv format to **C:\PACKARD\EXT PLATE MAPS\EXT LOGS** with the same name as the AUSLAB batch ID and click "**Apply**". (refer to Figure 9. below)

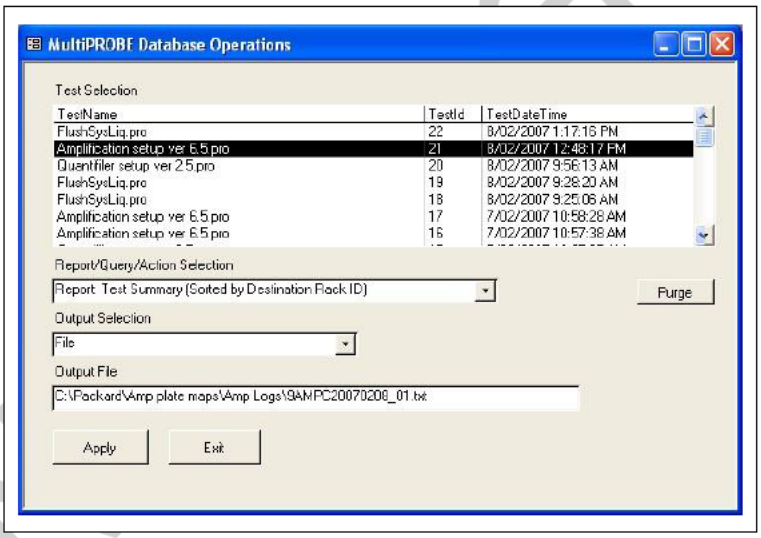

Figure 9. The MultiPROBE log database for collecting MP II run information

51. Open the log file and check for any errors that may have arisen during the Extraction process. Compare the listed errors to any that were encountered during the run. Report any critical errors to the line manager.
52. Copy the log file to **I:\EXTRACTION\EXT A MPII\LOGS or I:\ EXTRACTION\EXT B MPII\LOGS** for uploading to AUSLAB.
53. Log into the **AUSLAB Main Menu**.
54. Select **5.Workflow Management**.
55. Select **2. DNA Batch Details**.
56. Scan in the Extraction Batch ID barcode.
57. Press **[SF6]** ***Files***.
58. Press **[SF6]** ***Import Files***.
59. AUSLAB prompts "***Enter filename***"; enter the filename and extension and press **[Enter]**. (e.g. I:\EXTRACTION\EXT A MPII\Logs\CWIQEXT20071115_01.csv)
60. AUSLAB prompts "***Is this a result file Y/N?***" enter ***N*** and press **[Enter]**.
61. Press **[Esc]**.

Importing Extraction "Results" into AUSLAB

62. Log into the **AUSLAB Main Menu**.
63. Select **5. Workflow Management**.
64. Select **2. DNA Batch Details**.
65. Scan the Extraction batch ID barcode located on the worksheet.
66. Press **[SF6]** ***Files***.
67. Press **[SF6]** ***Import Files***.
68. AUSLAB prompts "***Enter filename***"; enter batch name and extension and press **[Enter]**.(e.g. CWIQEXT20071115_01.txt)
69. AUSLAB prompts "***Is this a results file y/n?***" enter "**y**" and press **[Enter]**.
70. The file will be imported into AUSLAB and appear in the DNA file table.
71. Highlight entry and press **[Enter]**, for access to the DNA results table.
72. Page down through the table and check that all sample results have been imported.
73. Press **[SF8]** ***Table Sort Order***, this sorts the table so any samples that have failed Autovalidation are sorted to the top of the table.
74. For all samples that have failed check the **Processing Comments,** by entering into the sample.
75. **a)** If processing comments state sample is to be sent to another batch type **other** than quant. Return the sample to the correct next batch type – e.g. microcon, nucleospin and pooling
    b) Press **[Esc]** to exit back to the DNA results table.
    c) Do not toggle accept.
76. **a)** If processing comment does not state next step for sample the sample will be processed as normal.
    b) Press **[Esc]** to exit back to the DNA results table.
    c) Highlight any entries to be changed and press **[SF7]** ***Toggle Accept***
77. Press **[F7]** ***Complete Batch***, all of the samples with a ***Yes*** in the Accept column will be transferred to the outstanding Quant Casework or Quant Reference lists.
78. File the Extraction worksheet into the relevant folder in Room 6117.

18.3.3 Sample Storage

Please refer to "Analytical Sample Storage" (QIS 24255) for how to store the old original 5 mL sample tubes, the DNA extract NUNC tubes, Slicprep with Basket and Axygen store plates.

18.4 Manual method for extraction using DNA IQ™

18.4.1 Sampling and Sample Preparation

Samples waiting to be extracted are stored in freezers as described in Table 9.

Table 9. Sample storage locations.

Sample type	Storage Device	Storage Location
Urgent/High/Medium Priority Samples	Freezer	6117-2
Medium Priority Samples	Walk in Freezer	6109
Low Priority Samples	N/A	

QC samples

All extraction batches require two controls to be registered. These controls, UR numbers and positions are listed below in Table 10.

Table 10. Extraction Quality Controls

QC	UR Number	Extraction types
Neg Control	FBOT277	All
QC swab (blood)	FBOT279	Blood

1. Log into the **AUSLAB Main Menu**.
2. Select **1. Request Registration**.
3. Select **2. Full Reception Entry**.
4. Scan in barcode of control.
5. Enter the UR number as per Table 4 and press **[Enter]**.
6. Enter the appropriate Specimen type (e.g. Blood for blood extraction).
7. Request a **9PLEX** test, when prompted to enter the processing comment, enter **EXTP** (Positive extraction control) or **EXTN** (Negative extraction control).
8. Press **[F7]** Enter **LAB** in the Billing code field.
9. Press **[F4]** ***Save*** to save the Billing details.
10. Press **[F4]** ***Save*** to save the registration details.

N.B Quality controls should not have a DNA priority allocated at time of registration to ensure they are included in the top positions of a batch

Create the Extraction Batch

14. Log into the **AUSLAB Main Menu**.
15. Select **5. Workflow management**.
16. Select **1. DNA workflow table**.
17. Highlight the appropriate Extraction batch type and press **[F5]** ***Batch Allocation***.
18. Press **[F6]** ***Create batch***.
19. Press **[F8]** ***Print menu***.
20. Press **[F6]** ***Print Batch label***. (for the deep well plate)
21. Press **[F7]** ***Print Sample labels***. (print four sets of labels for all extractions)
22. Press **[F8]** ***Print Worksheet***.
23. Press **[SF5]** ***Main menu***.
24. Press **[SF11]** ***Print***.
25. Press **[SF6]** ***Accept batch***.
26. Press **[Pause/Break]** to exit to the **Main Menu**.
27. Obtain worksheets (FBLASER3) and labels (FBLABEL13-16) from the Analytical Section printing bench (Room 6117).

Locating Samples

Determine the storage locations of the required samples using the Batch Creation table/Batch details table print out. The columns ***Rack*** and ***Pos*** respectively identify the rack and the grid location where the sample has been stored. Remove the samples from the storage rack and place in an orange rack (12x8).

When all samples have been located:

1. Log into the **AUSLAB Main Menu**.
2. Select **2. Sample Processing**.
3. Select **7. Search Sample storage**.
4. Scan in the sample barcode that is affixed to the sample tube.
5. Press **[F6]** ***Remove Sample***.
6. AUSLAB prompts "***Are you sure you want to remove XXXX-XXXX? (Y/N)***", Enter **Y** and press **[Enter]**.
7. AUSLAB prompts "***Please enter remove comment***", No comment is required. Press **[Enter]**.
8. Press **[Scroll lock]** to clear.
9. Repeat steps **5 - 8** until all of the samples have been removed from their rack.

Sequence Check the tubes

1. Thaw samples at room temperature and label 1.5mL sample tubes.
2. Sequence check the tubes.
3. Add the sequence check details into AUSLAB.
4. Log into **AUSLAB Main Menu**.
5. Select **5. Workflow Management**.
6. Select **2. DNA Batch Details**.
7. Scan in the appropriate extraction batch ID barcode.
8. Press **[F5]** ***Sequence Check***.
9. Scan in the appropriate extraction batch ID barcode.
10. Press **[Pause/Break]** to exit to **Main Menu**.

18.4.2 Procedure

1. Enter the number of samples to be extracted (including controls) into the "No of Samples" column of the DNA IQ Reagent Calculations Table to calculate the volumes of each reagent to be measured out for the extraction. Aliquot regents into either 5ml tubes or 50ml Falcon tubes. **Note:** The volume of Lysis Buffer calculated includes the volume used in the resin-lysis solution

2. Turn on the Eppendorf Thermo mixer and set the temperature to 37ºC.

3. Remove 1.5ml tube and retain the 5mL tube. Prepare the Spin baskets by placing a DNA IQ™ Spin basket into a 1.5mL Microtube. Label the spin baskets (for every tube except Ext. control), 2mL SSI tubes and Nunc storage tubes (for every sample) with the sample barcodes. Have a second operator perform a sequence check of all tubes. This person must Press **[F5] Sequence Check** against the batch in AUSLAB

4. Using the Reagents table, prepare Extraction Buffer, Lysis Buffer with DTT, & Resin Solution. Reagents need to be prepared fresh before each run.

5. Add 300 µL of Extraction Buffer to each tube. Vortex each tube before incubating the tubes at 37°C on the Thermomixer at 1000 rpm for 45 minutes.

6. Remove the tubes from the Thermo mixer and place into a rack, increase the temperature on the Thermomixer to 65°C (for use in the Elution steps).

7. Transfer the substrate from the original tube to a DNA IQ™ Spin Basket using autoclaved twirling sticks. Centrifuge the Spin basket for 2 minutes at room temperature at its maximum speed. Once completed, remove the spin basket & retain in the original 5ml tube. Ensuring minimal contamination and transfer the extract to a labelled 2mL SSI sterile screw tube.

8. Transfer the remaining extract from the original tube to the corresponding 2mL tube. Vortex the tube gently.

9. Add 500 µL of Lysis Buffer to each tube.

10. Into a separate, clean 2mL SSI tube, aliquot the required amount of Lysis Buffer for the Resin solution. Ensure that the DNA IQ™ Resin solution has been thoroughly mixed by vortexing the resin bottle before adding the required resin volume to the Lysis Buffer. Pipette mix the solution to clear the tip of any lingering resin beads. Mix the solution by inverting the tube to prevent bubbles forming.

11. Add 50µL of DNA IQ™ Resin-Lysis solution into each tube. Invert the resin-lysis tube at regular intervals to keep the resin suspended within the solution to ensure uniform results.

12. Vortex each tube for 3 seconds at high speed before placing the tubes in the Multitubeshaker set at 1200 rpm to incubate at room temperature for 5 minutes.

13. Remove from the Multitubeshaker and vortex the tubes for 2 seconds at high speed before placing the tubes in the magnetic stand. Separation will occur instantly.

Note: If resin does not form a distinct pellet on the side of the tube, or if the pellet has accidentally mixed with the solution while in the stand, vortex the tube and quickly place back in the stand.

14. Carefully remove all of the solution in the tube into the original 1.5mL tube (i.e. the tube which originally contained the substrate), ensuring that the resin is not disturbed from its place on the side of the tube.

Note: If some resin is drawn up in tip, gently expel resin back into tube to allow re-separation.

15. Add 125µL of prepared Lysis Buffer solution and vortex for 2 seconds at high speed. Return the tubes to the magnetic stand and allow for separation to occur. Once separation has occurred again remove the Lysis Buffer into the original 1.5mL tube (i.e. the tube which originally contained the substrate).

16. Add 100µL of prepared 1X Wash Buffer and vortex for 2 seconds at high speed. Return tube to the magnetic stand and once separation has occurred remove and discard all Wash Buffer.

17. Repeat Step 16 another two times for a total of three washes. Ensure that all of the solution has been removed after the last wash.

18. Wipe down a Biohazard hood with bleach followed by ethanol. Uncap the tubes, placing the lids inside down onto a clean rediwipe in consecutive order and place the tubes in the same order into a clean plastic rack. Air-dry the resin in the hood for 15

minutes at Room temperature. Do not dry for more than 20 minutes, as this may inhibit removal of DNA during the elution phase.

19. Once the resin is dry, replace the lids on the corresponding tubes and remove from the hood. Add 50µl of Elution Buffer to each of the samples by carefully pipetting the liquid to the side of the tube, above the pellet. Do not mix.

20. With the lids on, incubate the tubes in the Thermomixer at 65°C for 3 minutes. After the three minutes are up, continue to incubate for a further 3 minutes shaking at 1100 rpm.

21. Remove the tubes and vortex for 2 seconds at high speed. Immediately place the tube in the magnetic stand while hot to ensure maximum DNA yield during elution.

22. Carefully transfer the DNA containing supernatant to the corresponding labelled Nunc tubes.

23. Remove tubes from the magnetic stand and add carefully another 50 µL of Elution Buffer above the magnetic pellet.

24. Repeat step 20 to 22. The final volume after this elution should be approximately of 95 µL of DNA solution.

25. DNA can be stored at 4°C for short-term storage or at -20 or -70°C for long-term storage.

18.4.3 Sample storage

1. Log into **AUSLAB Main Menu**.
2. Select **2. Sample Processing**.
3. Select **6. Sample Storage**.
4. Scan in Rack barcode.
5. Press **[SF5]** ***Fill Rack***.
6. Scan in sample barcode and place in rack in scanned position.
7. Repeat for all samples.
8. Press **[Esc]**.
9. Press **[Pause/Break]** to return to the **Main Menu**.
10. Select **3. Patient Enquiry**.
11. Scan in Rack barcode.
12. Tab down to the next blank ***DNA Batch No*** field and press **[F2]** ***Edit***.
13. Scan in the Batch ID of the samples stored.
14. Press **[Pause/Break]** to return to the **Main Menu**.

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DNA IQ™ Method of Extracting DNA from Casework and Reference Samples

1 PURPOSE AND SCOPE

This method describes the routine method of DNA extraction using the PerkinElmer MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms and Promega DNA IQ™ kit. The manual method has been included as a back-up method should it be required.

This method applies to all DNA Analysis staff that are required to extract DNA from samples.

Reference samples and Casework samples must be extracted separately. If Casework and Reference samples are to be extracted on the same instrument, the instrument must be decontaminated between operations.

2 DEFINITIONS

Samples	Samples awaiting DNA extraction
Lysates	Samples that have had the Lysis step performed, but have not yet completed the entire extraction process
DNA Extracts	Samples that have had a DNA extraction processes performed
DNA IQ™ Resin	Magnetic Resin Beads used to bind DNA
MP II	MultiPROBE® II PLUS HT EX Platform
DTT	1,4 Dithiothreitol
Pro K	Proteinase K
Sarcosyl	N-Lauroylsarcosine sodium
TNE	Tris, NaCl and EDTA Buffer
EDTA	Ethylenediaminetetraacetate
EP-A	Extraction Platform A
EP-B	Extraction Platform B

3 PRINCIPLE

Sample Pre-lysis

The Extraction Buffer used in the pre-lysis of cellular material contains Proteinase K (Pro K), TNE (Tris, NaCl, EDTA) and Sarcosyl. TNE acts as a basic buffer with EDTA chelating ions in solution. Sarcosyl is a detergent that lyses open cell membranes. Proteinase K is added to digest cellular material, helping the DNA to be released. It also digests proteins that interfere with the DNA binding capacity of the resin. In addition it rapidly inactivates enzymatic activity that could potentially degrade DNA (e.g. nucleases).

Proteinase K (also known as Endoproteinase K) is a fungal enzyme from *Engyodontium album* (formerly *Tritirachium album*). It is a stable S8 family serine alkaline protease that has broad specificity and is inactivated by diisopropylfluorophosphate, phenyl-methane sulfonyl fluoride and Hg^{2+} ions. As a highly active endopeptidase, it cleaves peptide bonds adjacent/next to the carboxyl group of N-substituted (blocked alpha amino) hydrophobic, aliphatic and aromatic amino acids. Therefore, it actively hydrolyses proteins, glycoproteins, peptides and esters of amino acids. The smallest peptide that can be hydrolysed with Proteinase K is a tetrapeptide.

DNA IQ™ Kit

The DNA IQ ™ kit is designed to purify DNA from a number of different substrates and has been optimised for trace DNA samples. An in house validation was performed using a modified version of the PerkinElmer automated protocol. The protocol has been modified to incorporate a number of work practices used in DNA Analysis FSS. These are:

- The use of the Slicprep™ 96 device (Promega) for removing substrate from lysate.

- The increase of Extraction Buffer volume to 500µL for use with the Slicprep™ 96 device.
- The use of tubes and spin-baskets for the off-deck lysis of samples prior to extraction on MPII. Use of a 96-deepwell plate for completion of extraction on MPII.
- The provision of initial incubation with TNE Buffer and retention of a portion thereof for further testing (retained supernatant testing).
- The increase of Lysis Buffer volume to 957µL proportional to the increase of Extraction Buffer volume, according to the manufacturer's instructions.
- Double Elution step, with an Elution Buffer volume of 60µL for a final volume of 100µL.
- The use of Nunc™ Bank-It™ tubes for storage of final extracts.

Cell lysis is performed with Promega Lysis Buffer containing Dithiothreitol (DTT). 1,4 Dithiothreitol is a reducing agent used in extractions to break disulfide bonds of proteins. The Lysis Buffer used is a proprietary buffer containing chaotropic salts required for binding of DNA to the magnetic beads. According to the MSDS, the Lysis Buffer contains 50-75% guanidinium thiocyanate, < 2.5% EDTA, < 2% 3-[(3-Choalamidopropryl)dimethylammonio] propanesulfonic and < 2% polyethylene glycol tert-octylphenyl ether.

The basis of the DNA IQ™ kit is a magnetic bead resin which contains novel paramagnetic particles covered with silica. The magnetic bead resin usually has a DNA binding capacity of 100ng but the addition of Pro K will increase the binding capacity. Samples with small amounts of DNA are more efficient than samples with large amounts of DNA, and as a result a small sample size is critical to ensure efficient recovery of DNA.

The magnetic beads with silica have a negative charge at basic and near neutral pH. The Lysis Buffer changes the pH and salt concentration of the solution and the silica on the magnetic beads becomes positively charged which is then able to bind the DNA.

Several washing steps are employed in the protocol to remove inhibitors. The first washing step is performed with Lysis Buffer. This wash ensures the DNA is bound to the magnetic bead resin and washes out inhibitors. The next three washing procedures are with a Wash Buffer. This buffer contains an alcohol/aqueous mixture which ensures the DNA is not eluted during washing by keeping the DNA dehydrated, and aqueous phase washes out the inhibitor.

Elution Buffer removes the DNA from the magnetic bead resin. The Elution Buffer changes the salt content. Heating the complex to 65°C allows the DNA to be released from the magnetic beads. The Elution Buffer is a low ionic strength buffer that reduces the affinity of the DNA for the silica that covers the magnetic beads, by re-hydration of the phosphate backbone.

The DNA IQ™ kit isolates DNA greater than 80bp, smaller DNA is removed selectively to prevent PCR inhibition.

MultiPROBE® II HT EX PLUS with Gripper™ Integration Platform

Within DNA Analysis, routine DNA extractions are performed using either one of two MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms (Extraction Platform A, EP-A) and (Extraction Platform B, EP-B) perform casework or reference samples.
Each platform uses a computer – controlled Cartesian X-Y-Z liquid handling system designed for the efficient automation of sample preparation. Liquid transfer is performed by an 8-tip System with VersaTip® and VariSpan™ options. The VersaTip® option allows the use of fixed and/or disposable tips (both clear and conductive). The VariSpan™ option permits variable probe spacing between each of the individual probes so that a wide range of labware such as micro plates, tubes, vials and reagent troughs can be accessed. Each

sample probe is capable of independent motion in the Z direction due to independent Z drives.

The 8-tip System is also capable of multichannel liquid-level sensing by utilising Accusense™ technology. This technology works by each probe detecting a change in capacitance within the liquid. This capacitive mode of detection is also possible when using conductive disposable tips. Accusense™ also permits the detection of low or non-ionic polar solutions and solvents. Pipetting on the platforms is driven by 8 individually controlled syringes via the positive displacement of the system liquid (nanopure water) when transferring liquid.

The Gripper™ Integration on all the platforms (except for the Post – PCR MPII) allows for automated identification of labware via the scanning of barcodes and also allows for automated transfer of plates from one position on the deck to another. To increase deck space and capacity, all platforms include a left deck extension.

In this program a platemap is utilised to provide the necessary information for correct volumes and locations for pipetting. It also contains information regarding positions where pipetting must take place at various locations, the number and IDs of samples. Without a platemap the program will fail to work.

4 REAGENTS AND EQUIPMENT

4.1 Reagents

1. DNA IQ™ System Kit – 400 sample Kit
    - Resin
    - Lysis Buffer (LB)
    - 2x Wash Buffer (2xWB)
    - Elution Buffer (EB)
2. Tris/Sodium chloride/EDTA Buffer (TNE)
3. Proteinase K (Pro K) 20mg/mL
4. Dithiothreitol (DTT) 1M
5. 5% TriGene
6. 70% Ethanol
7. 1% Amphyl
8. 0.2% Amphyl
9. Isopropyl alcohol
10. AnalR 100 %Ethanol
11. 40% Sarcosyl
12. Decon® 90 solution
13. Nanopure H_2O

Table 1. Reagent storage locations.

Reagent	Device	Storage Location
Pro K	Freezer	Room 6120
DTT	Freezer	Room 6120
40% Sarcosyl	Shelf	Room 6122
Isopropyl alcohol	Shelf	Room 6122
TNE pH 8 Buffer	Shelf	Room 6122
DNA IQ™ Kit	Shelf	Room 6122
Amphyl (1% and 0.2%)	Shelf	Room 6127
Nanopure Water	Shelf	Room 6127
5% TriGene	Shelf	Room 6127
AnalR 100 %Ethanol	Shelf	Room 6127

Please see Table 2 for the volume of reagents for a full plate or half plate. Refer to "*Receipt, Storage and Preparation of Chemicals, Reagents and Kits*" (QIS 17165) for

preparation of the TNE Buffer. All reagents can be made on the bench in Room 6122, except for the Lysis Buffer with DTT which needs to be made in a fume hood. DNA IQ reagents are prepared by staff performing the DNA IQ method.

Table 2. Table of reagent volumes.

Reagent (volume per sample)	Constituent (volume per sample)	Volume req'd for 96 Samples (mL)	Volume req'd for 48 Samples (mL)
Extraction Buffer (500 µL/sample	TNE Buffer 462.5µL	54	27
	Prot K (20 mg/mL)25.0 µL	2.9	1.5
	Sarcosyl (40 %) 12.5µL	1.5	0.7
Lysis Buffer (with DTT) (1.127mL/sample)	Lysis Buffer (no DTT)	130	66
	DTT (add to Lysis Buffer)	1.3	0.66
Lysis Buffer (with DTT) Reagent Trough	As above	125	63
DNA IQ RESIN Solution (50µL/sample)	Lysis Buffer (with DTT) (from above) 43µL	5.536	3
	DNA IQ RESIN 7µL	0.901	0.5
DNA IQ 1X Wash Buffer (300µl/sample)	See Below for preparation	35	18
DNA IQ Elution Buffer (120µl/sample)	Use directly from Kit	14	8

NOTE: For batches not equal to either 96 or 48 samples samples, refer to Appendix 1 "*18.1 Reagents Calculation Tables*" Table 7 for batches of <48 samples, and Table 8 for <96 (but >48)

Extraction Buffer

Prepare the buffer just before extraction in a 100mL sterile glass bottle according to Table 2. Use one aliquot of 20mg/mL Proteinase K (1.5mL) for a 48 sample run and two aliquots for a 96 sample run. Remove aliquot/s of Proteinase K from the freezer, defrost, vortex and centrifuge before use. Ensure that the 40% (w/v) Sarcosyl is completely dissolved (clear) in the stock solution before making the Extraction Buffer. If not dissolved, invert the container a few times and leave longer at room temperature.

Lysis Buffer with DTT

Lysis Buffer is supplied with the kit. Lysis Buffer with DTT is prepared at the start of each run. Remove the DTT from the freezer, defrost, vortex and centrifuge before use. Into a sterilised glass bottle, add 1.3mL of DTT to 130ml of Lysis Buffer for 96 samples. If 48 samples are to be run, use 660µl of DTT to 66ml of Lysis Buffer, again, made up in a sterile glass bottle. Make up the Lysis Buffer with DTT within the Laminar Flow and ensure that full PPE is worn, including face shield. Warning: Lysis Buffer and DTT are toxic.

DNA IQ™ Resin

DNA IQ™ Resin is supplied with the kit. The resin is prepared at the start of each run in a 10mL sterile tube. Ensure the resin is properly mixed by vortexing before pipetting. Look for calculations in Table 2 for the correct volumes of resin and Lysis Buffer (with DTT). In the 10mL tube, mix by inversion before adding to column 4 in the 12-channel reagent plate.

1X Wash Buffer

2X Wash Buffer is supplied with the kit. Once a new kit has been opened, add 35mL of ***AnalR*** Ethanol and 35mL of Isopropyl alcohol to the 2X Wash Buffer. Once the reagents have been added, label the lid and side of the bottle with "1X Wash Buffer," initial and date.

4.2 Equipment

The following equipment (Table 3) and consumables (Table 4) are required for the DNA IQ extraction.

Table 3. Equipment used and location.

Equipment	Asset No.	Location
STORstar (B system)	10238493	6122
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (Ext A Platform)	10076438	6127
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (Ext B Platform)	10076437	6127
DPC shaker (Ext A Platform)	N/A	6127
DPC shaker (Ext B Platform)	N/A	6127
Automated Temperature Controller, Heat Block tiles and heat block adapters (Ext A Platform)	N/A	6127
Automated Temperature Controller, Heat Block tiles and heat block adapters (Ext B Platform)	N/A	6127
Eppendorf 5804 centrifuge	10238416	6127
Vortex	30087015	6127
Fridge	30433424	6127
Micro centrifuge	None	6127
Decapper	None	6127

Table 4. Consumables used for extraction

Consumables	Location
175uL Clear Non-Conductive Filter Tips – Pre-sterilized	6127
MβP Pure 1000uL Tips – Pre-Sterilized	6127
SlicPrep™ 96 device plate	6122
ABgene 96-deepwell plate	6120
Axygen 2mL Deep Well storage plate	6127
1.5ml or 2ml Eppendorf tubes with Spin baskets	6120
12 Channel plate	6127
Nunc™ Bank-it™ tubes	6120
Nunc™ Bank-it™ Caps	6127
Sterile 50mL Falcon tubes	6122
Sterile 10mL tubes	6122
Autoclaved 100mL glass bottles	6122
Autoclaved 250mL glass bottles	6122
Aluminium sealing film	6127
1000uL disposable tips	6120

5 SAFETY

As per the procedures in the QIS document "*Operational Practices in the DNA Dedicated Laboratories*" (QIS 17120), PPE is to be worn by all staff when performing this procedure.

The automated platforms, labware and associated equipment should be wiped with 5% TriGene™ followed by 70% ethanol before and after use. In addition, clean the work area around the MPII instrument with 10% (v/v) Bleach and 70% Ethanol.

While the MPII is running a procedure, refrain from placing any part of the body inside the cabinet. Either pause the program or use the emergency STOP button located at the front of the instrument before touching anything within the cabinet or on the deck surface.

Warning: Tris base, EDTA, Sarcosyl and Lysis Buffer are irritants. DTT is a reducing agent that destroys disulfide bonds. Chaotropic reagents such as guanidinium thiocyanate (GuSCN) are toxic. Do not breathe alcohol fumes. Handle carefully and wear appropriate PPE.

Lysis Buffer contains guanidinium thiocyanate which can be harmful if inhaled, swallowed or comes in contact with skin. Any left over Lysis Buffer with DTT is to be disposed of in a brown Winchester bottle in the fume hood. Never dispose down the sink. If spillage occurs, absorb with liquid-binding material (such as sand, kitty litter, etc) and dispose in a biohazard bin. Again, handle carefully and wear appropriate PPE, including face shield.

6 SAMPLING AND SAMPLE PREPARATION

Samples waiting to be extracted are stored in freezers as described in Table 5.

Table 5. Sample storage locations.

Sample type	Storage Device	Storage Location
Urgent/High/Medium Priority Samples	Freezer	6117-2
Medium Priority Samples	Walk in Freezer or Freezer in 6117	6109 or 6117-5
Low Priority Samples	Walk in Freezer or Freezer in 6117	6109 or 6117-5
Lysates in 1.5ml tubes	Fridge	6120
96 deep well plate containing lysates	Fridge	6127

QC samples

All extraction batches require two controls to be registered. These controls, UR numbers and positions are listed in Table 6.

Table 6. Extraction Quality Controls

QC Name	UR Number	Description
Negative Control	FBOT277	Negative Extraction control – Empty well
Positive Control	FBOT279	Positive Extraction control – Known Donor dried blood swab

Registration of QC

1. Log into the **AUSLAB Main Menu**.
2. Select **1. Request Registration**.
3. Select **2. Full Reception Entry**.
4. Scan in barcode of control.
5. Enter the UR number as per Table 6 and press **[Enter]**.
6. Enter the appropriate Specimen type (e.g. **Blood** for blood control).
7. Request a **9PLEX** test, when prompted to enter the processing comment, enter **EXTP** (Positive extraction control) or **EXTN** (Negative extraction control). Do no assign a priority.
8. Press **[F7]** ***Save*** to save the Billing details.
9. Enter **LAB** in the Billing code field and **t** in the date field and **FBQC** in the Loc/Client field.
10. Press **[F4]** ***Save twice*** to save the registration details.

Note 1: Quality controls should not have a DNA priority allocated at time of registration to ensure they are included in the top positions of a batch

Note 2: for DNA IQ Lysis batches with more than 46 samples (excluding controls) two sets of controls should be registered

Create the DNA IQ Lysis or Retain Supernatant Batch (as required)

1. Log into the **AUSLAB Main Menu**.
2. Select **5. Workflow management**.
3. Select **1. DNA workflow table**.
4. Highlight the appropriate batch type and press **[F5]** ***Batch Allocation***.
5. Press **[F6]** ***Create batch***.
6. Press **[F8]** ***Print menu***.
7. Press **[F7]** ***Print Sample Label***. (print 3 sets)
8. Press **[F8]** ***Print Worksheet***. (print 2 copies)
9. Press **[SF5]** ***Main menu***.

10. Press **[SF11]** ***Print***.
11. Press **[SF6]** ***Accept batch***.
12. Press **[Pause/Break]** to exit to the **Main Menu**.
13. Obtain worksheets **(FBLASER3)** and labels **(FBLABEL13-16)** from the Analytical Section printing bench **(Room 6117)**.

Locating Samples

To locate samples refer to "Analytical Sample Storage" (QIS 24255).

7 OFF-DECK LYSIS PROCEDURE (NO RETAINED SUPERNATANT)

1. Print or obtain a copy of Appendix 2. "*18.2 Reagent & Batch details recording tables (DNA IQ™ Lysis Batch & Extraction Batch)*".

2. Separate the batch into two smaller batches of 48 samples, including one set of controls. (If only a single operator the second batch can be started during step 11)

Note: Positions 1-4 will be the two sets of controls, positions 5-50 will be the first 46 samples and 51-96 will be the second set of 46 samples

3. Remove samples from 5mL storage tubes and label side with barcodes if necessary. If substrates are in a 0.5mL tube, transfer to a labelled 1.5mL tube. Label lid of each sample with position number within the lysis batch. Retain the original 5mL storage tube for substrate storage.

4. Prepare / assemble spin basket assembly or 1.5mL (or 2.0mL) tube and label side with a barcode and lid with position number as required.

Note: substrates from each sample need to be retained

   a. Samples requiring a spin basket include swabs, fabric, paper and cigarette butts.
   b. Samples requiring a 1.5mL include tapelifts, chewing gum, straws, fingernail clippings and toothbrush bristles.

5. Label the side of sterile 1.0mL Nunc™ Bank-It™ tubes with barcode.

6. Have a second operator sequence check all tubes and complete the sequence check in AUSLAB.

7. Prepare Extraction Buffer.

8. Using a multi-stepper pipette add 500µL of Extraction Buffer and vortex briefly. Ensure substrate is fully immersed in extraction buffer.

9. Incubate in a hotblock at 37°C for 45minutes (note temperature on worksheet).

10. Remove samples from hot block and vortex briefly then return to rack.

11. Transfer substrates to spin baskets if required using twirling sticks (if unable to remove with twirling sticks, use forceps. Forceps must be cleaned between each sample by rinsing in bleach followed by ethanol and flaming).

12. For samples not requiring spin baskets, transfer the lysate to the newly labeled 1.5mL tube. Then store original 1.5mL containing substrate in the original 5mL tube.

13. Centrifuge spin baskets at maximum speed (14000rpm) for 2 minutes.

14. Transfer flow through from spin baskets back to original lysis tube, retain spin basket in 5mL tube.

15. Vortex Lysates briefly, then incubate in hotblock at 65°C for 10 minutes (note temperature on worksheet)

16. Enter reagent details, temperatures etc. into AUSLAB.

17. Complete batch in AUSLAB.

18. Store lysates at 4°C (fridge in 6120).

19. Enter into completed batch in AUSLAB and determine the DNA IQ extraction batch ID the DNA lysates have progressed to.

20. Print the appropriate worksheets and 3 sets of batch labels for the DNA IQ extraction batch and note the lysis batch the samples have progressed from on the worksheets. Stamp as "Urgent" if necessary.

8 OFF-DECK LYSIS PROCEDURE (RETAINED SUPERNATANT)

1. Print or obtain a copy of Appendix 2. "*18.2 Reagent & Batch details recording tables (DNA IQ™ Lysis Batch & Extraction Batch)*".

2. Remove samples from 5mL storage tubes and label side with barcodes if necessary. If substrates are in a 0.5mL tube, transfer to a labelled 1.5mL tube. Label lid of each sample with position number. Retain 5mL tube for substrate storage.

3. Label the side of 1.5mL tubes with barcodes for retaining supernatant. Also label lid of 1.5mL tube indicating it contains supernatant.

4. Prepare spin basket assembly or a 1.5mL (or 2.0mL) tube and label side with a barcode and lid with position number as required. **Note:**
   a. Samples requiring a spin basket include swabs, fabric, paper and cigarette butts.
   b. Samples requiring a 1.5mL include tapelifts, chewing gum, straws, fingernail clippings and toothbrush bristles.

5. Label the side of sterile 1.0mL Nunc™ Bank-It™ tubes with barcode.

6. Have a second operator sequence check all tubes and complete the sequence check in AUSLAB.

7. Using a pipette add 650µL of TNE Buffer and vortex briefly.

8. Incubate at room temperature for 30 minutes.

9. Vortex, then centrifuge for 3 minutes at maximum speed (14000rpm).

10. Remove 150µL of supernatant and place into labelled 1.5ml tube (for further testing).

11. Add 25µL of 20ng/µL (mg/mL) Proteinase K and 12.5µL 40% (w/v) Sarcosyl to each original sample tube containing TNE Buffer. Vortex briefly.

12. Incubate in hotblock at 37°C for 45 minutes (note temperature on worksheet).

13. Remove samples from hotblock, vortex briefly and return to rack.

14. Transfer substrates to spin baskets if required using twirling sticks (if unable to remove with twirling sticks, use forceps. Forceps must be cleaned between each sample by rinsing in bleach followed by ethanol and flaming).

15. For samples not requiring spin baskets, transfer the lysate to the newly labeled 1.5mL tube. Then store original 1.5mL containing substrate in the original 5mL tube.

16. Centrifuge spin baskets at maximum speed (14000rpm) for 2 minutes.

17. Transfer flow through from spin baskets back to original lysis tube, retain spin basket in 5mL.

18. Vortex Lysates briefly, then incubate in hotblock at 65°C for 10minutes (note temperature on worksheet).

19. Enter reagent details, temperatures etc. into AUSLAB.

20. Complete batch in AUSLAB.

21. Store supernatants in Freezer 6117-2 (-20°C).

22. Store lysates at 4°C (Fridge in 6120).

23. Enter into completed batch in AUSLAB and determine the DNA IQ extraction batch ID the DNA lysates have progressed to.

24. Print the appropriate worksheets and 3 sets of batch labels for the DNA IQ extraction batch and note the retained supernatant batch the samples have progressed from on the worksheets. Stamp as "Urgent" if necessary.

9 MPII EXTRACTION PROCEDURE

Automated setup of DNA IQ extractions in 96-deep well format is performed using the MultiPROBE® II PLUS HT EX EP-A and EP- B platforms located in Room 6127.

Refer to "*Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform*" (QIS 23939) for instructions on the use and maintenance of the MultiPROBE® II PLUS HT EX platforms.

Summary of DNA IQ EXTRACTION Version 4.1 ODL

1. ***Binding of paramagnetic resin to DNA and further Lysis:*** add Resin solution (50µL) and Lysis Buffer (957µL). Automated mixing and shaking at room temperature for 5 minutes. (this occurs at steps 10-15 of the protocol)

2. ***Removing lysis reagents:*** Slicprep plate is moved to the PKI Magnet to separate beads. Removing of supernatant (1600µL) without disturbing resin, dispense this solution in the storage plate. (this occurs at steps 16-18 of the protocol)

3. ***Washing of the resin-DNA complex:*** To remove any inhibitors in solution. The first wash is with Lysis Buffer (125µL), shaking at room temperature for 1 minute. The plate is moved to the PKI Magnet and the supernatant is removed into the storage plate. The next three washes are with 1X Wash Buffer (100µL), shaking at room

temperature for 1 minute. During each wash cycle, the plate is moved to the PKI Magnet and the supernatant is discarded. (this occurs at steps 21-59 of the protocol)

4. ***Removing any excess of 1X Wash Buffer:*** air dry at room temperature for 5 minutes. (this occurs at step 60 of the protocol)

5. ***Elution of DNA from the Resin-DNA complex:*** Add Elution Buffer (60µL) and incubate at 65 °C for 6 minutes (3 minutes no shaking and 3 minutes shaking). The plate is moved to the PKI Magnet. The eluted solution (supernatant) is removed to the Nunc™ Bank-It™ tubes. Elution is repeated twice. (this occurs at steps 63-83 of the protocol)

6. ***Flushing of capillaries:*** The capillaries are washed with Amphyl and nanopure water.

Sequence Check the Nunc™ Bank-It™ tubes and Sample Lysates

To sequence check storage tubes and transfer DNA lysates to ABgene 96-deep well plates, please refer to method "*Procedure for the Use of the STORstar unit for automated sequence checking*" (QIS 24256).

ENSURE the Nunc™Bank-It™ tube rack is labelled with the AUSLAB Batch ID and barcode on the right hand side of the plate.

Preparation of Reagents & Lysates prior to extraction

1. Refer to table 2 for reagent volumes to make up the required amount of Lysis Buffer (with DTT) and Resin solution. Also measure the required amount of 1X Wash Buffer.

2. Record the Lot numbers of all the reagents used onto the worksheet (printed from appendix 2 and in the AUSLAB batch audit entry.

3. Remove the deep well plate containing Lysates from either storage (either freezer or fridge as case may be) to allow to come to room temperature before starting extraction procedure.

Setting up the EP-A or EP-B MPIIs

These steps are to be carried out in the Automated extraction Room (Room 6127)

4. Turn on the instrument PC.

5. Log onto the network using the **Robotics** login.

6. Double click the WinPrep® icon on the computer desktop (Figure 1).

Figure 1 The WinPrep® icon.

7. Log onto the WinPrep® software by entering your username and password, then press **"Enter"**.

8. Ensure the **System Liquid reservoir is FULL** and tubing is fully submerged in the system liquid before every run and perform a Flush/Wash.

9. Ensure that the daily/weekly start-up has been performed before running any program. If WinPrep® has been closed or been idle for a long period of time initialise the MP II platform as described in QIS 23939.

10. Open the Extraction setup MP II test file in WinPrep® by selecting:
    - **File**
    - **Open**, navigate to **C:\PACKARD\MULTIPROBE\BIN\QHSS PROTOCOLS**
    - Select "**DNA IQ Extraction_Ver 4.1_ODL.mpt**"
    - Click the "**Open**" button

11. Check the whole program there are no bold fonts (e.g. plates may lose gripability and the program will not run). See the line manager.

12. Open the required plate map from the network **I:\EXTRACTION**. Check that the plate map is complete. If there are blanks (samples that have been removed), make all volumes to 0. If it is not a full batch, delete the rows of blank samples at the end of the platemap, then save the changes. Copy the plate map to the following file path: **C:\PACKARD\EXT PLATE MAPS**

13. Decontaminate the required labware by wiping with 5% TriGene followed by 70% ethanol before placing the labware on to the designated grid positions as outlined in the virtual deck view within WinPrep® (Figure 2).

    - The white WALLAC Isoplates (catalogue #1450-514) that are used to support the ABgene 96-deep well plate must be placed into positions **D16** and **C19**.

    - Ensure that the PKI Magnet at **F16** is stable in its insert. This is critical so that beads and DNA are not lost during pipetting.

    - Ensure the DPC shaker is positioned properly

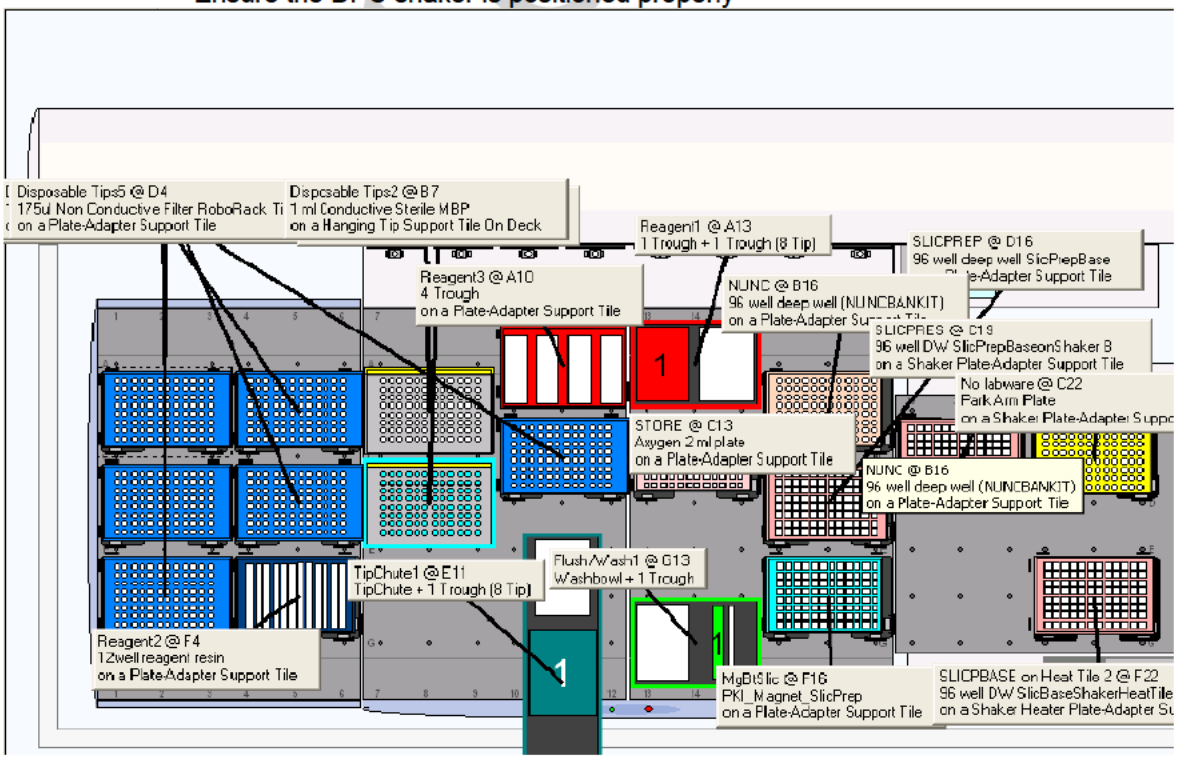

Figure 2. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

14. Ensure that the DPC shaker and Heater Controller Box are switched on.
    For EP-A: Tile 1 at F22 (85^{0}C).
    For EP-B: Tile 2 at F22 (85^{0}C).
    Note: Press the start/stop button twice at the front of the DPC shaker to ensure that it displays zero on the screen.

15. Ensure the heat transfer tile is clicked into the plate adapter tile properly. This is critical to ensure correct incubation temperatures.

16. To the Amphyl wash station at **A10**, add fresh 1% Amphyl to the trough on the far left hand side, 0.2% diluted Amphyl to the middle left and place an empty reagent tough in the middle right position of the station. The nanopure water goes to position **G13** into a 160mL trough in the Flush-Wash station.

17. Pour the required amount of Lysis Buffer into the labelled 150mL reagent trough. Place Lysis Buffer on the left hand side of the 2 trough holder located in position **A13**.
    Note: Ensure that full PPE is worn, including face shield when handling these reagents.

18. **Nunc™ Bank-It™ tube rack**: Check that is the same AUSLAB batch number as the platemap to be run. Ensure that the barcode of the first (A1) and last tube (H12) are the respective ones. Check that the batch label and batch barcode labels are attached to front side of rack. Add B1-Lite generated "**NUNC**" barcode to the right side of the Nunc™ Bank-It™ tube rack. Then place the rack into position **B16**.

19. On an Axygen 2ml deep well Storage plate, label the left side of the plate with both the Batch ID and barcode. Label the right side of the plate with a B1-Lite generated "**STORE**" barcode. Then place in position **C13**.

20. **ABgene 96-deep well plate containing lysates**: Centrifuge plate for 2 minutes at 3021rpm before gently removing adhesive seal and place into position **D16** ensuring the plate is oriented such that the long side of the plate with the words "Front" written on at time of STORstar processing is visible from the front. (This should correspond with the cut corner at H1 being visible to the front of the operator)

21. After ensuring that all the necessary labware has been positioned in the correct grid position as displayed within WinPrep®, click the "**EXECUTE TEST**" button. While the test is loading, record all run information in the Run Log book.

22. Message will appear (Figure 3 below):

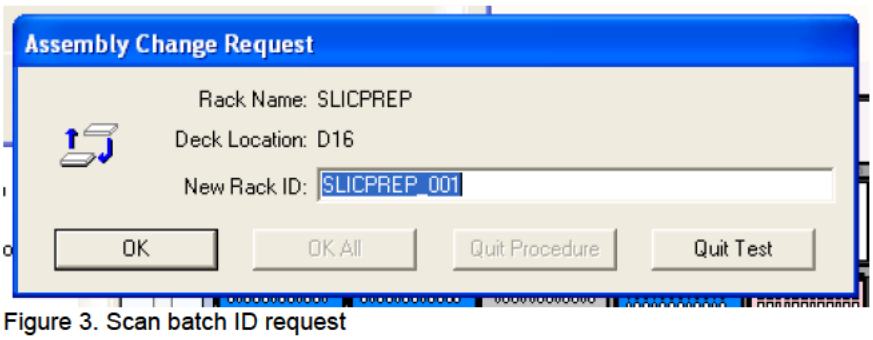

Figure 3. Scan batch ID request

Into "New Rack ID:" Scan barcode of ABgene 96-deep well plate (matches batch ID).

23. Click "**Reset Tip Boxes**" and ensure that the number of tips displayed in this window match those as available on the deck platform. Fill new tips if necessary. Click "**Close**" to accept the tip count, followed by clicking "**Next**"

24. Select the correct platemap by browsing to **C:\PACKARD\EXT PLATE MAPS**. Ensure that the platemap selected matches the batch ID affixed to the ABgene 96-deep well plate containing Lysates located in position **D16.** Once this has been done, click "**Start**", to continue.

25. After the barcodes have been read, a user prompt will appear as a reminder to:
"**Ensure**
1. Shaker and heat box are on.
2. Deck has been populated correctly.
3. The Lysis Buffer is on the left side at A13."
Click "**OK**" to continue.

26. Add the Resin to the 12 Channel plate. Pipette mix thoroughly. Then dispense 50uL of resin into each well of the ABgene 96-deep well plate containing lysates and return plate to position **D16**.

27. Place the 12 channel plate into position **F4** then add the Elution Buffer to the plate by splitting the amount of Elution Buffer in half between channels 11 and 12.

28. Place the Wash Buffer in the far right hand side of the 4 well reagent trough holder (Amphyl wash station at **A10)**

29. The next User prompt will appear with the following directions:
"**Ensure Wash Buffer has been added. Manually add 50uL of Resin and place the ABgene plate in position D16. Ensure Elution Buffer has been added.**" Press **"OK"** when steps 24-26 have been performed.

30. Once the lysis and wash steps have been completed, a message will appear waiting for the heating tile to reach 85^0C (real temp 65^0C). **DO NOT PRESS CONTINUE** it will continue automatically when temperature has reached 85^0C.

31. After the first elution step when the plate is moved to the PKI Magnet, a User message will appear requesting:
"**Push down the plate on the PKI Magnet, Check Nunc™ Bank-It™ tubes are uncapped at position B16, then press OK**."
Note: Allow the plate to cool slightly before pushing down on all four corners of the plate to lock it into place on the PKI Magnet.
Once this is done, do not open the cabinet doors unless absolutely necessary as this will increase the speed of cooling and DNA may re-bind to the resin.

32. After the second elution step, the above prompt will appear again. **Note:** Allow the plate to cool slightly before pushing down on all four corners of the plate to lock it into place on the PKI Magnet
Once this is done, do not open the cabinet doors unless absolutely necessary as this will increase the speed of cooling and DNA may re-bind to the resin.

33. Once the program is completed, a final User Message prompt appears asking to:
"**Remove plates and cover them with aluminium sealing film. Remove Nunc rack and recap Nunc tubes.**"

Once all plates are removed from the deck and sealed, place into small clipseal plastic bags. Click "**OK**" to proceed to the Amphyl wash step to decontaminate the system tubing. **Note:** review the supernatant storage plate for the transfer of beads.

Finalising the MP II run

34. Remove the resin-Lysis-DTT solution from the 12 channel plate in the glass Lysis-DTT bottle used. Discard the plate in the biohazard waste bin.

35. Remove Lysis Buffer with DTT (wear face shield) and dispose of left over reagent into the glass bottle used previously. Bring this bottle to the room 6122 and dispose in the brown Winchester bottle located in the fume hood.

36. Empty the contents of the Amphyl wash station into the sink and rinse the troughs with nanopure H_2O upon completion of the run.

37. Remove all labware from the deck and clean with 5% TriGene™ followed by 70% ethanol, and setup for the next run if necessary. In addition, clean the work area around the MPII instrument with 10% (v/v) Bleach and 70% Ethanol.

38. Remove the tip chute and funnel, rinse with warm tap water to help loosen and remove any residue inside the chute before cleaning with 5% TriGene and 70% ethanol. Hang the chute to dry on the rack beside the sink. If running another extraction straight after, replace the tip chute with another spare, clean chute.

39. Move the platemap to **C:\PACKARD\EXT PLATE MAPS** to the "**Completed Extractions**" folder.

Recording Reagent Details and other information in AUSLAB

40. To record reagent lot numbers, log into the **AUSLAB Main Menu.**
41. Select **5.Workflow Management**.
42. Select **2. DNA Batch Details**.
43. Scan in the Extraction Batch ID.
44. Press **[F6]** ***Reagents***.
45. Press **[SF8]** ***Audit***.
46. Press **[F5]** ***Insert Audit Entry***, enter the lot number details, operator name and Extraction platform the batch was run on and press **[Enter]**.

Importing the MP II log file into AUSLAB

47. To extract the MP II log file, click on the Microsoft Access icon in the WinPrep® main menu to open the MultiPROBE log database.
48. Click on the relevant run Test ID in the Test Selection box. In the Report/Query/Action Selection dropdown menu, select "Report: Test Summary (Sorted by Destination Rack ID)"
49. In the Output Selection dropdown menu, select "File". Save the output file as *.csv format to **C:\PACKARD\EXT PLATE MAPS\EXT LOGS** with the same name as the AUSLAB batch ID and click "**Apply**". (refer to Figure 4. below)

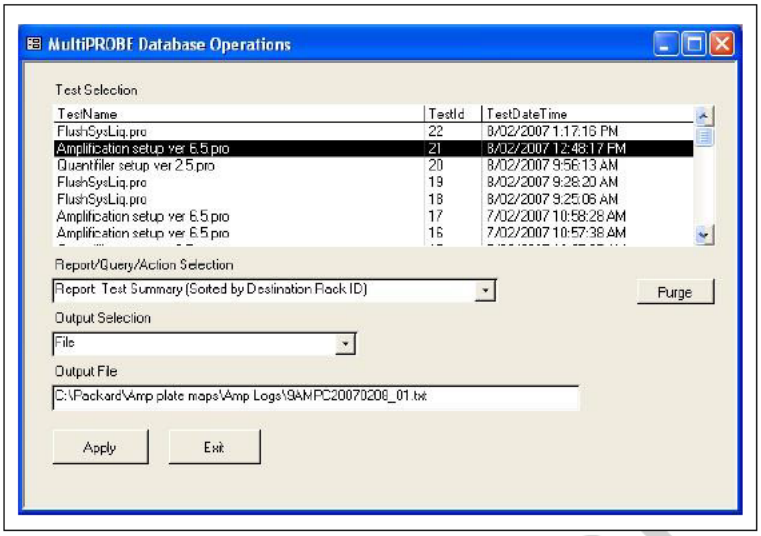

Figure 4. The MultiPROBE log database for collecting MP II run information

50. Open the log file and check for any errors that may have arisen during the Extraction process. Compare the listed errors to any that were encountered during the run. Report any critical errors to the line manager.
51. Copy the log file to **I:\EXTRACTION\EXT A MPII\LOGS or I:\ EXTRACTION\EXT B MPII\LOGS** for uploading to AUSLAB.
52. Log into the **AUSLAB Main Menu**.
53. Select **5.Workflow Management**.
54. Select **2. DNA Batch Details**.
55. Scan in the Extraction Batch ID barcode.
56. Press **[SF6]** ***Files***.
57. Press **[SF6]** ***Import Files***.
58. AUSLAB prompts "***Enter filename***"; enter the filename and extension and press **[Enter]**. (e.g. I:\EXTRACTION\EXT A MPII\Logs\CWIQEXT20071115_01.csv)
59. AUSLAB prompts "***Is this a result file Y/N?***" enter ***N*** and press **[Enter]**.
60. Press **[Esc]**.

Importing Extraction "Results" into AUSLAB

61. Log into the **AUSLAB Main Menu**.
62. Select **5. Workflow Management**.
63. Select **2. DNA Batch Details**.
64. Scan the Extraction batch ID barcode located on the worksheet.
65. Press **[SF6]** ***Files***.
66. Press **[SF6]** ***Import Files***.
67. AUSLAB prompts "***Enter filename***"; enter batch name and extension and press **[Enter]**.(e.g. CWIQEXT20071115_01.txt)
68. AUSLAB prompts "***Is this a results file y/n?***" enter "**y**" and press **[Enter]**.
69. The file will be imported into AUSLAB and appear in the DNA file table.
70. Highlight entry and press **[Enter]**, for access to the DNA results table.
71. Page down through the table and check that all sample results have been imported.
72. Press **[SF8]** ***Table Sort Order***, this sorts the table so any samples that have failed Autovalidation are sorted to the top of the table.
73. For all samples that have failed check the **Processing Comments,** by entering into the sample.
74. **a)** If processing comments state sample is to be sent to another batch type **other** than quant. Request the appropriate rework test code via the SF7 results history table and

the SF8 request rework functions (e.g. samples requiring Microcon, NucleoSpin and pooling)
b) Press **[Esc]** to exit back to the DNA results table.
c) Do not toggle accept.
d) add the extraction batch ID into the 9PLEX or 9FTAR completed date fields in AUSLAB

75. **a)** If processing comment does not state next step for sample the sample will be processed as normal.
b) Press [Esc] to exit back to the DNA results table.
c) Highlight any entries to be changed and press [SF7] Toggle Accept
76. Press **[F7]** ***Complete Batch***, all of the samples with a ***Yes*** in the Accept column will be transferred to the outstanding Quant Casework or Quant Reference lists.
77. File the Extraction worksheet into the relevant folder in Room 6117.

10 SAMPLE STORAGE

Please refer to "Analytical Sample Storage" (QIS 24255) for how to store the old original 5 mL sample tubes, the DNA extract Nunc™ Bank-It™ tubes, ABgene 96-deep well and Axygen store plates.

11 TROUBLESHOOTING

1. If the barcode reader is not reading the barcodes of the Nunc™ Bank-It™ tube rack, or the Slicprep Plate or the Store plate, manually scan the appropriate barcodes.
2. When reading the Nunc™ Bank-It™ tube rack barcode, if the Gripper is not picking up or placing the Nunc™ Bank-It™ tube rack properly on the deck, just manually place the rack properly on the plate adapter support tile.
3. When reading the Store plate barcode, if the Gripper is not picking up or placing the Store plate properly on the deck, just manually place the plate properly on the plate adapter support tile.
4. When reading the Slicprep plate barcode if the Gripper is not picking up the plate properly :
    a. if the plate was not properly placed on the plate adapter support tile with the Wallac Isoplate, just manually place the plate properly.
    b. if the plate was properly placed on the plate adapter support tile with the Wallac Isoplate on it: it means that the gripper needs to be initialised. Abort the run, Initialise the instrument and restart the run. If problem persists, shutdown the MPII and PC, restart and then initialise the whole instrument. Otherwise, contact your line manager.
    c. Calibrate relevant labware using the SlicPrep Calibration plate. This has preset standardised positions that need to be the same on all labware where the Slicprep plate is being moved. The same plate is used on both extraction platforms A and B.
    d. Check the calibrations against the run program DNAIQGripperTest.pro. This program moves the Slicprep across all the labware the gripper moves across. Start with the Slicprep at D16.

5. In steps 18 or 26, if a message is stating that the instrument is having a motor problem when picking up 1 mL tips and the only option is to Abort, abort, initialise and open program version **1.3a** (if the problem is in step 18) or version **1.3b** (if the problem is in step 26).

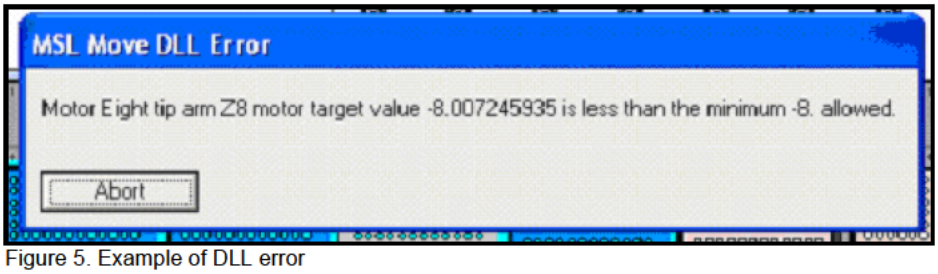

Figure 5. Example of DLL error

As the program will start the gripper will pick up the plates, it is not necessary that the Nunc™ Bank-It™ tube rack is in position (B16), only ensure that it is reading the correct barcode. It is **important not** to place the Slicprep in the original position (E13) as the Slicprep plate has the Spin basket part removed (ie keep at D16), ensure it will scan the correct batch barcode. The Store plate remains in the original position. If the problem persists even after restarting, replace the rack of disposable 1 mL conductive sterile MBP tips for a new one.

6. If the program has already started step 18 and the previous message is appearing, you need to abort. Initialise the instrument and open program version **1.3a.**
   Go to the plate map in C:\PACKARD\ EXT PLATE MAPS and change volume to 0 for all the samples that the Lysis Buffer have been dispensed (Column 6), ensure that the number of samples where the Lysis Buffer was added is the same as the ones where the volume needs to be changed.
   Save the changes and use this plate map for the restart of the program. Please read troubleshooting 5 for barcode reading of plates.
   If the problem persists even after restarting, replace the rack of disposable 1 mL conductive sterile MBP tips for a new one.

7. If the program has already started step 26 and the previous message is appearing, you need to abort. Initialise the instrument and open program version **1.3b**.
   Go to the plate map in C:\PACKARD\ EXT PLATE MAPS and change volume to 0 in all the samples that the Lysis Buffer and Extraction Buffer have been removed (Column 9), ensure that the number of samples where the solution was removed is the same that the ones the volume need to be changed.
   Save the changes and use this plate map for the restart of the program. Please read troubleshooting 5 for barcode reading of plates.
   If the problem persists even after restarting, replace the rack of disposable 1 mL conductive sterile MBP tips for a new one.

8. If a disposable tip gets stuck on the 8 tip arm during disposal of tips a user message will appear. Remove and press retry and then continue.

9. If the message Figure 6 below has appeared:

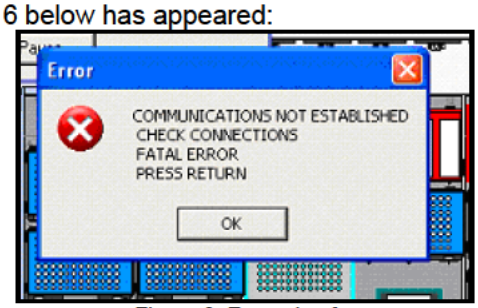

Figure 6. Example of error

press OK and the program will be aborted automatically. Check that all the connections to the instrument (DPC shaker, heater and computer) are properly plugged in. If

everything is OK, you need to close WinPrep, shut down the instrument, DPC shaker, heater and PC. After 2 min restart everything. Once Winprep has been opened, reinitialise the instrument and start the program (check version number according to which step the message has came up). Please read troubleshooting 5 for barcode reading of plates.

12 VALIDATION

- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 9. Automation: Report on the Evaluation of DNA Extraction Chemistries." June 2007.
- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 11: Report on the Validation of the Automated Extraction Chemistry Kit using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Casework Platform." 2007.
- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 13: Report on the Verification of the Automated Extraction Chemistry Kit using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Casework Platform." 2007.
- Muharam I., Hlinka V., Gallagher, B., Lundie, G., Iannuzzi, C., Nurthen T., McNevin A., Ientile V. "Project 21: A Modified DNA IQ™ Method Consisting of Off-Deck Lysis to Allow Supernatant Retention for Presumptive Identification of α-Amylase" 2008
- Muharam I., Hlinka V., Gallagher, B., Lundie, G., Iannuzzi, C., Nurthen T., McNevin A., Ientile V. "Project 22: A Modified DNA IQ™ Method for Off-Deck Lysis Prior to Performing Automated DNA Extraction" 2008

13 QUALITY ASSURANCE/ACCEPTANCE CRITERIA

- A negative control (also known as the reagent blank) is included with each batch of extractions. This reagent blank is processed as a normal sample through to completion. If any results are obtained from this sample, either at the quantitation step or the Genescan analysis step, then the possible source of the contamination is investigated. The samples extracted with this control are thoroughly checked and repeated if possible.
- Positive and negative controls are included in each extraction batch as per table 4.

14 REFERENCES

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7. Mandrekar, P., V., Flanagan, L., & Tereba, A., Forensic Extraction and Isolation of DNA Form Hair, Tissue and Bone. Profiles in DNA, 2002: p. 11.
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16. Promega, Tissue and hair Extraction Kit (for use with DNA IQ™) Protocol. Promega Technical Bulletin #TB307, 2006. Rev 5/06: p. 1-11.
17. Schiffner, L.A., Bajda, E. J., Prinz, M., Sebestyen, J., Shaler, R. & Caragine, T.A., Optimisation of a Simple, Automatable Extraction Method to Recover Sufficient DNA from Low Copy Number DNA Samples for Generation of Short Tandem Repeat Profiles. Croat Med J, 2005. 46(4): p. 578 -586.
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15 STORAGE OF DOCUMENTS

All worksheets are stored in the Analytical area (Room 6117).

16 ASSOCIATED DOCUMENTS

QIS 17120 Operational Practices in the DNA Dedicated Laboratories
QIS 17171 Method for Chelex Extraction
QIS 17165 Receipt, Storage and Preparation of Chemicals, Reagents and Test Kits
QIS 23939 Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform
QIS 24255 Analytical Sample Storage
QIS 24256 Sequence Checking with the STORstar Instrument
QIS 24469 Batch functionality in AUSLAB

17 AMENDMENT HISTORY

Revision	Date	Author/s	Amendments
0	23 Oct 2007	B. Gallagher, T. Nurthen, C. Iannuzzi, V. Hlinka, G. Lundie, I Muharam.	First Issue
1	12 Dec 2007	M Harvey, C Iannuzzi, A McNevin	Reviewed and updated after initial training
2	19 March 2008	M Harvey, B Andersen, C Iannuzzi, A McNevin	Addition of Off-deck Lysis procedure, Retention of fully automated method as Appendix, addition of reagent record tables into Appendix
3	April 2008	QIS2 Migration Project	Headers and Footers changed to new CaSS format. Amended Business references from QHSS to FSS, QHPSS to CaSS and QHPS to Pathology Queensland

18 APPENDIX

18.1 Reagents Calculation Tables

Table 7. **Less than 48 samples** (note difference is in DNA IQ RESIN Solution)

Lysis-DTT Buffer		Volume (in mL)
Lysis Buffer	(Nx1.35)+0.75	
DTT (1M)	Lysis Buffer/100	
Extraction Buffer		
TNE Buffer	Nx0.56	
Prot K (20 mg/L)	Nx0.03	
Sarcosyl (40 %)	Nx0.015	
DNA IQ RESIN Solution		
LYSIS Buffer	0.054x(N+8)	
DNA IQ RESIN	0.009x(N+8)	
DNA IQ 1X Wash Buffer	Nx0.36	
DNA IQ Elution Buffer	Nx0.144	

N = number of samples on batch including positive and negative controls, round up to nearest multiple of 8)

Table 8. **Greater than 48 samples** (note difference is in DNA IQ RESIN Solution)

Lysis-DTT Buffer		Volume (in mL)
Lysis Buffer	(Nx1.35)+0.75	
DTT (1M)	Lysis Buffer/100	
Extraction Buffer		
TNE Buffer	Nx0.56	
Prot K (20 mg/L)	Nx0.03	
Sarcosyl (40 %)	Nx0.015	
DNA IQ RESIN Solution		
LYSIS Buffer	0.054x(N+16)	
DNA IQ RESIN	0.009x(N+16)	
DNA IQ 1X Wash Buffer	Nx0.36	
DNA IQ Elution Buffer	Nx0.144	

N = number of samples on batch including positive and negative controls, round up to nearest multiple of 8)

18.2 Reagent & Batch details recording tables (DNA IQ™ Lysis Batch & Extraction Batch)

Off Deck Lysis Batch ID:	DNA IQ Extraction Batch ID:

Lysis batch:

Samples located by:	
Sample set 1	**Sample set 2**
Operator:	Operator:
Sequence check performed by:	Sequence check performed by:
Transfer tubes sequence checked:	Transfer tubes sequence checked:
37°C Incubation temp:	37°C Incubation temp:
65°C Incubation temp:	65°C Incubation temp:

Extraction Buffer made by:	TNE Buffer Lot#:
40% Sarcosyl Lot#:	Proteinase K Lot#:
Comments:	

Extraction batch:

Plate Lot#:	Lysate/STORstar Operators:
Nunc tube/STORstar Operators:	Lysate Logfile uploaded:
Nunc Logfile uploaded:	
Comments:	

MultiPROBE Platform:	Operator:
Date and Start time:	

Kit Lot#:	1xWash Buffer Lot#:
Lysis Buffer Lot#:	DTT Lot#:
Resin Lot#:	Elution Buffer Lot#:
MP II Logfile uploaded:	Results file uploaded:
Comments:	

18.3 Fully automated method for extraction using DNA IQ™

18.3.1 Sampling and Sample Preparation

FTA® Samples waiting for extraction will have been punched into a Slicprep™ 96 device according to "*FTA® Processing*" SOP (QIS document 24823) and stored in the Fridge located in room 6127.

18.3.2 Procedure

Preparation of Reagents prior to extraction

1. Defrost Prot K and DTT
2. Refer to table 2 for reagent volumes to make up the required amount of Extraction Buffer, Lysis Buffer (with DTT) and Resin solution. Also measure the required amount of 1X Wash Buffer.
3. Record the Lot numbers of all the reagents used onto the AUSLAB worksheet.

Setting up the EP-A or EP-B MPIIs

These steps are to be carried out in the Automated extraction Room (Room 6127)

4. Turn on the instrument PC.
5. Log onto the network using the **Robotics** login.
6. Double click the WinPrep® icon on the computer desktop (Figure 7).
7. Log onto the WinPrep® software by entering your username and password, then press **"Enter"**.

Figure 7 The WinPrep® icon.

8. Ensure the **System Liquid reservoir is FULL** and fully submerged in the system liquid before every run and perform a Flush/Wash.
9. Ensure that the daily/weekly start-up has been performed before running any program. If WinPrep® has been closed or been idle for a long period of time initialise the MP II platform as described in QIS 23939.
10. Open the Extraction setup MP II test file in WinPrep® by selecting:
    - **File**
    - **Open**, navigate to **C:\PACKARD\MULTIPROBE\BIN\QHSS PROTOCOLS**
    - Select "**DNA IQ Extraction_Ver1.3.mpt.**"
    - Click the "**Open**" button
11. Check the whole program there are no bold fonts (e.g. plates may lose gripability and the program will not run). See the line manager.
12. Decontaminate the required labware by wiping with 5% TriGene followed by 70% ethanol before placing the labware on to the designated grid positions as outlined in the virtual deck view within WinPrep® (Figure 2).

    - The white WALLAC Isoplates (catalogue #1450-514) that are used to support the SlicPrep™ 96 device plate must be placed into positions **E13**, **D16** and **C19**.

- Ensure that the PKI Magnet at **F16** is stable in its insert. This is critical so that beads and DNA are not lost during pipetting.

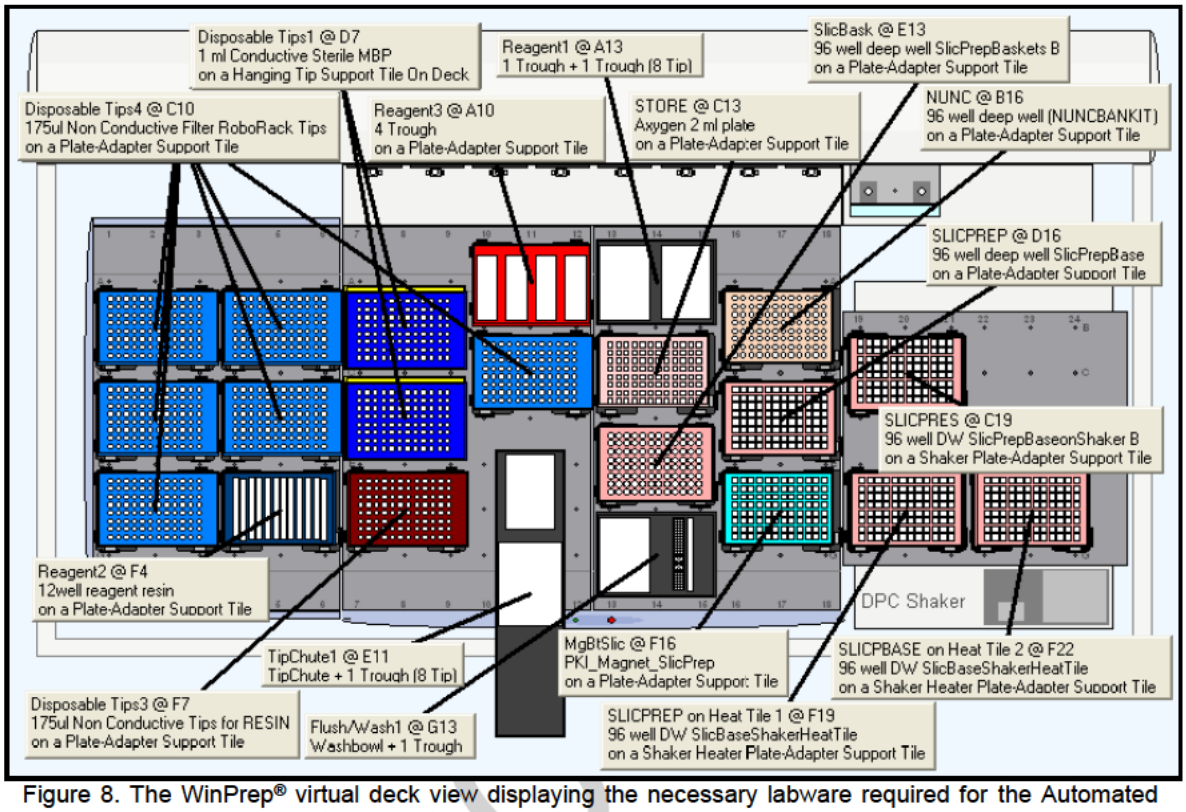

Figure 8. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

13. Ensure that the DPC shaker and Heater Controller Box are switched on.
    For EP-A: Tile 3 should be at F19 (50^{0}C), Tile 1 at F22 (85^{0}C).
    For EP-B: Tile 1 should be at F19 (50^{0}C), Tile 2 at F22 (85^{0}C).
    Note: Press the start/stop button twice at the front of the DPC shaker to ensure that it displays zero on the screen.

14. To the Amphyl wash station at **A10**, add fresh 1% Amphyl to the trough on the far left hand side, 0.2% diluted Amphyl to the middle left and place an empty reagent tough in the middle right position of the station. The nanopure water goes to position **G13** into a 160mL trough in the Flush-Wash station.

15. Pour the required amounts of Extraction Buffer and Lysis Buffer into the labelled 150mL reagent troughs. Place Lysis Buffer on the left hand side and the Extraction Buffer on the right hand side of the 2 trough holder located in position **A13**.
    Note: Ensure that full PPE is worn, including face shield when handling these reagents

16. **Nunc™ Bank-It™ tube rack**: Check that is the same AUSLAB batch number as the platemap to be run. Ensure that the barcode of the first (A1) and last tube (H12) are the respective ones. Attach batch label and batch barcode label to front side of rack. Add B1-Lite generated "**NUNC**" barcode to the right side of the Nunc™ Bank-It™ tube rack. Then place the rack into position **B16**

17. On an Axygen 2ml deep well Storage plate, label the left side of the plate with both the Batch ID and barcode. Label the right side of the plate with a B1-Lite generated "**STORE**" barcode. Then place in position **C13**.

18. **Slicprep™ 96 device**: Gently remove septa mat from Slicprep™ 96 device and check that substrates are at the bottom of the Spin baskets, if not push them down with a sterile disposable tip and place the Slicprep™ 96 device into position **E13**.

19. In I drive from Extraction folder open the required plate map. Check that the plate map is complete. If there are blanks (samples that have been removed), make all volumes to 0. If it is not a full batch, delete the rows of blank samples at the end of the platemap, then save the changes. Copy the plate map to the following file path: **C:\PACKARD\EXT PLATE MAPS**

20. After ensuring that all the necessary labware has been positioned in the correct grid position as displayed within WinPrep®, click the "**EXECUTE TEST"** button. While the test is loading, record all run information in the Run Log book.

21. Click "**Reset Tip Boxes**" and ensure that the number of tips displayed in this window match those as available on the deck platform. Fill new tips if necessary. Click "**Close**" to accept the tip count, followed by clicking "**Next**"

22. Select the correct platemap by browsing to **C:\PACKARD\EXT PLATE MAPS**. Ensure that the platemap selected matches the batch ID affixed to the 96-well Slicprep™ 96 device in position **D16.** Once this has been done, click "**Start**", to continue.

23. After the barcodes have been read, a user prompt will appear as a reminder to:
"**Ensure**
1. Shaker and heat box are on.
2. Deck has been populated correctly.
3. The Lysis Buffer is on the left side and Extraction Buffer is on the right at A13."
Click "**OK"** to continue.

24. Once the Extraction Buffer has been added to the plate, a message will appear waiting for the heating tile to reach 50^{0}C (real temp 37^{0}C). When current temperature reaches 50^{0}C click "**Continue**".

25. The next prompt that appears will request the following:
"Cover Slicprep with the Aluminium sealing film, then place in position F19. Press "OK."

26. After shaking, a User Prompt will appear with the following directions:
"**Remove plate, add white plastic collar and centrifuge 5mins at 3021rpm, then in the cabinet, remove the spin basket part and place it in the empty 1 ml tip container**."
Place the Slicprep™ 96 device into the plate centrifuge and ensure the correct balance plate is used. Once the plate has been centrifuged, carry the plate to the hood and remove the basket of the Slicprep™ 96 device, storing the basket in an empty 1mL tip box, discard the Collar. Complete the step by clicking "**OK**".

27. Add the Resin to the 12 Channel plate. Pipette mix thoroughly. Then dispense 50uL of resin into each well of the Slicprep™ 96 device.

28. Place the 12 channel plate into position **F4** then add the Elution Buffer to the plate by splitting the amount of Elution Buffer in half between channels 11 and 12.

29. Place the Wash Buffer in the far right hand side of the 4 well reagent trough holder (Amphyl wash station at **A10)**

30. The next User prompt will appear with the following directions:
"**Ensure Wash Buffer has been added to trough 4 at A10.**
Manually add 50uL resin to each well of the SlicPrep plate
Place the plate in position D16.
Add the Elution Buffer to the 12 channel plate.
THEN
Press OK when ready." Press **"OK"** when steps 27-29 have been performed.

31. Once the lysis and wash steps have been completed, a message will appear waiting for the heating tile to reach 85^0C (real temp 65^0C). **DO NOT PRESS CONTINUE** it will continue automatically when temperature has reached 85^0C.

32. After the first elution step when the plate is moved to the PKI Magnet, a User message will appear requesting:
"**Check Nunc tubes are uncapped at position B16**
Push down the Slicprep on the PKI Magnet then press OK."
Note: Allow the plate to cool slightly before pushing down on all four corners of the plate to lock it into place on the PKI Magnet.

33. After the second elution step, the above prompt will appear again. **Note:** Allow the plate to cool slightly before pushing down on all four corners of the plate to lock it into place on the PKI Magnet

34. Once the program is completed, a final User Message prompt appears asking to:
"**Remove all the plates starting with the Slicprep plate, place the Spin Basket into the Slicprep plate.**
Cover the Storage plate with the aluminium sealing film."
Recap the NUNC tubes
Once all plates are removed from the deck and sealed, place into small clipseal plastic bags. Click "**OK**" to proceed to the Amphyl wash step to decontaminate the system tubing.

Finalising the MP II run

35. Discard the 12-channel plate used for Resin and Elution Buffer in the biohazard waste bin.
36. Remove Lysis Buffer with DTT and dispose of left over reagent into the glass bottle used previously. Bring this bottle to the room 6122 and transfer into the brown Winchester bottle located in the fume hood.
37. Empty the contents of the Amphyl wash station into the sink and rinse the troughs with nanopure H_2O upon completion of the run.
38. Remove all labware from the deck and clean with 5% TriGene™ followed by 70% ethanol, and setup for the next run if necessary. In addition, clean the work area around the MPII instrument with 10% (v/v) Bleach and 70% Ethanol.
39. Remove the tip chute and funnel, rinse with warm tap water to help loosen and remove any residue inside the chute before cleaning with 5% TriGene and 70% ethanol. Hang the chute to dry on the rack beside the sink. If running another extraction straight after, replace the tip chute with another spare, clean chute.
40. Move the platemap to **C:\PACKARD\EXT PLATE MAPS** to the "**Completed Extractions**" folder.

Recording Reagent Details and other information in AUSLAB

41. To record reagent lot numbers, log into the **AUSLAB Main Menu.**
42. Select **5.Workflow Management**.
43. Select **2. DNA Batch Details**.
44. Scan in the Extraction Batch ID.
45. Press **[F6]** ***Reagents***.
46. Press **[SF8]** ***Audit***.
47. Press **[F5]** ***Insert Audit Entry***, enter the lot number details, operator name and Extraction platform the batch was run on and press **[Enter]**.

Importing the MP II log file into AUSLAB

48. To extract the MP II log file, click on the Microsoft Access icon in the WinPrep® main menu to open the MultiPROBE log database.
49. Click on the relevant run Test ID in the Test Selection box. In the Report/Query/Action Selection dropdown menu, select "Report: Test Summary (Sorted by Destination Rack ID)"
50. In the Output Selection dropdown menu, select "File". Save the output file as *.csv format to **C:\PACKARD\EXT PLATE MAPS\EXT LOGS** with the same name as the AUSLAB batch ID and click "**Apply**". (refer to Figure 9. below)

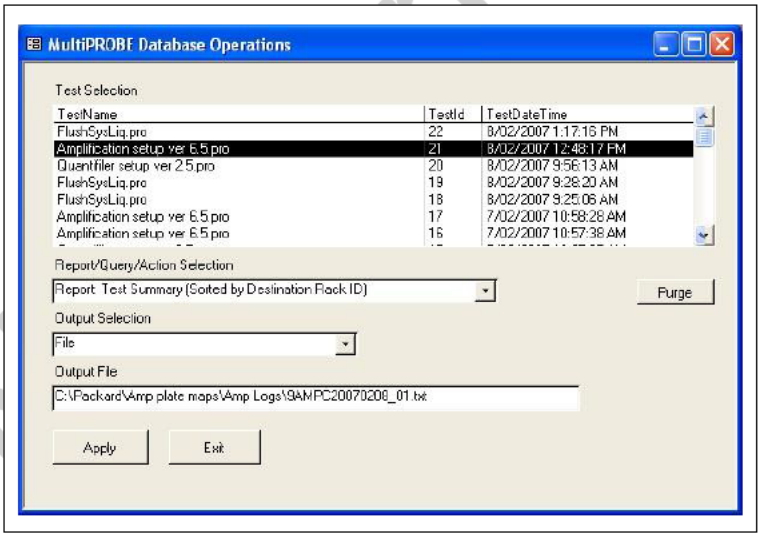

Figure 9. The MultiPROBE log database for collecting MP II run information

51. Open the log file and check for any errors that may have arisen during the Extraction process. Compare the listed errors to any that were encountered during the run. Report any critical errors to the line manager.
52. Copy the log file to **I:\EXTRACTION\EXT A MPII\LOGS or I:\ EXTRACTION\EXT B MPII\LOGS** for uploading to AUSLAB.
53. Log into the **AUSLAB Main Menu**.
54. Select **5.Workflow Management**.
55. Select **2. DNA Batch Details**.
56. Scan in the Extraction Batch ID barcode.
57. Press **[SF6]** ***Files***.
58. Press **[SF6]** ***Import Files***.
59. AUSLAB prompts "***Enter filename***"; enter the filename and extension and press **[Enter]**. (e.g. I:\EXTRACTION\EXT A MPII\Logs\CWIQEXT20071115_01.csv)

60. AUSLAB prompts "***Is this a result file Y/N?***" enter ***N*** and press **[Enter]**.
61. Press **[Esc]**.

Importing Extraction "Results" into AUSLAB

62. Log into the **AUSLAB Main Menu**.
63. Select **5. Workflow Management**.
64. Select **2. DNA Batch Details**.
65. Scan the Extraction batch ID barcode located on the worksheet.
66. Press **[SF6]** ***Files***.
67. Press **[SF6]** ***Import Files***.
68. AUSLAB prompts "***Enter filename***"; enter batch name and extension and press **[Enter]**.(e.g. CWIQEXT20071115_01.txt)
69. AUSLAB prompts "***Is this a results file y/n?***" enter "**y**" and press **[Enter]**.
70. The file will be imported into AUSLAB and appear in the DNA file table.
71. Highlight entry and press **[Enter]**, for access to the DNA results table.
72. Page down through the table and check that all sample results have been imported.
73. Press **[SF8]** ***Table Sort Order***, this sorts the table so any samples that have failed Autovalidation are sorted to the top of the table.
74. For all samples that have failed check the **Processing Comments,** by entering into the sample.
75. **a)** If processing comments state sample is to be sent to another batch type **other** than quant. Return the sample to the correct next batch type – e.g. microcon, nucleospin and pooling
    b) Press **[Esc]** to exit back to the DNA results table.
    c) Do not toggle accept.
76. **a)** If processing comment does not state next step for sample the sample will be processed as normal.
    b) Press **[Esc]** to exit back to the DNA results table.
    c) Highlight any entries to be changed and press **[SF7]** ***Toggle Accept***
77. Press **[F7]** ***Complete Batch***, all of the samples with a ***Yes*** in the Accept column will be transferred to the outstanding Quant Casework or Quant Reference lists.
78. File the Extraction worksheet into the relevant folder in Room 6117.

18.3.3 Sample Storage

Please refer to "Analytical Sample Storage" (QIS 24255) for how to store the old original 5 mL sample tubes, the DNA extract Nunc™ Bank-It™ tubes, Slicprep with Basket and Axygen store plates.

18.4 Manual method for extraction using DNA IQ™

18.4.1 Sampling and Sample Preparation

Samples waiting to be extracted are stored in freezers as described in Table 9.

Table 9. Sample storage locations.

Sample type	Storage Device	Storage Location
Urgent/High/Medium Priority Samples	Freezer	6117-2
Medium Priority Samples	Walk in Freezer	6109
Low Priority Samples	N/A	

QC samples

All extraction batches require two controls to be registered. These controls, UR numbers and positions are listed below in Table 10.

Table 10. Extraction Quality Controls

QC	UR Number	Extraction types
Neg Control	FBOT277	All
QC swab (blood)	FBOT279	Blood

1. Log into the **AUSLAB Main Menu**.
2. Select **1. Request Registration**.
3. Select **2. Full Reception Entry**.
4. Scan in barcode of control.
5. Enter the UR number as per Table 4 and press **[Enter]**.
6. Enter the appropriate Specimen type (e.g. Blood for blood extraction).
7. Request a **9PLEX** test, when prompted to enter the processing comment, enter **EXTP** (Positive extraction control) or **EXTN** (Negative extraction control).
8. Press **[F7]** Enter **LAB** in the Billing code field.
9. Press **[F4]** ***Save*** to save the Billing details.
10. Press **[F4]** ***Save*** to save the registration details.

N.B Quality controls should not have a DNA priority allocated at time of registration to ensure they are included in the top positions of a batch

Create the Extraction Batch

14. Log into the **AUSLAB Main Menu**.
15. Select **5. Workflow management**.
16. Select **1. DNA workflow table**.
17. Highlight the appropriate Extraction batch type and press **[F5]** ***Batch Allocation***.
18. Press **[F6]** ***Create batch***.
19. Press **[F8]** ***Print menu***.
20. Press **[F6]** ***Print Batch label***. (for the deep well plate)
21. Press **[F7]** ***Print Sample labels***. (print four sets of labels for all extractions)
22. Press **[F8]** ***Print Worksheet***.
23. Press **[SF5]** ***Main menu***.
24. Press **[SF11]** ***Print***.
25. Press **[SF6]** ***Accept batch***.
26. Press **[Pause/Break]** to exit to the **Main Menu**.
27. Obtain worksheets (FBLASER3) and labels (FBLABEL13-16) from the Analytical Section printing bench (Room 6117).

Locating Samples

Determine the storage locations of the required samples using the Batch Creation table/Batch details table print out. The columns ***Rack*** and ***Pos*** respectively identify the rack and the grid location where the sample has been stored. Remove the samples from the storage rack and place in an orange rack (12x8).

When all samples have been located:

1. Log into the **AUSLAB Main Menu**.
2. Select **2. Sample Processing**.
3. Select **7. Search Sample storage**.
4. Scan in the sample barcode that is affixed to the sample tube.
5. Press **[F6]** ***Remove Sample***.
6. AUSLAB prompts "***Are you sure you want to remove XXXX-XXXX? (Y/N)***", Enter **Y** and press **[Enter]**.
7. AUSLAB prompts "***Please enter remove comment***", No comment is required. Press **[Enter]**.
8. Press **[Scroll lock]** to clear.
9. Repeat steps **5 - 8** until all of the samples have been removed from their rack.

Sequence Check the tubes

1. Thaw samples at room temperature and label 1.5mL sample tubes.
2. Sequence check the tubes.
3. Add the sequence check details into AUSLAB.
4. Log into **AUSLAB Main Menu**.
5. Select **5. Workflow Management**.
6. Select **2. DNA Batch Details**.
7. Scan in the appropriate extraction batch ID barcode.
8. Press **[F5]** ***Sequence Check***.
9. Scan in the appropriate extraction batch ID barcode.
10. Press **[Pause/Break]** to exit to **Main Menu**.

18.4.2 Procedure

1. Enter the number of samples to be extracted (including controls) into the "No of Samples" column of the DNA IQ Reagent Calculations Table to calculate the volumes of each reagent to be measured out for the extraction. Aliquot regents into either 5ml tubes or 50ml Falcon tubes. **Note:** The volume of Lysis Buffer calculated includes the volume used in the resin-lysis solution

2. Turn on the Eppendorf Thermo mixer and set the temperature to 37ºC.

3. Remove 1.5ml tube and retain the 5mL tube. Prepare the Spin baskets by placing a DNA IQ™ Spin basket into a 1.5mL Microtube. Label the spin baskets (for every tube except Ext. control), 2mL SSI tubes and Nunc™ Bank-It™ storage tubes (for every sample) with the sample barcodes. Have a second operator perform a sequence check of all tubes. This person must Press **[F5] Sequence Check** against the batch in AUSLAB

4. Using the Reagents table, prepare Extraction Buffer, Lysis Buffer with DTT, & Resin Solution. Reagents need to be prepared fresh before each run.

5. Add 300 µL of Extraction Buffer to each tube. Vortex each tube before incubating the tubes at 37°C on the Thermomixer at 1000 rpm for 45 minutes.

6. Remove the tubes from the Thermo mixer and place into a rack, increase the temperature on the Thermomixer to 65°C (for use in the Elution steps).

7. Transfer the substrate from the original tube to a DNA IQ™ Spin Basket using autoclaved twirling sticks. Centrifuge the Spin basket for 2 minutes at room temperature at its maximum speed. Once completed, remove the spin basket & retain in the original 5ml tube. Ensuring minimal contamination and transfer the extract to a labelled 2mL SSI sterile screw tube.

8. Transfer the remaining extract from the original tube to the corresponding 2mL tube. Vortex the tube gently.

9. Add 500 µL of Lysis Buffer to each tube.

10. Into a separate, clean 2mL SSI tube, aliquot the required amount of Lysis Buffer for the Resin solution. Ensure that the DNA IQ™ Resin solution has been thoroughly mixed by vortexing the resin bottle before adding the required resin volume to the Lysis Buffer. Pipette mix the solution to clear the tip of any lingering resin beads. Mix the solution by inverting the tube to prevent bubbles forming.

11. Add 50µL of DNA IQ™ Resin-Lysis solution into each tube. Invert the resin-lysis tube at regular intervals to keep the resin suspended within the solution to ensure uniform results.

12. Vortex each tube for 3 seconds at high speed before placing the tubes in the Multitube shaker set at 1200 rpm to incubate at room temperature for 5 minutes.

13. Remove from the Multitube shaker and vortex the tubes for 2 seconds at high speed before placing the tubes in the magnetic stand. Separation will occur instantly.

Note: If resin does not form a distinct pellet on the side of the tube, or if the pellet has accidentally mixed with the solution while in the stand, vortex the tube and quickly place back in the stand.

14. Carefully remove all of the solution in the tube into the original 1.5mL tube (i.e. the tube which originally contained the substrate), ensuring that the resin is not disturbed from its place on the side of the tube.

Note: If some resin is drawn up in tip, gently expel resin back into tube to allow re-separation.

15. Add 125µL of prepared Lysis Buffer solution and vortex for 2 seconds at high speed. Return the tubes to the magnetic stand and allow for separation to occur. Once separation has occurred again remove the Lysis Buffer into the original 1.5mL tube (i.e. the tube which originally contained the substrate).

16. Add 100µL of prepared 1X Wash Buffer and vortex for 2 seconds at high speed. Return tube to the magnetic stand and once separation has occurred remove and discard all Wash Buffer.

17. Repeat Step 16 another two times for a total of three washes. Ensure that all of the solution has been removed after the last wash.

18. Wipe down a Biohazard hood with bleach followed by ethanol. Uncap the tubes, placing the lids inside down onto a clean rediwipe in consecutive order and place the tubes in the same order into a clean plastic rack. Air-dry the resin in the hood for 15 minutes at Room temperature. Do not dry for more than 20 minutes, as this may inhibit removal of DNA during the elution phase.

19. Once the resin is dry, replace the lids on the corresponding tubes and remove from the hood. Add 50µl of Elution Buffer to each of the samples by carefully pipetting the liquid to the side of the tube, above the pellet. Do not mix.

20. With the lids on, incubate the tubes in the Thermomixer at 65°C for 3 minutes. After the three minutes are up, continue to incubate for a further 3 minutes shaking at 1100 rpm.

21. Remove the tubes and vortex for 2 seconds at high speed. Immediately place the tube in the magnetic stand while hot to ensure maximum DNA yield during elution.

22. Carefully transfer the DNA containing supernatant to the corresponding labelled Nunc™ Bank-It™ tubes.

23. Remove tubes from the magnetic stand and add carefully another 50 µL of Elution Buffer above the magnetic pellet.

24. Repeat step 20 to 22. The final volume after this elution should be approximately of 95 µL of DNA solution.

25. DNA can be stored at 4°C for short-term storage or at -20 or -70°C for long-term storage.

18.4.3 Sample storage

1. Log into **AUSLAB Main Menu**.
2. Select **2. Sample Processing**.
3. Select **6. Sample Storage**.
4. Scan in Rack barcode.
5. Press **[SF5]** ***Fill Rack***.
6. Scan in sample barcode and place in rack in scanned position.
7. Repeat for all samples.
8. Press **[Esc]**.
9. Press **[Pause/Break]** to return to the **Main Menu**.
10. Select **3. Patient Enquiry**.
11. Scan in Rack barcode.
12. Tab down to the next blank ***DNA Batch No*** field and press **[F2]** ***Edit***.
13. Scan in the Batch ID of the samples stored.
14. Press **[Pause/Break]** to return to the **Main Menu**.

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DNA IQ™ Method of Extracting DNA from Casework and Reference Samples

1. PURPOSE AND SCOPE

This method describes the routine method for the extraction of DNA using the DNA IQ™ kit (Promega Corp., Madison, WI, USA). The automated method is the preferred procedure, utilising the MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms (PerkinElmer BioDiscovery, Downers Grove, IL, USA). The manual method has also been included. This method applies to all DNA Analysis staff members that are required to extract DNA from samples.

Reference samples and casework samples must be extracted separately. If casework and reference samples are to be extracted on the same instrument, the instrument (including all required labware) must be decontaminated between operations.

2. DEFINITIONS

DNA IQ™ Resin	Magnetic resin beads used to bind DNA
DTT	1,4 Dithiothreitol
EDTA	Ethylenediaminetetraacetatic acid
EP-A	Extraction Platform A
EP-B	Extraction Platform B
Extracts	Samples that have had a DNA extraction processes performed
Lysates	Samples that have had the off-deck lysis step performed, but have not yet completed the entire extraction process
MPII	MultiPROBE® II PLUS HT EX Platform
Paramagnetic	To become magnetic with the application of a magnetic force
Pro K	Proteinase K
Samples	Sample substrates (in tubes) awaiting DNA extraction
Sarcosyl	N-Lauroylsarcosine sodium
TNE	Tris, NaCl and EDTA buffer (10mM Tris, 100mM NaCl, 1mM EDTA, pH 8.0)

3. PRINCIPLE

Sample Pre-lysis

The Extraction Buffer used in the pre-lysis of cellular material contains Proteinase K (Pro K), TNE buffer (10mM Tris, 100mM NaCl, 1mM EDTA, pH 8.0) and Sarcosyl. TNE acts as a basic buffer with EDTA chelating ions in solution. Sarcosyl is a detergent that lyses open cell membranes. Proteinase K is added to digest cellular material, helping the DNA to be released. It also digests proteins that interfere with the DNA binding capacity of the resin. In addition, Proteinase K rapidly inactivates enzymatic activity that could potentially degrade DNA (e.g. nucleases).

Proteinase K (or Endoproteinase K) is a fungal enzyme from *Engyodontium album* (formerly *Tritirachium album*). It is a stable S8 family serine alkaline protease that has broad specificity and is inactivated by diisopropylfluorophosphate, phenyl-methane sulfonyl

fluoride and Hg^{2+} ions. As a highly active endopeptidase, it cleaves peptide bonds adjacent to the carboxyl group of N-substituted (blocked alpha amino) hydrophobic, aliphatic and aromatic amino acids. Therefore, it actively hydrolyses proteins, glycoproteins, peptides and esters of amino acids. The smallest peptide that can be hydrolysed with Proteinase K is a tetrapeptide.

DNA IQ™ Kit

The DNA IQ™ kit (Promega Corp., Madison, WI, USA) is designed to purify DNA from a number of different substrates and has been optimised for trace DNA samples. An in-house validation was performed using a modified version of the PerkinElmer automated protocol.

The in-house protocol includes:

- Off-deck lysis steps with the option to retain a portion of the supernatant for further testing;
- The use of 300µL Extraction Buffer containing TNE (10mM Tris, 100mM NaCl, 1mM EDTA, pH 8.0) and Proteinase K to lyse cellular material prior to performing the DNA IQ process;
- The use of tubes and spin baskets for off-deck lysis of samples prior to extraction on the MPII platform. At the conclusion of off-deck lysis, lysates are transferred to individual Nunc Bank-It™ tubes;
- Nunc Bank-It™ tubes (arranged in sequence using STORstar) containing lysates are presented to the MPII platform for automated transfer of lysates into a 96-deep well plate;
- DNA IQ™ Resin is added using the MPII platform, followed by addition of two volumes of DNA IQ™ Lysis Buffer;
- The 96-deep well plate containing DNA IQ™ Resin and Lysis Buffer is sealed using an adhesive aluminium film and is placed on a MixMate to mix the contents of each well. The plate is centrifuged and the aluminium film is then pierced using a 96 well half skirt PCR microplate and the plate is returned to the MPII platform;
- A double elution step is performed using two dispenses of DNA IQ™ Elution Buffer at 60µL, resulting in a final DNA extract volume of 100µL;
- DNA extracts are automatically transferred into Nunc Bank-It™ tubes for storage.

Cell lysis is performed using DNA IQ™ Lysis Buffer containing Dithiothreitol (DTT). 1,4 Dithiothreitol is a reducing agent used in extractions to break disulfide bonds of proteins. The Lysis Buffer used is a proprietary buffer containing chaotropic salts required for binding of DNA to the magnetic beads. According to the MSDS (Promega Corporation 2006), the Lysis Buffer contains 50-75% guanidinium thiocyanate, < 2.5% EDTA, < 2% 3-[(3-Choalamidopropryl)dimethylammonio] propanesulfonic and < 2% polyethylene glycol tert-octylphenyl ether.

The basis of the DNA IQ™ kit is a magnetic bead resin which contains novel paramagnetic particles covered with silica. The magnetic bead resin usually has a DNA binding capacity of 100ng but the addition of Proteinase K increases the binding capacity. Samples with small amounts of DNA are more efficient than samples with large amounts of DNA, and as a result a small sample size is critical to ensure efficient recovery of DNA.

The magnetic beads with silica have a negative charge at basic and near neutral pH. The Lysis Buffer changes the pH and salt concentration of the solution and the silica on the magnetic beads becomes positively charged, which is then able to bind the DNA.

Several washing steps are employed in the protocol to remove inhibitors. The first washing step is performed using Lysis Buffer. This wash ensures the DNA is bound to the magnetic bead resin and washes out inhibitors. The next three washing procedures incorporate the use of DNA IQ™ Wash Buffer. This buffer contains an alcohol/aqueous mixture which

ensures the DNA is not eluted during washing by keeping the DNA dehydrated, and the aqueous phase washes out the inhibitor.

The DNA IQ™ Elution Buffer removes the DNA from the magnetic bead resin. The Elution Buffer changes the salt content. Heating the complex to 65ºC allows the DNA to be released from the magnetic beads. The Elution Buffer is a low ionic strength buffer that reduces the affinity of the DNA for the silica that covers the magnetic beads, by re-hydration of the phosphate backbone.

The DNA IQ™ kit isolates DNA greater than 80bp, smaller DNA is removed selectively to prevent PCR inhibition.

MultiPROBE® II HT EX PLUS with Gripper™ Integration Platform
Within DNA Analysis, routine DNA extractions are performed on casework or reference samples using two MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms (EP-A or EP-B) located in Room 6127.

Each platform uses a computer-controlled Cartesian XYZ liquid handling system designed for the efficient automation of sample preparation. Liquid transfer is performed by an 8-tip system with VersaTip® and VariSpan™ options. The VersaTip® option allows the use of both fixed and disposable tips (conductive and non-conductive). The VariSpan™ option permits variable probe spacing between each of the individual probes so that a wide range of labware such as micro plates, tubes, vials and reagent troughs can be accessed. Each sample probe is capable of independent motion in the Z direction due to independent Z drives.

The 8-tip system is also capable of multichannel liquid-level sensing by utilising Accusense™ technology. This technology works by each probe detecting a change in capacitance within the liquid. This capacitive mode of detection is also possible when using conductive disposable tips. Accusense™ also permits the detection of low or non-ionic polar solutions and solvents. Pipetting on the platforms is driven by 8 individually controlled syringes via the positive displacement of the system liquid (nanopure water) when transferring liquid.

The Gripper™ Integration allows for automated identification of labware via the scanning of barcodes and also allows for automated transfer of plates from one position on the deck to another. To increase deck space and capacity, the platforms include a left deck extension.

For automated DNA extraction using the DNA IQ™ kit, a platemap is utilised to provide the necessary information for correct volumes and locations for pipetting. It also contains information regarding batch and plate identification, sample positions, lab numbers of samples, and lists all the samples in the batch, providing the total number of samples. The program will fail to work without a platemap.

4. REAGENTS AND EQUIPMENT

4.1. Reagents

- DNA IQ™ System Kit (400 sample kit)
  - DNA IQ™ Resin
  - Lysis Buffer (LB)
  - 2x Wash Buffer (2xWB)
  - Elution Buffer (EB)
- TNE (10mM Tris, 100mM NaCl, 1mM EDTA, pH 8.0)
- Proteinase K (Pro K) 20mg/mL
- Dithiothreitol (DTT) 1M

- 5% TriGene
- 70% Ethanol
- 10% Bleach 7x Solution
- 1% Amphyl
- 0.2% Amphyl
- Isopropyl Alcohol
- AnalR 100% Ethanol
- 40% Sarcosyl
- Nanopure Water

These reagents are stored in locations as per Table 1.

Table 1. Reagent storage locations.

Reagent	Device	Storage Location
Pro K	Freezer	Room 6120
DTT	Freezer	Room 6120
40% Sarcosyl	Shelf	Room 6122
Isopropyl Alcohol	Shelf	Room 6122
AnalR 100 %Ethanol	Shelf	Room 6122
TNE pH 8 Buffer	Shelf	Room 6122
DNA IQ™ Kit	Shelf	Room 6122
Amphyl (1% and 0.2%)	Shelf	Room 6127
Nanopure Water	Shelf	Room 6127
5% TriGene	Shelf	Room 6127
10% Bleach 7x Solution	Shelf	Room 6127

Table 2 shows the volume of reagents for a full plate or half plate. All reagents can be made on the bench, with the exception of the Lysis Buffer-DTT which needs to be made in a fume hood. DNA IQ™ reagents are prepared by staff performing the method. Refer to "*Receipt, Storage and Preparation of Chemicals, Reagents and Kits*" (QIS 17165) for preparation of TNE Buffer.

Table 2. Table of reagent volumes.

Reagent	Volume for 96 samples (mL)	Volume for 48 samples (mL)	Volume for 24 samples (mL)
Extraction Buffer			
TNE buffer	33.3	20	10
Proteinase K (20mg/mL)	1.8	1.08	0.54
Sarcosyl (40%)	0.9	0.54	0.27
Lysis-DTT Buffer			
DNA IQ™ Lysis Buffer	90.0	50	N/A
DTT (1M)	0.9	0.5	N/A
DNA IQ™ Resin solution			
Lysis-DTT Buffer	6.0	3	N/A
DNA IQ™ Resin	1.0	0.5	N/A
DNA IQ™ 1x Wash Buffer	35.0	18	N/A
DNA IQ™ Elution Buffer	14.0	8	N/A

Note: Do not directly pipette from reagent stock bottles. Stock reagents should be decanted into working containers where appropriate. Volume for 24 samples is for off-deck lysis samples only.

4.2. Extraction Buffer

Note: Prepare Extraction Buffer just prior to commencing the off-deck lysis or extraction procedure.

1. Determine the required volumes of reagents by using Table 2.
2. Remove the required amount of 20mg/mL Proteinase K from the freezer and thaw. Vortex and centrifuge before use.

3. Ensure that the 40% (w/v) Sarcosyl is completely dissolved and appears clear in the stock solution. If not dissolved, invert the container a few times and leave at room temperature.
4. Retrieve an aliquot of TNE buffer of the appropriate volume size from the falcon tube storage container in Room 6122.
5. Add the appropriate volumes of 20mg/mL Proteinase K and 40% (w/v) Sarcosyl to the falcon tube containing TNE buffer, and invert gently to mix.
6. Label the tube with "Extraction Buffer", your initials and the date.

4.3. Lysis Buffer with DTT Solution

Note: Lysis Buffer is supplied with the DNA IQ™ kit. The Lysis Buffer with DTT solution is prepared just prior to commencing the extraction procedure.

Warning: Lysis Buffer and DTT are toxic, use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling Lysis Buffer.

1. Determine whether a half- or full-plate of reagents are required (Table 2).
2. Remove the required amount of DTT from the freezer and thaw. Vortex and centrifuge before use.
3. In the fume hood add the required volume of Lysis Buffer to a sterilised glass Schott bottle and then add the required volume of DTT.
4. Label the glass Schott bottle with "Lysis Buffer + DTT", your initials and the date.

4.4. DNA IQ™ Resin

Note: DNA IQ™ Resin is supplied with the DNA IQ™ kit. The resin is prepared at the start of each run. Ensure the resin is properly mixed by *vortexing* prior to use.

1. Determine whether a half- or full-plate of reagents are required (Table 2).
2. Into a 10mL (or 5mL) sterile tube, add the required volume of Lysis Buffer with DTT solution (from 4.1.2) followed by the required volume of DNA IQ™ Resin.
3. Mix by gentle inversion.
4. Label the tube with "Resin", your initials and the date.

4.5. 1x Wash Buffer

Note: 2x Wash Buffer is supplied with the DNA IQ™ kit. To prepare 1x Wash Buffer, add 35mL of *AnalR* Ethanol and 35mL of Isopropyl Alcohol to the 2x Wash Buffer bottle. Then label the lid and side of the bottle with "1x Wash Buffer," your initials and the date. Also fill out the Reagent Log (DNA IQ Reagents).

1. Determine whether a half- or full-plate of reagents are required (Table 2).
2. Into a Falcon tube, add the required volume of 1x Wash Buffer.
3. Label the falcon tube with "Wash Buffer", your initials and the date.

4.6. Elution Buffer

Note: Elution Buffer is supplied with the DNA IQ™ kit. The Elution Buffer can be used directly from the kit. The Elution Buffer is removed from the kit and stored in the automated extraction room (6127).

5. EQUIPMENT

The following equipment (Table 3) and consumables (Table 4) are required for the DNA IQ™ extraction process.

Table 3. Equipment used and location.

Equipment	Asset No.	Location
STORstar (B system)	10238493	6122
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (EP-A)	10076438	6127
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (EP-B)	10076437	6127
DPC shaker (EP-A)	N/A	6127
DPC shaker (EP-B)	N/A	6127
Automated Temperature Controller, Heat Block tiles and heat block adapters (EP-A)	N/A	6127
Automated Temperature Controller, Heat Block tiles and heat block adapters (EP-B)	N/A	6127
Eppendorf 5804 centrifuge	10238416	6127
Vortex	30087015	6127
Fridge	30433424	6127
Micro centrifuge	None	6127
MixMate		6127
Decapper	None	6127
4titude 4seal Sealer	30512847	6127

Table 4. Consumables used for extraction.

Consumables	Location
175µL Clear Non-Conductive Filter RoboRack tips – Pre-Sterilised	6127
MβP Pure 1000uL Tips – Pre-Sterilised	6127
SlicPrep™ 96 device plate	6122
ABgene 96-deep well plate	6120
Axygen 2mL deep well storage plate	6127
96 well Half Skirt PCR Microplate	6127
1.5mL or 2mL Eppendorf tubes with spin baskets	6120
12 Channel plate	6127
Nunc Bank-it™ tubes	6120
Nunc Bank-it™ caps	6127
Sterile 50mL Falcon tubes	6122
Sterile 10mL or 5mL tubes	6122
Autoclaved 100mL glass bottles	6122
Autoclaved 250mL glass bottles	6122
Aluminium sealing film	6127
300µL ART tips	6120
1000µL ART tips	6120

6. SAFETY

As per the procedures in the QIS document "*Operational Practices in the DNA Dedicated Laboratories*" (QIS 17120), PPE is to be worn by all staff when performing this procedure. This includes the use of safety glasses.

The automated platforms, labware and associated equipment should be wiped with 5% TriGene™ followed by 70% Ethanol before and after use. In addition, clean the work area around the MPII instrument with 10% (v/v) Bleach 7x and 70% Ethanol.

While the MPII is running a procedure, refrain from placing any part of the body inside the cabinet. Either pause the program or use the emergency STOP button located at the front of the instrument before touching anything within the cabinet or on the deck surface.

Warning: Tris base, EDTA, Sarcosyl and Lysis Buffer are irritants. DTT is a reducing agent that destroys disulfide bonds. Handle carefully and wear appropriate PPE.

Lysis Buffer contains guanidinium thiocyanate (GuSCN) which is toxic and can be harmful if inhaled, swallowed or comes in contact with skin. Left over Lysis Buffer-DTT is disposed of in a brown Winchester bottle in the fume hood. Never dispose down the sink. If spillage

occurs, absorb with liquid-binding material (such as sand or kitty litter) and dispose in a biohazard bin. Do not combine bleach with Lysis Buffer and do not wipe areas of Lysis Buffer spillage with bleach, as the two reagents produce cyanide gas when combined. Handle carefully and wear appropriate PPE, including safety glasses, when handling Lysis Buffer. If Lysis Buffer is spilt onto PPE (eg. gloves, gowns), discard the PPE and obtain new PPE.

7. SAMPLING AND SAMPLE PREPARATION

7.1. Sample Locations

Samples waiting to be extracted are stored in freezers as described in Table 5.

Table 5. Sample storage locations.

Sample type	Storage Device	Storage Location
Urgent/High/Medium Priority Samples	Freezer	6117-2
Lysates in 1.0mL Nunc Bank-it™ tubes	Freezer	6117
Extracts in 1.0mL Nunc Bank-It™ tubes	Freezer	6117

Note: Some Medium and Low Priority storage boxes may be located in the Exh bit Room (6106).

7.2. QC Samples

For all off-deck lysis batches (with 48 samples or less) and extraction batches; one negative control and one positive control is required to be registered. For all off-deck lysis batches with > 48 samples; two negative and two positive controls is required to be registered.

Table 6. Extraction Quality Controls

QC Name	Batch Type	Description
Negative Control	Off-Deck Lysis	Negative Extraction control – empty well
Positive Control	Off-Deck Lysis	Positive Extraction control – dried blood swab from a known donor
Negative Control	IQ Extraction	Negative Extraction control – empty well
Positive Control	IQ Extraction	Internal IQ Efficiency Control

7.2.1. Registration of QC Samples

The registration of control samples is covered in the DNA Analysis workflow procedure (QIS 24919)

7.3. Create the DNA IQ™ Lysis or Retain Supernatant batch

Creation of Lysis and retain supernatant batches is covered in the DNA Analysis Workflow Procedure (QIS 24919).

7.4. Locating Samples

To locate samples refer to “Analytical Sample Storage” (QIS 24255).

8. OFF-DECK LYSIS PROCEDURE

8.1. Off-Deck Lysis (No Retained Supernatant)

1. For batches of 48 samples or less, one set of controls and one operator is required. For larger batches, separate the batch into two smaller batches of 48 samples, including one set of controls in each. If a single operator is performing the whole procedure, the second batch can be started during an appropriate incubation step.

   Note: For full batches positions 1-4 will be the two sets of controls, positions 5-50 will be the first 46 samples and 51-96 will be the second set of 46 samples.

2. For each sample label:
   - Original sample tube
   - Spin basket or 1.5mL tube
   - 1.0mL Nunc Bank-It™ tube

   Note 1: Samples that require a spin basket are: swabs, fabric, paper and cigarette butts. Samples that require a 1.5mL tube are: tapelifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

   Note 2: If samples are in a 2mL QPS tube and require a spin basket, label a new 5mL tube for the substrate to be retained in.

3. If substrate is in a 0.5mL tube, transfer to a labelled 1.5mL tube.

4. Have a second operator sequence check the tubes and enter the sequence check in AUSLAB.

5. Prepare Extraction Buffer as per Section 4.1.1.

6. Add 300µL of Extraction Buffer and vortex briefly. Ensure that substrates are immersed in the Extraction Buffer.

7. Incubate on a Thermomixer at 37°C for 45 minutes at 1000rpm or on the hotblock (if using hotblock vortex samples at 22-23 minutes and again at the end of the incubation). Record temperature on worksheet.

8. Remove from the Thermomixer/hotblock. Transfer substrate to spin basket if required or transfer the lysate to the labelled 1.5mL tube. Store original 1.5mL tube containing the substrate in the 5mL tube.

9. Centrifuge spin basket at maximum speed (14,000rpm) for 2 minutes.

10. Retain spin basket containing the substrate in the corresponding 5mL tube and transfer flow through back to original lysis tube.

11. Vortex lysate, then incubate in hotblock/Thermomixer at 65°C for 10 minutes. Record temperature on worksheet.

12. Centrifuge at maximum speed (14,000rpm) for 1 minute.

13. Transfer 300uL of lysate to the corresponding Nunc Bank-It™ tube.

Note: If more than 300uL of lysate is present, retain the remaining lysate in the 1.5mL tube. In AUSLAB, write a specimen note for that sample (eg. “extra lysate retained from sample XXXXXXXXX.”). Store the retained 1.5mL lysate tube in appropriate box in freezer.

14. In AUSLAB, enter reagent and hotblock/Thermomixer temperature details and complete the batch.

15. Store lysates in temporary storage boxes in freezer 6117-2 (-20°C).

16. Store 5mL tubes containing substrates in “Spin Basket boxes” in freezer 6117-5 (-20°C).

8.2. Off-Deck Lysis (Retained Supernatant)

1. For batches of 48 samples or less, one set of controls and one operator is required. For larger batches, separate the batch into two smaller batches of 48 samples, including one set of controls in each. If a single operator is performing the whole procedure, the second batch can be started during step 7.

   Note: For full batches positions 1-4 will be the two sets of controls, positions 5-50 will be the first 46 samples and 51-96 will be the second set of 46 samples.

2. For each sample label:
   - Original sample tube
   - Spin basket or 1.5mL tube
   - 1.5mL tube (also labelled with "**sup**" to indicate supernatant)
   - 1.0mL Nunc Bank-It™ tube

   Note 1: Samples that require a spin basket are: swabs, fabric, paper and cigarette butts. Samples that require a 1.5mL tube are: tapelifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

   Note 2: If samples are in a 2mL QPS tube and require a spin basket, label a new 5mL tube for the substrate to be retained in.

3. If substrate is in a 0.5mL tube, transfer to a labelled 1.5mL tube.

4. Have a second operator sequence check the tubes and enter the sequence check in AUSLAB.

5. Add 450µL of TNE buffer and vortex.

6. Incubate at room temperature for 30 minutes.

7. Vortex, then centrifuge at maximum speed (14,000rpm) for 3 minutes.

8. Remove 150µL of supernatant and place into the respective 1.5mL tube labelled with "**sup**" (for further testing).

9. Add 14µL of 20mg/mL Proteinase K and 7µL 40% (w/v) Sarcosyl to the original sample tube containing substrate and TNE buffer, then vortex.

10. Incubate in Thermomixer at 37°C for 45 minutes at 1000rpm or on the hotblock (if using hotblock vortex samples at 22-23 minutes and again at the end of the incubation). Record temperature on worksheet.

11. Remove from hotblock/Thermomixer. Transfer substrate to spin basket if required or transfer the lysate to the labelled 1.5mL tube. Store original 1.5mL tube containing substrate in the 5mL tube.

12. Centrifuge spin basket at maximum speed (14,000rpm) for 2 minutes.

13. Retain spin basket containing the substrate in the corresponding 5mL tube and transfer flow through back to original lysis tube.

14. Vortex Lysate, then incubate in hotblock/Thermomixer at 65°C for 10 minutes. Record temperature on worksheet.

15. Centrifuge at maximum speed (14,000rpm) for 1 minute.

16. Transfer 300uL of lysate to the corresponding Nunc Bank-It™ tube.

Note: If more than 300uL of lysate is present, retain the remaining lysate in the 1.5mL tube. In AUSLAB, write a specimen note for that sample (eg. "extra lysate retained from sample XXXXXXXXX."). Store the retained 1.5mL lysate tube in appropriate box in freezer.

17. In AUSLAB, enter reagent and hotblock/Thermomixer temperature details and complete the batch.

18. Store supernatants in the "S/N Retention" boxes in Freezer 6117-2 (-20°C).

17. Store lysates in temporary storage boxes in freezer 6117-2 (-20°C).

19. Store 5mL tubes containing substrates in "Spin Basket boxes" in freezer 6117-5 (-20°C).

9. AUTOMATED EXTRACTION OF LYSED SAMPLES

9.1. Create the DNA IQ Extraction batch

Creation of extraction batch is covered in the DNA Analysis Workflow Procedure (QIS 24919).

9.2. Locating samples

To locate samples refer to "Analytical Sample Storage" (QIS 24255).

9.3. Sequence checking the Nunc Bank-It™ tubes

The procedure for the automated checking of sample tubes is covered in the Procedure for the use of the STORstar unit for automated sequence checking (QIS 24256)

9.4. MPII Extraction Procedure

Automated setup of DNA IQ extractions in 96-deep well format is performed using the MultiPROBE® II PLUS HT EX EP-A and EP-B platforms located in Room 6127.

Refer to "*Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform*" (QIS 23939) for instructions on the use and maintenance of the MultiPROBE® II PLUS HT EX platforms.

9.5. Summary of DNA IQ™ Extraction Version 6.4_ODL (following off-deck lysis)

1. ***Transfer of lysates from Nunc Bank-It™ tubes into the ABgene 96-deep well plate***
   Lysates from the off-deck lysis protocol, contained in individual Nunc Bank-It™ tubes, are transferred automatically into an ABgene 96-deep well plate prior to commencing automated sample processing. Instead of a 96-deep well plate, the use of individual Bank-It™ tubes for storage of lysates removes the need for an adhesive film and therefore reduces the risk of well-to-well contamination from the action of peeling back an adhesive plate cover.

2. ***Automated addition of DNA IQ™ Resin and Lysis Buffer***
   DNA IQ™ Resin is added automatically into the ABgene 96-deep well plate on the platform. The program uses a waste mode dispensing step to add 53µL DNA IQ™ Resin-Lysis Buffer solution to each well in either half the plate or the whole plate. Because of this, batch sizes are restricted to either 48 or 96 samples in order to maintain efficiency and economy. Two volumes of Lysis Buffer are then added to promote binding of DNA to the paramagnetic resin.

3. ***Mixing using a MixMate to bind DNA to resin***
   Manual intervention is required to seal the ABgene 96-deep well plate with a 4titude Pierce Seal and sealing plate using 4titude sealer pre-heated at 175 °C. The plate is then transferred onto a MixMate instrument for mixing at 1100rpm for 5 minutes, followed by centrifugation on an Eppendorf 5804 centrifuge at 3000rpm for 2 minutes. The seal is then carefully pierced with a 96 well PCR microplate and the ABgene plate is returned to the Applied Biosystems magnet on the MPII platform.

4. ***Removing lysis reagents for storage***
   At this point, most of the DNA is bound to the paramagnetic resin. With the positioning of the ABgene plate on the ABI magnet, DNA IQ™ Resin becomes immobile at the bottom of the plate. The lysis reagents from each well are transferred automatically to a storage plate on the MPII platform without disturbing the DNA IQ™ Resin. The purpose

of the storage plate is for retaining supernatant that may potentially still contain DNA material. The storage plate may also become useful in quality investigations.

5. ***Washing of the resin-DNA complex***
   Washing steps are performed to remove any inhibitors in solution. The first wash uses 125µL Lysis Buffer with shaking at room temperature for 1 minute on the DPC shaker to ensure that the DNA is bound to the paramagnetic resin. The plate is moved to the ABI magnet and the supernatant is transferred into the storage plate. The next three washes incorporate 100µL of 1x Wash Buffer with shaking at room temperature for 1 minute on the DPC shaker. During each wash cycle, the ABgene 96-deep well plate is moved to the ABI magnet and the supernatant is discarded into the tip chute.

6. ***Removing any excess of 1x Wash Buffer***
   The samples are allowed to air dry at room temperature for 5 minutes in order to remove all traces of Ethanol from the Wash Buffer. The presence of Ethanol may potentially inhibit both the elution process and also downstream PCR.

7. ***Elution of DNA from the resin-DNA complex***
   A double elution method is employed in this procedure. At each elution step, 60µL of Elution Buffer is added to each sample, followed by incubation at 65°C for 6 minutes (3 minutes without shaking followed by 3 minutes shaking on the DPC shaker). The ABgene plate is moved to the ABI magnet and the eluted solution (supernatant containing eluted DNA) is transferred to fresh Nunc™ Bank-It™ tubes.

8. ***Flushing of capillaries***
   As a decontamination measure, the MPII capillaries and liquid pathway are washed with Amphyl and Nanopure water at the conclusion of the automated extraction process.

9.6. Preparation of reagents for the automated extraction process

Note: Reagents are prepared during the setting up of the MPII platforms (Section 14.3).

9.7. Setting up the MPII platforms for automated DNA IQ™ processing

The following steps are carried out in the automated extraction room (Room 6127).

1. Remove the Nunc Bank-It™ tubes containing lysates from the fridge to allow to come to room temperature before commencing the extraction procedure.

   Note: If the lysates are frozen, remove them from the freezer and thaw in Room 6127. Also remove the required amount of DTT and Pro K to thaw.

2. Restart or turn on the instrument PC.

3. Log onto the network using the **Robotics** login.

4. Open WinPrep® by double clicking icon on the computer desktop (Figure 1).

5. Log onto the WinPrep® software by entering your username and password, then press **"Enter"**.

Figure 1 The WinPrep® icon.

6. Ensure that the daily/weekly start-up and maintenance has been performed before running any program. If WinPrep® has been closed or been idle for a long period of time initialise the MP II platform as descr bed in QIS 23939.

7. Ensure the **System Liquid reservoir is FULL** and tubing is fully submerged in the system liquid before every run and perform a Flush/Wash. If visible air bubbles have appeared in tubing or in syringes between setting up the deck and executing the test, another flush wash will need to be performed before starting the MPII extraction run.

8. Open the Extraction setup MP II test file in WinPrep® by selecting:
   - **File**
   - **Open**, navigate to **C:\PACKARD\MULTIPROBE\BIN\QHSS PROTOCOLS**
   - Select "**DNA IQ Extraction_Ver 6.4_ODL.mpt**"
   - Click the "**Open**" button

9. Check the tree pane of the whole program for any bold fonts. See the Analytical Senior Scientist if bold fonts are present.

10. Copy the required plate map from the network folder **I:\EXTRACTION** into the local folder **C:\PACKARD\EXT PLATE MAPS**. Open the plate map using Excel and check that the plate map is complete, and make changes if necessary. If there are blanks (samples that have been removed), make all volumes to 0. If it is not a full batch, delete the rows of blank samples at the end of the platemap. Save all changes made to the platemap (as a .txt file).

11. Decontaminate the required labware with 5% TriGene followed by 70% Ethanol and place onto the instrument deck in the designated grid positions (as shown in the WinPrep® software). Additionally, ensure the DPC shaker is positioned properly.

12. Ensure that the DPC shaker and Heater Controller Box are switched on.
    For EP-A: Tile 1 at F22 (85^0C).
    For EP-B: Tile 2 at F22 (85^0C).
    Note: Press the start/stop button twice at the front of the DPC shaker to ensure that it displays zero on the screen.

13. Ensure the heat transfer tile is clicked into the plate adapter tile properly.
    Note: This is critical to ensure correct incubation temperatures.

14. To the Amphyl wash station in position **A10**, add 1% Amphyl to the far left hand side, 0.2% diluted Amphyl to the middle left and place an empty (waste) reagent trough in the middle right position of the station.

15. Refer to section 4.1 for the preparation of reagents. Record all lot numbers onto the worksheet and in AUSLAB.

16. Check the syringes and tubing and perform a Flush/Wash if required.

17. Add Wash Buffer to the far right hand side trough of the Amphyl wash station in position **A10**. Add Lysis Buffer to the labelled 150mL reagent trough on the left hand side of the 2 trough holder in position **A13**.

18. Place the 12 channel plate into position **A16**. Add Elution Buffer to the plate by dividing the Elution Buffer between channels 11 and 12.

19. **Nunc Bank-It™ lysate tubes:** The lysates should now be at room temperature. Ensure that the rack is labelled with the correct **AUSLAB batch ID** on the **front** of the Nunc™ Bank-It™ tube rack and that the label matches the batch ID on the worksheet and platemap. Additionally, randomly check some visible barcodes on the side of lysate tubes against the positions on the worksheet to ensure the correct batch of samples is to be processed.

    a. Add a B1-Lite generated **'LYSATE'** barcode on the **right hand side** of the Nunc™ Bank-It™ tube rack.
    b. Place the rack onto the MixMate to shake for 1 minute at 1000rpm.
    c. Centrifuge the rack at 3000rpm for 2 minutes in the Eppendorf 5804 centrifuge and then place into position **C13**.

    Note: Do not uncap lids until Step 33.

20. **ABgene 96-deep well plate:** Label the **left hand side** of the plate with both the correct **AUSLAB batch ID** and **batch ID barcode**. With a marker, print the word "FRONT-lysate" on the front of the plate. Place the plate in its correct orientation in position **E13**.

21. **2mL 96-deep well storage plate:** Label the **left hand side** of the plate with both the correct **AUSLAB batch ID** and **batch ID barcode**. Label the **right hand side** of the plate with a B1-Lite generated "**STORE**" barcode. With a marker, print the word "FRONT-store" on the front of the plate. Place the plate in its correct orientation in position **E16**.

22. **Nunc Bank-It™ extract tubes:** Ensure that the rack is labelled with the correct **AUSLAB batch ID** on the **front** of the Nunc™ Bank-It™ tube rack. Label the **right hand side** of the plate with a B1-Lite generated "**EXTRACT**" barcode. Additionally, randomly check some visible barcodes on the side of lysate tubes against the positions on the worksheet to ensure the correct batch of samples is to be processed. Place the rack in position **G16**.
    Note 1: Do not uncap lids during this step.
    Note 2: If B1-Lite generated barcodes are not available hand-write the labels.

23. Add Nanopure water to the 160mL trough in the Flush/Wash station in position **G13**.

    Ensure that all necessary labware have been positioned correctly as displayed within WinPrep®, then click "**EXECUTE TEST**". Record run information in the Run Log book.

24. The following message will appear (Figure 2 below):

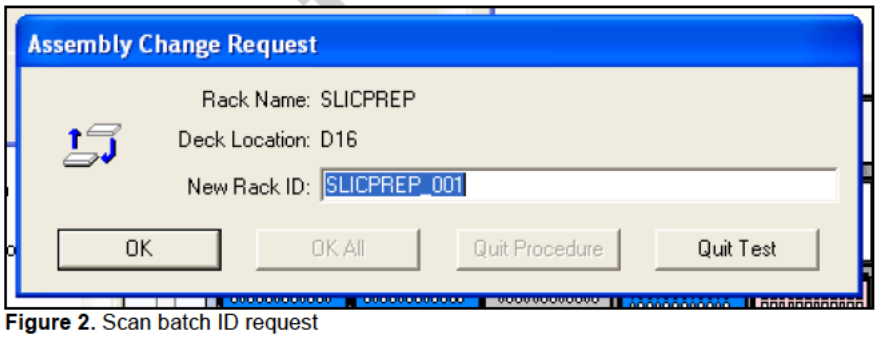

Figure 2. Scan batch ID request

    Into "New Rack ID:" scan barcode off the worksheet. It is important this corresponds to the labelling of labware on the deck and the plate maps used.

25. Click "**Reset Tip Boxes**" and ensure that the number of tips displayed in this window match those as available on the deck platform. Fill new tips if necessary. Click "**Close**" to accept the tip count, and then click "**Next**".

26. Select the correct platemap by browsing to **C:\PACKARD\EXT PLATE MAPS**. Ensure that the platemap selected corresponds to the labelling of labware on the deck, the paperwork used and the "New Rack ID" entered above.

27. For a full batch of 96 samples, ensure that all nodes are checked. For a batch of 48 samples or less, uncheck the node: "Add resin to second half of plate". Click "**Next**" to check all other nodes.

28. Click "**Start**" to continue.

29. The MPII instrument will proceed to scan the required plates on the platform deck in the below order. If barcode reading fails or if B1-Lite barcodes are not available (and hand-written labels have been used), the user is prompted to enter a plate ID. A plate ID can be entered manually into the "Read failed" prompt window for:
    a. Nunc extract tubes, type in **EXTRACT** and press **"Enter".**
    b. 96-deep well storage plate, type in **STORE** and press **"Enter".**
    c. Nunc lysate tubes, type in **LYSATE** and press **"Enter".**

30. After the plates have been identified, two user prompts will appear as a reminder to confirm the deck setup.
    a. Ensure all steps on the first prompt have been complete, Click **OK** to continue.
    Note: At this stage the DNA IQ™ Resin solution is added to the deck. Pipette mix the DNA IQ™ Resin and then add to channel 1 of the 12 channel plate in position **A16.** It is important to add the resin in a uniform fashion to ensure equal distribution of resin along the channel.
    b. Ensure all steps on the second prompt have been complete, Click **OK** to continue.

31. The program will progress to transfer the lysates followed by automated addition of the Lysis-DTT buffer and the DNA IQ™ Resin solution. The next user prompt will appear. Follow the steps as outlined in the user prompt. Then click **OK** when ready.
    Note: Ensure that plate is sealed properly with the Pierce Seal. Once the Pierce Seal film is pierced, the PCR Microplate is then discarded (new plate used each time).

32. Once lysis steps have been completed, a user prompt will appear. Follow the directions as outlined in the user prompt. Then click **OK** when ready.
    Note: Nunc lysate tubes (including the rack) should be placed into a clipseal bag, sealed and discarded into a biohazard waste bin.

33. Once the wash steps have been completed, a user prompt will appear. Follow the directions as outlined in the user prompt. Then click **OK** when ready.
    Note: The Nunc extract tubes can be decapped on the bench in the MPII cabinet while the samples are drying and the heating tile is stabilising. At this point, the operator has approximately **12 minutes** to decap the tubes. If the operator has been interrupted and requires more time to decap the tubes, the program may be paused at this step.

34. A message will appear waiting for the heating tile to reach 85°C (for incubation at 65°C). **DO NOT PRESS CONTINUE** as the program will continue automatically when the temperature has been reached with sufficient stability.

35. A user prompt will appear. Follow the directions as outlined in the user prompt. Then click OK when ready.

36. Once the elution is completed, a user prompt appears. Follow the directions as outlined in the user prompt. Then click OK when ready.

37. Once all plates are removed from the deck and sealed, place into a clipseal plastic bag. Click "**OK**" to proceed to the Amphyl wash step.
    Note: Before placing the supernatant storage plate into a clipseal bag, check the plate for the transfer of beads. To do this, place the plate onto the ABI magnet (if beads are present they will settle to the bottom of the plate) and then inspect the bottom of the plate. If beads are present refer to the Section 15, Troubleshooting.

38. A final message will advise that the run has completed. Click "**OK**".

9.8. Finalising the MP II Run

1. Transfer left over Resin solution from the 12 channel plate and the Lysis Buffer-DTT (wearing safety glasses) into the glass Lysis-DTT bottle previously used. Discard the 12 channel plate in the biohazard waste bin. Take the bottle to room 6122 and transfer left over reagents into the brown Winchester bottle located in the fume hood.
2. Discard the contents of the Amphyl wash station into the sink and rinse the troughs with Nanopure water.
3. Remove all labware from the deck and clean with 5% TriGene™ followed by 70% Ethanol, and setup for the next run if necessary. In addition, clean the work area around the MPII instrument with 10% (v/v) Bleach 7x and 70% Ethanol.
4. Remove the tip chute and funnel, rinse with warm tap water to remove any residue inside the chute before cleaning with 5% TriGene and 70% Ethanol.
5. Move the platemap to **C:\PACKARD\EXT PLATE MAPS**\Completed Extractions.

9.9. Importing MP II Log File into AUSLAB

1. Click on the Microsoft Access icon in the WinPrep® main menu to open the MultiPROBE log database.
2. Click on the relevant run Test ID in the Test Selection box. In the Report/Query/Action Selection dropdown menu, select "Report: Test Summary (Sorted by Destination Rack ID)"
3. In the Output Selection dropdown menu, select "File". Save the output file as *.csv format to **C:\PACKARD\EXT PLATE MAPS\EXT LOGS** with the same name as the AUSLAB batch ID and click "**Apply**".
4. Open the log file and check for any errors that may have arisen during the extraction process. Compare the listed errors to any that were encountered during the run. Report any critical errors to the line manager.
5. Copy the log file to **I:\EXTRACTION\EXT A MPII\LOGS or I:\ EXTRACTION\EXT B MPII\LOGS** for uploading to AUSLAB.
6. Import the log file, entering the path, filename and extension (e.g. I:\EXTRACTION\EXT A MPII\Logs\CWIQEXT20071115_01.csv) and press **[Enter]**. For more details on importing files into AUSLAB refer to Batch functionality in AUSLAB SOP (QIS 24469).

9.10. Importing Extraction "results" into AUSLAB

1. Import the results file, entering the filename and extension. For more details on importing files into AUSLAB refer to Batch functionality in AUSLAB SOP (QIS 24469).
2. The file will be imported into AUSLAB and appear in the DNA file table.
3. Highlight entry and press **[Enter]**, for access to the DNA results table.
4. Page down through the table and check that all sample results have been imported.
5. Press **[SF8]** ***Table Sort Order***, this sorts the table, sorting samples that have failed Autovalidation to the top. Samples that have failed are those that have processing comments present.
6. For samples that have failed, check the **Processing Comments,** by entering into the sample.
7. If processing comments state sample is to be sent to another batch type **other** than quant. Proceed with the following steps:
   a. Request the appropriate rework test code via the **[SF7]** results history table and the **[SF8]** request rework functions (e.g. samples requiring Microcon, NucleoSpin and pooling).
   b. Press **[Esc]** to exit back to the DNA results table. Do not toggle accept.
   c. Add the extraction batch ID into the 9PLEX or 9FTAR completed date fields in AUSLAB.
8. If processing comments do not state next step the sample will be processed as normal:
   a. Press **[Esc]** to exit back to the DNA results table.
   b. Highlight any entries to be changed and press **[SF7]** ***Toggle Accept.***

9. Press **[F7]** ***Complete Batch***, all of the samples with a ***Yes*** in the Accept column will be transferred to the outstanding Quant Casework or Quant Reference lists.
10. File the extraction worksheet into the relevant folder in Room 6117.

9.11. Sample Storage

Refer to "Analytical Sample Storage" (QIS 24255) for how to store the DNA extract Nunc™ Bank-It™ tubes, ABgene 96-deep well and Axygen store plates.

10. TROUBLESHOOTING

1. If the resin is not pipette mixing correctly (eg. resin is not being drawn up into the pipette tip), pause the Winprep program and check that the support tile is clicked onto the MPII deck correctly. Alternatively, pipette and mix resin manually one more time from the corresponding column in the 12 channel plate.

2. If the Gripper is not placing the rack/plate properly on the deck, pause the program and manually adjust the rack/plate properly on the adapter support tile.

3. If the Gripper appears to be not gripping a plate correctly, or the pipetting alignment of a labware appears to be incorrect, the deck and / or labware can be re-calibrated. This is covered in the Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform SOP (QIS 23939)

11. VALIDATION

- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 9. Automation: Report on the Evaluation of DNA Extraction Chemistries." June 2007.
- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 11: Report on the Validation of the Automated Extraction Chemistry Kit using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Casework Platform." 2007.
- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 13: Report on the Verification of the Automated Extraction Chemistry Kit using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Casework Platform." 2007.
- Muharam I., Hlinka V., Gallagher, B., Lundie, G., Iannuzzi, C., Nurthen T., McNevin A., Ientile V. "Project 21: A Modified DNA IQ™ Method Consisting of Off-Deck Lysis to Allow Supernatant Retention for Presumptive Identification of α-Amylase" 2008
- Muharam I., Hlinka V., Gallagher, B., Lundie, G., Iannuzzi, C., Nurthen T., McNevin A., Ientile V. "Project 22: A Modified DNA IQ™ Method for Off-Deck Lysis Prior to Performing Automated DNA Extraction" 2008

12. QUALITY ASSURANCE/ACCEPTANCE CRITERIA

- Positive and negative (reagent blank) controls are included in each extraction batch as per Table 6. These controls are processed as normal samples through to completion.
- If any results are obtained from the reagent blank, either at quantification or during the CE QC check, then the possible source of the contamination is investigated. The samples extracted with this control are thoroughly checked and repeated if possible.

13. REFERENCES

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7. Mandrekar, P., V., Flanagan, L., & Tereba, A., Forensic Extraction and Isolation of DNA Form Hair, Tissue and Bone. Profiles in DNA, 2002: p. 11.
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14. STORAGE OF DOCUMENTS

All worksheets are stored in the Analytical area (Room 6117).

15. ASSOCIATED DOCUMENTS

QIS 17120 Operational Practices in the DNA Dedicated Laboratories
QIS 17171 Method for Chelex Extraction
QIS 17165 Receipt, Storage and Preparation of Chemicals, Reagents and Test Kits
QIS 23939 Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform
QIS 24255 Analytical Sample Storage
QIS 24256 Sequence Checking with the STORstar Instrument
QIS 24469 Batch functionality in AUSLAB

QIS 24919 DNA Analysis Workflow Procedure

16. AMENDMENT HISTORY

Version	Date	Author/s	Amendments
R0	23 Oct 2007	B. Gallagher, T. Nurthen, C. Iannuzzi, V. Hlinka, G. Lundie, I Muharam.	First Issue
R1	12 Dec 2007	M Harvey, C Iannuzzi, A McNevin	Reviewed and updated after initial training
R2	19 March 2008	M Harvey, B Andersen, C Iannuzzi, A McNevin	Addition of Off-deck Lysis procedure, Retention of fully automated method as Appendix, addition of reagent record tables into Appendix
R3	April 2008	QIS2 Migration Project	Headers and Footers changed to new CaSS format. Amended Business references from QHSS to FSS, QHPSS to CaSS and QHPS to Pathology Queensland
4	13 March 2009	QIS2 migration	Version incremented by one on migration to QIS2
5	03 June 2009	M Aguilera, B Micic, C Iannuzzi, A. Cheng, V. Hlinka, I. Muharam, G. Lundie, C. Weber	Major changes to reflect new procedure. Updated to reflect changes in procedure as an outcome of internal and external audits. Created ver.6.4 ODL in MPII Platforms. Minor changes in procedures using 4titude 4seal heat sealer to seal plates.

17. APPENDIX

17.1. Manual method for extraction using DNA IQ™

17.1.1. Sampling and Sample Preparation

Refer to section 9 above.

17.1.2. QC samples

All extraction batches require two controls to be registered. The registration of control samples is covered in the DNA Analysis workflow procedure (QIS 24919)

17.1.3. Creating the Extraction Batch and Locating Samples

Refer to "DNA Analy i Workflow Procedure" (QIS 24919)

17.1.4. Procedure (No Retain Supernatant)

1. Turn on the Eppendorf Thermomixer and set the temperature to 37ºC. Alternatively an appropriately cal brated hot block may be used.

2. Remove DTT 1M (small aliquot) and Prot K 20mg/mL (small aliquot) from freezer to thaw.

3. Label for each sample:
   - Original sample tube; 2mL SSI tube (if original sample is not in a 2mL tube); 1.5mL tube (for supernatant) these tubes should not be in contact with the substrate; Spin basket or 2mL tube; Nunc™ Bank-It™ storage tube.

Note: Spin baskets are not required for the Negative Extraction control, tape lifts, nails and other non absorbent substrates. For these samples, excluding the Negative Extraction control, label a 2mL tube instead of a spin basket.

4. Have a second operator perform a sequence check of all tubes. This person must enter the sequence check in AUSLAB.

5. Using Table 7, prepare Extraction Buffer, Lysis Buffer & Resin solution. Ensure that the DNA IQ™ Resin solution is thoroughly vortexed prior to use.
   Note: Reagents need to be prepared fresh before each run and Lysis Buffer-DTT solution and Resin solution need to be prepared in the fume hood.

Table 7. Table of reagent volumes for DNA IQ Manual Extraction

Reagent (volume per sample)	Constituent	Volume per sample (µL)	Volume for 12 Samples (mL)
Extraction Buffer (300µL/sample)	**TNE Buffer**	277.5	4.0
	Prot K (20mg/mL)	15.0	0.216
	Sarcosyl (40% w/v)	7.5	0.108
Lysis Buffer – DTT (726µL/sample)	**Lysis Buffer**	660	10.0
	DTT	6.6	0.1
Resin-Lysis Solution (50µL/sample)	**Lysis Buffer with DTT** (from above)	43	0.645
	DNA IQ RESIN	7	0.105
DNA IQ 1X Wash Buffer (300µL/sample)	See Reagent preparation		4.0
DNA IQ Elution Buffer (100µL/sample)	Use directly from Kit		1.4

Note: The volume of Lysis-DTT Buffer calculated includes the volume used in the Resin-Lysis solution preparation.

6. Add 300µL of Extraction Buffer. Ensure that large substrates including tape lifts are fully submerged. Vortex, then incubate at 37°C on the Thermomixer at 1000 rpm for 45 minutes. If no thermomixer available, incubate samples within a hotblock, vortex mix at the start and end of the incubation and at least one during the incubation.

7. Remove from the Thermomixer and increase the temperature on the Thermomixer to 65°C (for use in the Elution steps).

8. Transfer the substrate to spin basket. Centrifuge spin basket for 2 minutes at maximum speed. Retain the spin basket in the original 5ml tube and transfer the flow through back into sample tube.

Note: If original sample tube is not a 2mL tube, transfer flow through from spin basket and the supernatant from the original tube into a 2mL tube.

9. For samples that do not require a spin basket, pulse spin and transfer supernatant to the labelled 2mL tube.

10. Add 550µL of Lysis-DTT Buffer solution.

11. Add 50µL of DNA IQ™ Resin-Lysis solution. Pipette mix the Resin-Lysis solution between each addition to keep the resin suspended in solution.

12. Vortex and place on the Multitube shaker set at 1200 rpm for 5 minutes.

13. Remove from the Multitube shaker, vortex and immediately place into the magnetic stand. Separation will occur instantly.
    Note: If resin does not form a distinct pellet on the side of the tube, or if the magnetic beads have re-suspended while in the stand, vortex the tube and quickly place back in the stand.

14. Carefully transfer the solution (supernatant) into the 1.5mL supernatant tube, ensuring that the resin is not disturbed. Remove from the magnetic stand.
    Note: If some resin is drawn up in the pipette tip, gently expel resin back into tube to allow re-separation.

15. Add 125µL of Lysis-DTT Buffer solution. Vortex and place into the magnetic stand.

16. Once separation has occurred, transfer the Lysis-DTT Buffer to the supernatant 1.5mL tube. Remove from the magnetic stand.

17. Add 100µL of 1X Wash Buffer, vortex and place into the magnetic stand. Once separation has occurred remove and discard the Wash Buffer. Remove from the magnetic stand.

18. Repeat the Wash Buffer step (step 17) two times for a total of three washes. Ensure that all of the solution has been removed after the last wash.

19. In a Biohazard hood, uncap the tubes and place the lids down onto a clean rediwipe. Air-dry the resin in the hood for 15 minutes and then recap tubes and remove from the Biohazard hood. Remove from the magnetic stand.

Note: Do not dry for more than 20 minutes, as this may inhibit the elution of DNA.

20. Add 50µl of Elution Buffer by carefully pipetting the solution to the side of the tube, above the pellet. **Do not mix**.

21. Incubate in the Thermomixer at 65°C for 3 minutes and then continue to incubate for a further 3 minutes shaking at 1100 rpm. If no thermomixer available, incubate samples within a hotblock, vortex mix at the start and end of the incubation. Remove samples.

22. Vortex and immediately place into the magnetic stand while hot to ensure maximum DNA yield during elution.

23. Carefully transfer the DNA extract to the corresponding labelled Nunc™ Bank-It™ tube.

24. Remove from the magnetic stand and repeat the Elution Buffer steps (step 20-23). The final volume after the double elution is approximately 95µL of DNA extract.

25. DNA extracts are stored in temporary storage in freezer 6117-2 (-20°C) located in the workflow area. Tubes containing magnetic beads and supernatants are stored in the allocated boxes in freezer 6117-2 (-20°C) located in the workflow area.

26. 5mL tubes containing the origingal substrate are to be stored in spin basket boxes in freezer 6117-5 located in the workflow area.

17.1.5. Procedure (Retain Supernatant)

1. Turn on the Eppendorf Thermomixer and set the temperature to 37ºC. Alternatively an appropriately cal brated hot block may be used.

2. Remove DTT 1M (small aliquot) and Prot K 20mg/mL (small aliquot) from freezer to thaw.

3. Label for each sample: Original sample tube; 2mL SSI tube (if original sample is not in a 2mL tube); 1.5mL tube (for supernatant) these tubes should not be in contact with the substrate; Spin basket or 2mL tube; Nunc™ Bank-It™ storage tube.

4. Note: Spin baskets are not required for the Negative Extraction control, tape lifts, nails and other non absorbent substrates. For these samples, excluding the Negative Extraction control, label a 2mL tube instead of a spin basket.

5. Have a second operator perform a sequence check of all tubes. This person must enter the sequence check in AUSLAB.

6. Using Table 8, prepare Lysis Buffer & Resin solution. Ensure that the DNA IQ™ Resin solution is thoroughly vortexed prior to use.
   Note: Reagents need to be prepared fresh before each run and Lysis Buffer-DTT solution and Resin solution need to be prepared in the fume hood.

Table 7. Table of reagent volumes for DNA IQ Manual Extraction

Reagent (volume per sample)	Constituent	Volume per sample (µL)	Volume for 12 Samples (mL)
Lysis Buffer – DTT (726µL/sample)	**Lysis Buffer**	660	10.0
	DTT	6.6	0.1
Resin-Lysis Solution (50µL/sample)	**Lysis Buffer with DTT** (from above)	43	0.645
	DNA IQ RESIN	7	0.105
DNA IQ 1X Wash Buffer (300µL/sample)	See Reagent preparation		4.0
DNA IQ Elution Buffer (100µL/sample)	Use directly from Kit		1.4

Note: The volume of Lysis-DTT Buffer calculated includes the volume used in the Resin-Lysis solution preparation.

7. Add 450µL of TNE buffer and vortex.

8. Incubate at room temperature for 30 minutes.

9. Vortex, then centrifuge at maximum speed (14,000rpm) for 3 minutes.

10. Remove 150µL of supernatant and place into the respective 1.5mL tube labelled with “sup” (for further testing).

11. Add 14µL of 20mg/mL Proteinase K and 7µL 40% (w/v) Sarcosyl to the original sample tube containing substrate and TNE buffer, then vortex.Add 300µL of Extraction Buffer. Ensure that large substrates including tape lifts are fully submerged.

12. Vortex, then incubate at 37°C on the Thermomixer at 1000 rpm for 45 minutes. If no thermomixer available, incubate samples within a hotblock, vortex mix at the start and end of the incubation and at least one during the incubation.

13. Remove from the Thermomixer and increase the temperature on the Thermomixer to 65°C (for use in the Elution steps).

14. Transfer the substrate to spin basket. Centrifuge spin basket for 2 minutes at maximum speed. Retain the spin basket in the original 5ml tube and transfer the flow through back into sample tube.
    Note: If original sample tube is not a 2mL tube, transfer flow through from spin basket and the supernatant from the original tube into a 2mL tube.

15. For samples that do not require a spin basket, pulse spin and transfer supernatant to the labelled 2mL tube.

16. Add 550µL of Lysis-DTT Buffer solution.

17. Add 50µL of DNA IQ™ Resin-Lysis solution. Pipette mix the Resin-Lysis solution between each addition to keep the resin suspended in solution.

18. Vortex and place on the Multitube shaker set at 1200 rpm for 5 minutes.

19. Remove from the Multitube shaker, vortex and immediately place into the magnetic stand. Separation will occur instantly.
    Note: If resin does not form a distinct pellet on the side of the tube, or if the magnetic beads have re-suspended while in the stand, vortex the tube and quickly place back in the stand.

20. Carefully transfer the solution (supernatant) into the 1.5mL supernatant tube, ensuring that the resin is not disturbed. Remove from the magnetic stand.
    Note: If some resin is drawn up in the pipette tip, gently expel resin back into tube to allow re-separation.

21. Add 125µL of Lysis-DTT Buffer solution. Vortex and place into the magnetic stand.

22. Once separation has occurred, transfer the Lysis-DTT Buffer to the supernatant 1.5mL tube. Remove from the magnetic stand.

23. Add 100µL of 1X Wash Buffer, vortex and place into the magnetic stand. Once separation has occurred remove and discard the Wash Buffer. Remove from the magnetic stand.

24. Repeat the Wash Buffer step (step 17) two times for a total of three washes. Ensure that all of the solution has been removed after the last wash.

25. In a Biohazard hood, uncap the tubes and place the lids down onto a clean rediwipe. Air-dry the resin in the hood for 15 minutes and then recap tubes and remove from the Biohazard hood. Remove from the magnetic stand.

Note: Do not dry for more than 20 minutes, as this may inhibit the elution of DNA.

26. Add 50µl of Elution Buffer by carefully pipetting the solution to the side of the tube, above the pellet. **Do not mix**.

27. Incubate in the Thermomixer at 65°C for 3 minutes and then continue to incubate for a further 3 minutes shaking at 1100 rpm. If no thermomixer available, incubate samples within a hotblock, vortex mix at the start and end of the incubation. Remove samples.

28. Vortex and immediately place into the magnetic stand while hot to ensure maximum DNA yield during elution.

29. Carefully transfer the DNA extract to the corresponding labelled Nunc™ Bank-It™ tube.

30. Remove from the magnetic stand and repeat the Elution Buffer steps (step 20-23). The final volume after the double elution is approximately 95µL of DNA extract.

31. DNA extracts are stored in temporary storage in freezer 6117-2 (-20°C) located in the workflow area. Tubes containing magnetic beads and supernatants are stored in the allocated boxes in freezer 6117-2 (-20°C) located in the workflow area.

32. 5mL tubes containing the origingal substrate are to be stored in spin basket boxes in freezer 6117-5 located in the workflow area.

17.1.6. Sample storage

Refer to “DNA Analysis Workflow Procedure” (QIS 24919).

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DNA IQ™ Method of Extracting DNA from Casework and Reference Samples

1. PURPOSE AND SCOPE

This method describes the routine method for the extraction of DNA using the DNA IQ™ kit (Promega Corp., Madison, WI, USA). The automated method is the preferred procedure, utilising the MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms (PerkinElmer BioDiscovery, Downers Grove, IL, USA). The manual method has also been included. This method applies to all DNA Analysis staff members that are required to extract DNA from samples.

Reference samples and casework samples must be extracted separately. If casework and reference samples are to be extracted on the same instrument, the instrument (including all required labware) must be decontaminated between operations.

2. DEFINITIONS

DNA IQ™ Resin	Magnetic resin beads used to bind DNA
DTT	1,4 Dithiothreitol
EDTA	Ethylenediaminetetraacetatic acid
EP-A	Extraction Platform A
EP-B	Extraction Platform B
Extracts	Samples that have had a DNA extraction processes performed
Lysates	Samples that have had the off-deck lysis step performed, but have not yet completed the entire extraction process
MPII	MultiPROBE® II PLUS HT EX Platform
Paramagnetic	To become magnetic with the application of a magnetic force
Pro K	Proteinase K
Samples	Sample substrates (in tubes) awaiting DNA extraction
Sarcosyl	N-Lauroylsarcosine sodium
TNE	Tris, NaCl and EDTA buffer (10mM Tris, 100mM NaCl, 1mM EDTA, pH 8.0)

3. PRINCIPLE

Sample Pre-lysis

The Extraction Buffer used in the pre-lysis of cellular material contains Proteinase K (Pro K), TNE buffer (10mM Tris, 100mM NaCl, 1mM EDTA, pH 8.0) and Sarcosyl. TNE acts as a basic buffer with EDTA chelating ions in solution. Sarcosyl is a detergent that lyses open cell membranes. Proteinase K is added to digest cellular material, helping the DNA to be released. It also digests proteins that interfere with the DNA binding capacity of the resin. In addition, Proteinase K rapidly inactivates enzymatic activity that could potentially degrade DNA (e.g. nucleases).

Proteinase K (or Endoproteinase K) is a fungal enzyme from *Engyodontium album* (formerly *Tritirachium album*). It is a stable S8 family serine alkaline protease that has broad specificity and is inactivated by diisopropylfluorophosphate, phenyl-methane sulfonyl fluoride and Hg^{2+} ions. As a highly active endopeptidase, it cleaves peptide bonds adjacent to the carboxyl group of N-substituted (blocked alpha amino) hydrophobic, aliphatic and aromatic amino acids. Therefore, it actively hydrolyses proteins, glycoproteins, peptides and esters of amino acids. The smallest peptide that can be hydrolysed with Proteinase K is a tetrapeptide.

DNA IQ™ Kit

The DNA IQ™ kit (Promega Corp., Madison, WI, USA) is designed to purify DNA from a number of different substrates and has been optimised for trace DNA samples. An in-house validation was performed using a modified version of the PerkinElmer automated protocol.

The in-house protocol includes:

- Off-deck lysis steps with the option to retain a portion of the supernatant for further testing;
- The use of 300µL Extraction Buffer containing TNE (10mM Tris, 100mM NaCl, 1mM EDTA, pH 8.0) and Proteinase K to lyse cellular material prior to performing the DNA IQ process;
- The use of tubes and spin baskets for off-deck lysis of samples prior to extraction on the MPII platform. At the conclusion of off-deck lysis, lysates are transferred to individual Nunc Bank-It™ tubes;
- Nunc Bank-It™ tubes (arranged in sequence using STORstar) containing lysates are presented to the MPII platform for automated transfer of lysates into a 96-deep well plate;
- DNA IQ™ Resin is added using the MPII platform, followed by addition of two volumes of DNA IQ™ Lysis Buffer;
- The 96-deep well plate containing DNA IQ™ Resin and Lysis Buffer is sealed using an adhesive aluminium film and is placed on a MixMate to mix the contents of each well. The plate is centrifuged and the aluminium film is then pierced using a 96 well half skirt PCR microplate and the plate is returned to the MPII platform;
- A double elution step is performed using two dispenses of DNA IQ™ Elution Buffer at 60µL, resulting in a final DNA extract volume of 100µL;
- DNA extracts are automatically transferred into Nunc Bank-It™ tubes for storage.

Cell lysis is performed using DNA IQ™ Lysis Buffer containing Dithiothreitol (DTT). 1,4 Dithiothreitol is a reducing agent used in extractions to break disulfide bonds of proteins. The Lysis Buffer used is a proprietary buffer containing chaotropic salts required for binding of DNA to the magnetic beads. According to the MSDS (Promega Corporation 2006), the Lysis Buffer contains 50-75% guanidinium thiocyanate, < 2.5% EDTA, < 2% 3-[(3-Choalamidopropryl)dimethylammonio] propanesulfonic and < 2% polyethylene glycol tert-octylphenyl ether.

The basis of the DNA IQ™ kit is a magnetic bead resin which contains novel paramagnetic particles covered with silica. The magnetic bead resin usually has a DNA binding capacity of 100ng but the addition of Proteinase K increases the binding capacity. Samples with small amounts of DNA are more efficient than samples with large amounts of DNA, and as a result a small sample size is critical to ensure efficient recovery of DNA.

The magnetic beads with silica have a negative charge at basic and near neutral pH. The Lysis Buffer changes the pH and salt concentration of the solution and the silica on the magnetic beads becomes positively charged, which is then able to bind the DNA.

Several washing steps are employed in the protocol to remove inhibitors. The first washing step is performed using Lysis Buffer. This wash ensures the DNA is bound to the magnetic bead resin and washes out inhibitors. The next three washing procedures incorporate the use of DNA IQ™ Wash Buffer. This buffer contains an alcohol/aqueous mixture which ensures the DNA is not eluted during washing by keeping the DNA dehydrated, and the aqueous phase washes out the inhibitor.

The DNA IQ™ Elution Buffer removes the DNA from the magnetic bead resin. The Elution Buffer changes the salt content. Heating the complex to 65°C allows the DNA to be released from the magnetic beads. The Elution Buffer is a low ionic strength buffer that

reduces the affinity of the DNA for the silica that covers the magnetic beads, by re-hydration of the phosphate backbone.

The DNA IQ™ kit isolates DNA greater than 80bp, smaller DNA is removed selectively to prevent PCR inhibition.

MultiPROBE® II HT EX PLUS with Gripper™ Integration Platform
Within DNA Analysis, routine DNA extractions are performed on casework or reference samples using two MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms (EP-A or EP-B) located in Room 6127.

Each platform uses a computer-controlled Cartesian XYZ liquid handling system designed for the efficient automation of sample preparation. Liquid transfer is performed by an 8-tip system with VersaTip® and VariSpan™ options. The VersaTip® option allows the use of both fixed and disposable tips (conductive and non-conductive). The VariSpan™ option permits variable probe spacing between each of the individual probes so that a wide range of labware such as micro plates, tubes, vials and reagent troughs can be accessed. Each sample probe is capable of independent motion in the Z direction due to independent Z drives.

The 8-tip system is also capable of multichannel liquid-level sensing by utilising Accusense™ technology. This technology works by each probe detecting a change in capacitance within the liquid. This capacitive mode of detection is also possible when using conductive disposable tips. Accusense™ also permits the detection of low or non-ionic polar solutions and solvents. Pipetting on the platforms is driven by 8 individually controlled syringes via the positive displacement of the system liquid (nanopure water) when transferring liquid.

The Gripper™ Integration allows for automated identification of labware via the scanning of barcodes and also allows for automated transfer of plates from one position on the deck to another. To increase deck space and capacity, the platforms include a left deck extension.

For automated DNA extraction using the DNA IQ™ kit, a platemap is utilised to provide the necessary information for correct volumes and locations for pipetting. It also contains information regarding batch and plate identification, sample positions, lab numbers of samples, and lists all the samples in the batch, providing the total number of samples. The program will fail to work without a platemap.

4. REAGENTS AND EQUIPMENT

4.1. Reagents

- DNA IQ™ System Kit (400 sample kit)
  - DNA IQ™ Resin
  - Lysis Buffer (LB)
  - 2x Wash Buffer (2xWB)
  - Elution Buffer (EB)
- TNE (10mM Tris, 100mM NaCl, 1mM EDTA, pH 8.0)
- Proteinase K (Pro K) 20mg/mL
- Dithiothreitol (DTT) 1M
- 5% TriGene
- 70% Ethanol
- 10% Bleach 7x Solution
- 1% Amphyl
- 0.2% Amphyl
- Isopropyl Alcohol
- AnalR 100% Ethanol

- 40% Sarcosyl
- Nanopure Water

These reagents are stored in locations as per Table 1.

Table 1. Reagent storage locations.

Reagent	Device	Storage Location
Pro K	Freezer	Room 6120
DTT	Freezer	Room 6120
40% Sarcosyl	Shelf	Room 6122
Isopropyl Alcohol	Shelf	Room 6122
AnalR 100 %Ethanol	Shelf	Room 6122
TNE pH 8 Buffer	Shelf	Room 6122
DNA IQ™ Kit	Shelf	Room 6122
Amphyl (1% and 0.2%)	Shelf	Room 6127
Nanopure Water	Shelf	Room 6127
5% TriGene	Shelf	Room 6127
10% Bleach 7x Solution	Shelf	Room 6127

Table 2 shows the volume of reagents for a full plate or half plate. All reagents can be made on the bench, with the exception of the Lysis Buffer-DTT which needs to be made in a fume hood. DNA IQ™ reagents are prepared by staff performing the method. Refer to "*Receipt, Storage and Preparation of Chemicals, Reagents and Kits*" (QIS 17165) for preparation of TNE Buffer.

Table 2. Table of reagent volumes.

Reagent	Volume for 96 samples (mL)	Volume for 48 samples (mL)	Volume for 24 samples (mL)
Extraction Buffer			
TNE buffer	33.3	20	10
Proteinase K (20mg/mL)	1.8	1.08	0.54
Sarcosyl (40%)	0.9	0.54	0.27
Lysis-DTT Buffer			
DNA IQ™ Lysis Buffer	90.0	50	N/A
DTT (1M)	0.9	0.5	N/A
DNA IQ™ Resin solution			
Lysis-DTT Buffer	6.0	3	N/A
DNA IQ™ Resin	1.0	0.5	N/A
DNA IQ™ 1x Wash Buffer	35.0	18	N/A
DNA IQ™ Elution Buffer	14.0	8	N/A

Note: Do not directly pipette from reagent stock bottles. Stock reagents should be decanted into working containers where appropriate. Volume for 24 samples is for off-deck lysis samples only.

4.2. Extraction Buffer

Note: Prepare Extraction Buffer just prior to commencing the off-deck lysis or extraction procedure.

1. Determine the required volumes of reagents by using Table 2.
2. Remove the required amount of 20mg/mL Proteinase K from the freezer and thaw. Vortex and centrifuge before use.
3. Ensure that the 40% (w/v) Sarcosyl is completely dissolved and appears clear in the stock solution. If not dissolved, invert the container a few times and leave at room temperature.
4. Retrieve an aliquot of TNE buffer of the appropriate volume size from the falcon tube storage container in Room 6122.
5. Add the appropriate volumes of 20mg/mL Proteinase K and 40% (w/v) Sarcosyl to the falcon tube containing TNE buffer, and invert gently to mix.
6. Label the tube with "Extraction Buffer", your initials and the date.

4.3. Lysis Buffer with DTT Solution

Note: Lysis Buffer is supplied with the DNA IQ™ kit. The Lysis Buffer with DTT solution is prepared just prior to commencing the extraction procedure.

Warning: Lysis Buffer and DTT are toxic, use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling Lysis Buffer.

1. Determine whether a half- or full-plate of reagents are required (Table 2).
2. Remove the required amount of DTT from the freezer and thaw. Vortex and centrifuge before use.
3. In the fume hood add the required volume of Lysis Buffer to a sterilised glass Schott bottle and then add the required volume of DTT.
4. Label the glass Schott bottle with "Lysis Buffer + DTT", your initials and the date.

4.4. DNA IQ™ Resin

Note: DNA IQ™ Resin is supplied with the DNA IQ™ kit. The resin is prepared at the start of each run. Ensure the resin is properly mixed by *vortexing* prior to use.

1. Determine whether a half- or full-plate of reagents are required (Table 2).
2. Into a 10mL (or 5mL) sterile tube, add the required volume of Lysis Buffer with DTT solution (from 4.1.2) followed by the required volume of DNA IQ™ Resin.
3. Mix by gentle inversion.
4. Label the tube with "Resin", your initials and the date.

4.5. 1x Wash Buffer

Note: 2x Wash Buffer is supplied with the DNA IQ™ kit. To prepare 1x Wash Buffer, add 35mL of *AnalR* Ethanol and 35mL of Isopropyl Alcohol to the 2x Wash Buffer bottle. Then label the lid and side of the bottle with "1x Wash Buffer," your initials and the date. Also fill out the Reagent Log (DNA IQ Reagents).

1. Determine whether a half- or full-plate of reagents are required (Table 2).
2. Into a Falcon tube, add the required volume of 1x Wash Buffer.
3. Label the falcon tube with "Wash Buffer", your initials and the date.

4.6. Elution Buffer

Note: Elution Buffer is supplied with the DNA IQ™ kit. The Elution Buffer can be used directly from the kit. The Elution Buffer is removed from the kit and stored in the automated extraction room (6127).

5. EQUIPMENT

The following equipment (Table 3) and consumables (Table 4) are required for the DNA IQ™ extraction process.

Table 3. Equipment used and location.

Equipment	Asset No.	Location
STORstar (B system)	10238493	6122
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (EP-A)	10076438	6127
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (EP-B)	10076437	6127
DPC shaker (EP-A)	N/A	6127
DPC shaker (EP-B)	N/A	6127
Automated Temperature Controller, Heat Block tiles and heat block adapters (EP-A)	N/A	6127
Automated Temperature Controller, Heat Block tiles and heat block adapters (EP-B)	N/A	6127
Eppendorf 5804 centrifuge	10238416	6127
Vortex	30087015	6127
Fridge	30433424	6127
Micro centrifuge	None	6127
MixMate		6127
Decapper	None	6127
4titude 4seal Sealer	30512847	6127

Table 4. Consumables used for extraction.

Consumables	Location
175µL Clear Non-Conductive Filter RoboRack tips – Pre-Sterilised	6127
MβP Pure 1000uL Tips – Pre-Sterilised	6127
SlicPrep™ 96 device plate	6122
ABgene 96-deep well plate	6120
Axygen 2mL deep well storage plate	6127
96 well Half Skirt PCR Microplate	6127
1.5mL or 2mL Eppendorf tubes with spin baskets	6120
12 Channel plate	6127
Nunc Bank-it™ tubes	6120
Nunc Bank-it™ caps	6127
Sterile 50mL Falcon tubes	6122
Sterile 10mL or 5mL tubes	6122
Autoclaved 100mL glass bottles	6122
Autoclaved 250mL glass bottles	6122
Aluminium sealing film	6127
300µL ART tips	6120
1000µL ART tips	6120

6. SAFETY

As per the procedures in the QIS document "*Operational Practices in the DNA Dedicated Laboratories*" (QIS 17120), PPE is to be worn by all staff when performing this procedure. This includes the use of safety glasses.

The automated platforms, labware and associated equipment should be wiped with 5% TriGene™ followed by 70% Ethanol before and after use. In addition, clean the work area around the MPII instrument with 10% (v/v) Bleach 7x and 70% Ethanol.

While the MPII is running a procedure, refrain from placing any part of the body inside the cabinet. Either pause the program or use the emergency STOP button located at the front of the instrument before touching anything on the deck surface. Pressing the emergency STOP button may cause the program to pause or abort.

Warning: Tris base, EDTA, Sarcosyl and Lysis Buffer are irritants. DTT is a reducing agent that destroys disulfide bonds. Handle carefully and wear appropriate PPE.

Lysis Buffer contains guanidinium thiocyanate (GuSCN) which is toxic and can be harmful if inhaled, swallowed or comes in contact with skin. Left over Lysis Buffer-DTT is disposed of

in a brown Winchester bottle in the fume hood. Never dispose down the sink. If spillage occurs, absorb with liquid-binding material (such as sand or kitty litter) and dispose in a biohazard bin. Do not combine bleach with Lysis Buffer and do not wipe areas of Lysis Buffer spillage with bleach, as the two reagents produce cyanide gas when combined. Handle carefully and wear appropriate PPE, including safety glasses, when handling Lysis Buffer. If Lysis Buffer is spilt onto PPE (eg. gloves, gowns), discard the PPE and obtain new PPE.

7. SAMPLING AND SAMPLE PREPARATION

7.1. Sample Locations

Samples waiting to be extracted are stored in freezers as described in Table 5.

Table 5. Sample storage locations.

Sample type	Storage Device	Storage Location
Urgent/High/Medium Priority Samples	Freezer	6117-2
Lysates in 1.0mL Nunc Bank-it™ tubes	Freezer	6117
Extracts in 1.0mL Nunc Bank-It™ tubes	Freezer	6117

Note: Some Medium and Low Priority storage boxes may be located in the Exhibit Room (6106).

7.2. QC Samples

For all off-deck lysis batches (with 48 samples or less) and extraction batches; one negative control and one positive control is required to be registered. For all off-deck lysis batches with > 48 samples; two negative and two positive controls is required to be registered.

Table 6. Extraction Quality Controls

QC Name	Batch Type	Description
Negative Control	Off-Deck Lysis	Negative Extraction control – empty well
Positive Control	Off-Deck Lysis	Positive Extraction control – dried blood swab from a known donor
Negative Control	IQ Extraction	Negative Extraction control – empty well
Positive Control	IQ Extraction	Internal IQ Efficiency Control

7.2.1. Registration of QC Samples

The registration of control samples is covered in the DNA Analysis workflow procedure (QIS 24919)

7.3. Create the DNA IQ™ Lysis or Retain Supernatant batch

Creation of Lysis and retain supernatant batches is covered in the DNA Analysis Workflow Procedure (QIS 24919).

7.4. Locating Samples

To locate samples refer to “Analytical Sample Storage” (QIS 24255).

8. OFF-DECK LYSIS PROCEDURE

8.1. Off-Deck Lysis (No Retained Supernatant)

1. For batches of 48 samples or less, one set of controls and one operator is required. For larger batches, separate the batch into two smaller batches of 48 samples, including one set of controls in each. If a single operator is performing the whole procedure, the second batch can be started during an appropriate incubation step.

   Note: For full batches positions 1-4 will be the two sets of controls, positions 5-50 will be the first 46 samples and 51-96 will be the second set of 46 samples.

2. For each sample label:
   - Original sample tube
   - Spin basket or 1.5mL tube as required
   - 1.0mL Nunc Bank-It™ tube

   Note 1: Samples that require a spin basket are: swabs, fabric, paper and cigarette butts. Samples that require a 1.5mL tube are: tapelifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

   Note 2: If samples are in a 2mL QPS tube and require a spin basket, label a new tube for the substrate to be retained in.

3. If substrate is in a 0.5mL tube, transfer to a labelled 1.5mL tube.

4. Have a second operator sequence check the tubes and enter the sequence check in AUSLAB.

5. Prepare Extraction Buffer as per Section 4.1.1.

6. Add 300µL of Extraction Buffer and vortex briefly. Ensure that substrates are immersed in the Extraction Buffer.

7. Incubate on a Thermomixer at 37°C for 45 minutes at 1000rpm or on the hotblock (if using hotblock vortex samples at 22-23 minutes and again at the end of the incubation). Record temperature on worksheet.

8. Remove from the Thermomixer/hotblock. Transfer substrate to spin basket if required or transfer the lysate to an appropriately labelled 1.5mL tube. Retain original tube containing the substrate in if no spin basket used.

9. Centrifuge spin basket at maximum speed (14,000rpm) for 2 minutes.

10. Retain spin basket containing the substrate and transfer flow through back to original lysis tube.

11. Vortex lysate, then incubate in hotblock/Thermomixer at 65°C for 10 minutes. Record temperature on worksheet.

12. Centrifuge at maximum speed (14,000rpm) for 1 minute.

13. Transfer 300uL of lysate to the corresponding Nunc Bank-It™ tube.

Note: If more than 300uL of lysate is present, retain the remaining lysate in the 1.5mL tube. In AUSLAB, write a specimen note for that sample (eg. “extra lysate retained from sample XXXXXXXXX.”). Store the retained 1.5mL lysate tube in appropriate box in freezer.

14. In AUSLAB, enter reagent and hotblock/Thermomixer temperature details and complete the batch.

15. Transfer substrates from spin baskets to an appropratiely labelled tube (may use original sample tube if no remaining lysate)

16. Store lysates in temporary storage boxes in freezer 6117-2 (-20°C). Store tubes containing substrates in “Spin Basket boxes” in freezer 6117-5 (-20°C).

8.2. Off-Deck Lysis (Retained Supernatant)

1. For batches of 48 samples or less, one set of controls and one operator is required. For larger batches, separate the batch into two smaller batches of 48 samples, including one set of controls in each. If a single operator is performing the whole procedure, the second batch can be started during step 7.

   Note: For full batches positions 1-4 will be the two sets of controls, positions 5-50 will be the first 46 samples and 51-96 will be the second set of 46 samples.

2. For each sample label:
   - Original sample tube
   - Spin basket or 1.5mL tube as required
   - 1.5mL tube (also labelled with "**sup**" to indicate supernatant)
   - 1.0mL Nunc Bank-It™ tube

   Note 1: Samples that require a spin basket are: swabs, fabric, paper and cigarette butts. Samples that require a 1.5mL tube are: tapelifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

   Note 2: If samples are in a 2mL QPS tube and require a spin basket, label a new 5mL tube for the substrate to be retained in.

3. If substrate is in a 0.5mL tube, transfer to a labelled 1.5mL tube.

4. Have a second operator sequence check the tubes and enter the sequence check in AUSLAB.

5. Add 450µL of TNE buffer and vortex.

6. Incubate at room temperature for 30 minutes.

7. Vortex, then centrifuge at maximum speed (14,000rpm) for 3 minutes.

8. Remove 150µL of supernatant and place into the respective 1.5mL tube labelled with "**sup**" (for further testing).

9. Add 14µL of 20mg/mL Proteinase K and 7µL 40% (w/v) Sarcosyl to the original sample tube containing substrate and TNE buffer, then vortex.

10. Incubate in Thermomixer at 37°C for 45 minutes at 1000rpm or on the hotblock (if using hotblock vortex samples at 22-23 minutes and again at the end of the incubation). Record temperature on worksheet.

11. Remove from the Thermomixer/hotblock. Transfer substrate to spin basket if required or transfer the lysate to an appropriately labelled 1.5mL tube. Retain original tube containing the substrate in if no spin basket used.

12. Centrifuge spin basket at maximum speed (14,000rpm) for 2 minutes.

13. Retain spin basket containing the substrate and transfer flow through back to original lysis tube.

14. Vortex Lysate, then incubate in hotblock/Thermomixer at 65°C for 10 minutes. Record temperature on worksheet.

15. Centrifuge at maximum speed (14,000rpm) for 1 minute.

16. Transfer 300uL of lysate to the corresponding Nunc Bank-It™ tube.

Note: If more than 300uL of lysate is present, retain the remaining lysate in the 1.5mL tube. In AUSLAB, write a specimen note for that sample (eg. "extra lysate retained from sample XXXXXXXXX."). Store the retained 1.5mL lysate tube in appropriate box in freezer.

17. In AUSLAB, enter reagent and hotblock/Thermomixer temperature details and complete the batch.

18. Transfer substrates from spin baskets to an appropratiely labelled tube (may use original sample tube if no remaining lysate)

19. Store supernatants in the "S/N Retention" boxes in Freezer 6117-2 (-20°C). Store lysates in temporary storage boxes in freezer 6117-2 (-20°C). Store tubes containing substrates in "Spin Basket boxes" in freezer 6117-5 (-20°C).

9. AUTOMATED EXTRACTION OF LYSED SAMPLES

9.1. Create the DNA IQ Extraction batch

Creation of extraction batch is covered in the DNA Analysis Workflow Procedure (QIS 24919).

9.2. Locating samples

To locate samples refer to "Analytical Sample Storage" (QIS 24255).

9.3. Sequence checking the Nunc Bank-It™ tubes

The procedure for the automated checking of sample tubes is covered in the Procedure for the use of the STORstar unit for automated sequence checking (QIS 24256)

9.4. MPII Extraction Procedure

Automated setup of DNA IQ extractions in 96-deep well format is performed using the MultiPROBE® II PLUS HT EX EP-A and EP-B platforms located in Room 6127.

Refer to "*Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform*" (QIS 23939) for instructions on the use and maintenance of the MultiPROBE® II PLUS HT EX platforms.

9.5. Summary of DNA IQ™ Extraction Version 6.5_ODL (following off-deck lysis)

1. ***Transfer of lysates from Nunc Bank-It™ tubes into the ABgene 96-deep well plate***
   Lysates from the off-deck lysis protocol, contained in individual Nunc Bank-It™ tubes, are transferred automatically into an ABgene 96-deep well plate prior to commencing automated sample processing. Instead of a 96-deep well plate, the use of individual Bank-It™ tubes for storage of lysates removes the need for an adhesive film and therefore reduces the risk of well-to-well contamination from the action of peeling back an adhesive plate cover.

2. ***Automated addition of DNA IQ™ Resin and Lysis Buffer***
   DNA IQ™ Resin is added automatically into the ABgene 96-deep well plate on the platform. The program uses a waste mode dispensing step to add 53µL DNA IQ™ Resin-Lysis Buffer solution to each well in either half the plate or the whole plate. Because of this, batch sizes are restricted to either 48 or 96 samples in order to

maintain efficiency and economy. Two volumes of Lysis Buffer are then added to promote binding of DNA to the paramagnetic resin.

3. ***Mixing using a MixMate to bind DNA to resin***
   Manual intervention is required to seal the ABgene 96-deep well plate with a 4titude Pierce Seal and sealing plate using 4titude sealer pre-heated at 175 °C. The plate is then transferred onto a MixMate instrument for mixing at 1100rpm for 5 minutes, followed by centrifugation on an Eppendorf 5804 centrifuge at 3000rpm for 2 minutes. The seal is then carefully pierced with a 96 well PCR microplate and the ABgene plate is returned to the Applied Biosystems magnet on the MPII platform.

4. ***Removing lysis reagents for storage***
   At this point, most of the DNA is bound to the paramagnetic resin. With the positioning of the ABgene plate on the ABI magnet, DNA IQ™ Resin becomes immobile at the bottom of the plate. The lysis reagents from each well are transferred automatically to a storage plate on the MPII platform without disturbing the DNA IQ™ Resin. The purpose of the storage plate is for retaining supernatant that may potentially still contain DNA material. The storage plate may also become useful in quality investigations.

5. ***Washing of the resin-DNA complex***
   Washing steps are performed to remove any inhibitors in solution. The first wash uses 125µL Lysis Buffer with shaking at room temperature for 1 minute on the DPC shaker to ensure that the DNA is bound to the paramagnetic resin. The plate is moved to the ABI magnet and the supernatant is transferred into the storage plate. The next three washes incorporate 100µL of 1x Wash Buffer with shaking at room temperature for 1 minute on the DPC shaker. During each wash cycle, the ABgene 96-deep well plate is moved to the ABI magnet and the supernatant is discarded into the tip chute.

6. ***Removing any excess of 1x Wash Buffer***
   The samples are allowed to air dry at room temperature for 5 minutes in order to remove all traces of Ethanol from the Wash Buffer. The presence of Ethanol may potentially inhibit both the elution process and also downstream PCR.

7. ***Elution of DNA from the resin-DNA complex***
   A double elution method is employed in this procedure. At each elution step, 60µL of Elution Buffer is added to each sample, followed by incubation at 65°C for 6 minutes (3 minutes without shaking followed by 3 minutes shaking on the DPC shaker). The ABgene plate is moved to the ABI magnet and the eluted solution (supernatant containing eluted DNA) is transferred to fresh Nunc™ Bank-It™ tubes.

8. ***Flushing of capillaries***
   As a decontamination measure, the MPII capillaries and liquid pathway are washed with Amphyl and Nanopure water at the conclusion of the automated extraction process.

9.6. Preparation of reagents for the automated extraction process

Note: Reagents are prepared during the setting up of the MPII platforms (Section 4.3).

9.7. Setting up the MPII platforms for automated DNA IQ™ processing

The following steps are carried out in the automated extraction room (Room 6127).

1. Remove the Nunc Bank-It™ tubes containing lysates from the fridge to allow to come to room temperature before commencing the extraction procedure.

   Note: If the lysates are frozen, remove them from the freezer and thaw in Room 6127. Also remove the required amount of DTT to thaw.

2. Restart or turn on the instrument PC.

3. Log onto the network using the **Robotics** login.

4. Open WinPrep® by double clicking icon on the computer desktop (Figure 1).

Figure 1. The WinPrep® icon.

5. Log onto the WinPrep® software by entering your username and password, then press **"Enter"**.

6. Ensure that the daily/weekly start-up and maintenance has been performed before running any program. If WinPrep® has been closed or been idle for a long period of time initialise the MP II platform as described in QIS 23939.

7. Ensure the **System Liquid reservoir is FULL** and tubing is fully submerged in the system liquid before every run and perform a Flush/Wash. If visible air bubbles have appeared in tubing or in syringes between setting up the deck and executing the test, another flush wash will need to be performed before starting the MPII extraction run.

8. Open the Extraction setup MP II test file in WinPrep® by selecting:
   - **File**
   - **Open**, navigate to **C:\PACKARD\MULTIPROBE\BIN\QHSS PROTOCOLS**
   - Select "**DNA IQ Extraction_Ver 6.5_ODL.mpt**"
   - Click the "**Open**" button

9. Check the tree pane of the whole program for any bold fonts. See the Analytical Senior Scientist if bold fonts are present.

10. Copy the required plate map from the network folder **I:\EXTRACTION** into the local folder **C:\PACKARD\EXT PLATE MAPS.** Open the plate map using Excel and check that the plate map is complete, and make changes if necessary. If there are blanks (samples that have been removed), make all volumes to 0. If it is not a full batch, delete the rows of blank samples at the end of the platemap. Save all changes made to the platemap (as a .txt file).

11. Decontaminate the required labware with 5% TriGene followed by 70% Ethanol and place onto the instrument deck in the designated grid positions (as shown in the WinPrep® software). Additionally, ensure the DPC shaker is positioned properly.

12. Ensure that the DPC shaker and Heater Controller Box are switched on.
    For EP-A: Tile 1 at F22 (85^0C).
    For EP-B: Tile 2 at F22 (85^0C).
    Note: Press the start/stop button twice at the front of the DPC shaker to ensure that it displays zero on the screen.

13. Ensure the heat transfer tile is clicked into the plate adapter tile properly.
    Note: This is critical to ensure correct incubation temperatures.

14. To the Amphyl wash station in position **A10**, add 1% Amphyl to the far left hand side, 0.2% diluted Amphyl to the middle left and place an empty (waste) reagent trough in the middle right position of the station.

15. Refer to section 4.1 for the preparation of reagents. Record all lot numbers onto the worksheet and in AUSLAB. Note, for batches of <48 samples, use volumes for 48 samples.

16. Check the syringes and tubing and perform a Flush/Wash if required.

17. Add Wash Buffer to the far right hand side trough of the Amphyl wash station in position **A10**. Add Lysis Buffer to the labelled 150mL reagent trough on the left hand side of the 2 trough holder in position **A13**.

18. Place the 12 channel plate into position **A16**. Add Elution Buffer to the plate by dividing the Elution Buffer between channels 11 and 12. Add Resin to channel 1. It is important to add the resin in a uniform fashion to ensure equal distribution of resin along the channel.

19. **Nunc Bank-It™ lysate tubes:** The lysates should now be at room temperature. Ensure that the rack is labelled with the correct **AUSLAB batch ID** on the **front** of the Nunc™ Bank-It™ tube rack and that the label matches the batch ID on the worksheet and platemap. Additionally, randomly check some visible barcodes on the side of lysate tubes against the positions on the worksheet to ensure the correct batch of samples is to be processed.

    a. Add a B1-Lite generated **'LYSATE'** barcode on the **right hand side** of the Nunc™ Bank-It™ tube rack.
    b. Place the rack onto the MixMate to shake for 1 minute at 1000rpm.
    c. Centrifuge the rack at 3000rpm for 2 minutes in the Eppendorf 5804 centrifuge and then place into position **C13**.

    Note: Do not uncap lids until prompted by program.

20. **ABgene 96-deep well plate:** Label the **left hand side** of the plate with both the correct **AUSLAB batch ID** and **batch ID barcode**. With a marker, print the word "FRONT-lysate" on the front of the plate. Place the plate in its correct orientation in position **E13**.

21. **2mL 96-deep well storage plate:** Label the **left hand side** of the plate with both the correct **AUSLAB batch ID** and **batch ID barcode**. Label the **right hand side** of the plate with a B1-Lite generated "**STORE**" barcode. With a marker, print the word "FRONT-store" on the front of the plate. Place the plate in its correct orientation in position **E16**.

22. **Nunc Bank-It™ extract tubes:** Ensure that the rack is labelled with the correct **AUSLAB batch ID** on the **front** of the Nunc™ Bank-It™ tube rack. Label the **right hand side** of the plate with a B1-Lite generated "**EXTRACT**" barcode. Additionally, randomly check some visible barcodes on the side of lysate tubes against the positions on the worksheet to ensure the correct batch of samples is to be processed. Place the rack in position **G16**.
    Note 1: Do not uncap lids during this step.
    Note 2: If B1-Lite generated barcodes are not available hand-write the labels.

23. Add Nanopure water to the 160mL trough in the Flush/Wash station in position **G13**.

24. Ensure that all necessary labware have been positioned correctly as displayed within WinPrep®, then click "**EXECUTE TEST**". Record run information in the Run Log book.

25. The following message will appear (Figure 2 below):

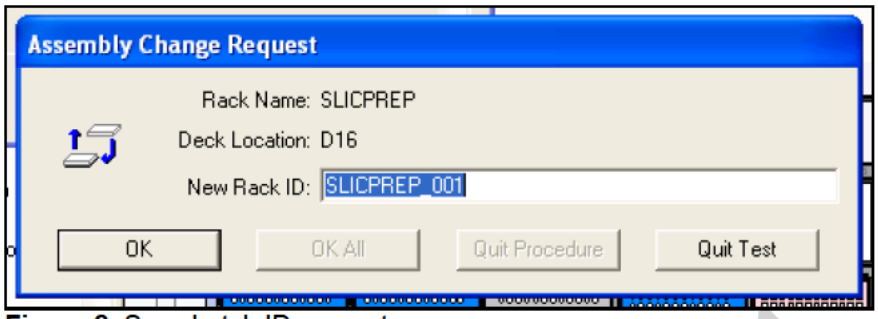

Figure 2. Scan batch ID request

Into "New Rack ID:" scan barcode off the worksheet. It is important this corresponds to the labelling of labware on the deck and the plate maps used.

26. Click "**Reset Tip Boxes**" and ensure that the number of tips displayed in this window match those as available on the deck platform. Fill new tips if necessary. Click "**Close**" to accept the tip count, and then click "**Next**".

27. Select the correct platemap by browsing to **C:\PACKARD\EXT PLATE MAPS**. Ensure that the platemap selected corresponds to the labelling of labware on the deck, the paperwork used and the "New Rack ID" entered above.

28. For a full batch of 96 samples, ensure that all nodes are checked. For a batch of 48 samples or less, uncheck the node: "Add resin to second half of plate". Click "**Next**" to check all other nodes.

29. Click "**Start**" to continue.

30. The MPII instrument will proceed to scan the required plates on the platform deck in the below order. If barcode reading fails or if B1-Lite barcodes are not available (and hand-written labels have been used), the user is prompted to enter a plate ID. A plate ID can be entered manually into the "Read failed" prompt window for:
    a. Nunc extract tubes, type in **EXTRACT** and press **"Enter".**
    b. 96-deep well storage plate, type in **STORE** and press **"Enter".**
    c. Nunc lysate tubes, type in **LYSATE** and press **"Enter".**

31. After the plates have been identified, two user prompts will appear as a reminder to confirm the deck setup. **Always decap tubes from positions H1 to A1, H2 to A2 etc.**
    a. Ensure all steps on the first prompt have been complete, Click **OK** to continue.
    b. Ensure all steps on the second prompt have been complete, Click **OK** to continue.

32. The program will progress to transfer the lysates followed by automated addition of the Lysis-DTT buffer and the DNA IQ™ Resin solution. The next user prompt will appear. Follow the steps as outlined in the user prompt. Then click **OK** when ready.
    Note: Ensure that plate is sealed properly with the Pierce Seal. Once the Pierce Seal film is pierced, the PCR Microplate is then discarded (new plate used each time).

33. Once lysis steps have been completed, a user prompt will appear. Follow the directions as outlined in the user prompt. Then click **OK** when ready.
    Note: Nunc lysate tubes (including the rack) should be placed into a clipseal bag, sealed and discarded into a biohazard waste bin.

34. Once the wash steps have been completed, a user prompt will appear. Follow the directions as outlined in the user prompt. Then click **OK** when ready.
    Note: The Nunc extract tubes can be decapped on the bench in the MPII cabinet while the samples are drying and the heating tile is stabilising. At this point, the operator has approximately **12 minutes** to decap the tubes. If the operator has been interrupted and requires more time to decap the tubes, the program may be paused at this step.

35. A message will appear waiting for the heating tile to reach 85°C (for incubation at 65°C). **DO NOT PRESS CONTINUE** as the program will continue automatically when the temperature has been reached with sufficient stability.

36. A user prompt will appear. Follow the directions as outlined in the user prompt. Then click OK when ready.

37. Once the elution is completed, a user prompt appears. Follow the directions as outlined in the user prompt. Then click OK when ready.

38. Once all plates are removed from the deck and sealed, place into a clipseal plastic bag. Click "**OK**" to proceed to the Amphyl wash step.
    Note: Before placing the supernatant storage plate into a clipseal bag, check the plate for the transfer of beads. To do this, place the plate onto the ABI magnet (if beads are present they will settle to the bottom of the plate) and then inspect the bottom of the plate. If beads are present refer to the Section 15, Troubleshooting.

39. A final message will advise that the run has completed. Click "**OK**".

9.8. Finalising the MP II Run

1. Transfer left over Resin solution from the 12 channel plate and the Lysis Buffer-DTT (wearing safety glasses) into the glass Lysis-DTT bottle previously used. Discard the 12 channel plate in the biohazard waste bin. Take the bottle to room 6122 and transfer left over reagents into the brown Winchester bottle located in the fume hood.
2. Discard the contents of the Amphyl wash station into the sink and rinse the troughs with Nanopure water.
3. Remove all labware from the deck and clean with 5% TriGene™ followed by 70% Ethanol, and setup for the next run if necessary. In addition, clean the work area around the MPII instrument with 10% (v/v) Bleach 7x and 70% Ethanol.
4. Remove the tip chute and funnel, rinse with warm tap water to remove any residue inside the chute before cleaning with 5% TriGene and 70% Ethanol.
5. Move the platemap to **C:\PACKARD\EXT PLATE MAPS**Completed Extractions.

9.9. Importing MP II Log File into AUSLAB

1. Click on the Microsoft Access icon in the WinPrep® main menu to open the MultiPROBE log database.
2. Click on the relevant run Test ID in the Test Selection box. In the Report/Query/Action Selection dropdown menu, select "Report: Test Summary (Sorted by Destination Rack ID)"
3. In the Output Selection dropdown menu, select "File". Save the output file as *.csv format to **C:\PACKARD\EXT PLATE MAPS\EXT LOGS** with the same name as the AUSLAB batch ID and click "**Apply**".
4. Open the log file and check for any errors that may have arisen during the extraction process. Compare the listed errors to any that were encountered during the run. Report any critical errors to the line manager.
5. Copy the log file to **I:\EXTRACTION\EXT A MPII\LOGS or I:\ EXTRACTION\EXT B MPII\LOGS** for uploading to AUSLAB.

6. Import the log file, entering the path, filename and extension (e.g. I:\EXTRACTION\EXTA MPII\Logs\CWIQEXT20071115_01.csv) and press **[Enter]**. For more details on importing files into AUSLAB refer to Batch functionality in AUSLAB SOP (QIS 24469).

9.10. Importing Extraction "results" into AUSLAB

1. Import the results file, entering the filename and extension. For more details on importing files into AUSLAB refer to Batch functionality in AUSLAB SOP (QIS 24469).
2. The file will be imported into AUSLAB and appear in the DNA file table.
3. Highlight entry and press **[Enter]**, for access to the DNA results table.
4. Page down through the table and check that all sample results have been imported.
5. Press **[SF8]** ***Table Sort Order***, this sorts the table, sorting samples that have failed Autovalidation to the top. Samples that have failed are those that have processing comments present.
6. For samples that have failed, check the **Processing Comments,** by entering into the sample.
7. If processing comments state sample is to be sent to another batch type **other** than quant. Proceed with the following steps:
   a. Request the appropriate rework test code via the **[SF7]** results history table and the **[SF8]** request rework functions (e.g. samples requiring Microcon, NucleoSpin and pooling).
   b. Press **[Esc]** to exit back to the DNA results table. Do not toggle accept.
   c. Add the extraction batch ID into the 9PLEX or 9FTAR completed date fields in AUSLAB.
8. If processing comments do not state next step the sample will be processed as normal:
   a. Press **[Esc]** to exit back to the DNA results table.
   b. Highlight any entries to be changed and press **[SF7]** ***Toggle Accept.***
9. Press **[F7]** ***Complete Batch***, all of the samples with a ***Yes*** in the Accept column will be transferred to the outstanding Quant Casework or Quant Reference lists.
10. File the extraction worksheet into the relevant folder in Room 6117.

9.11. Sample Storage

Refer to "Analytical Sample Storage" (QIS 24255) for how to store the DNA extract Nunc™ Bank-It™ tubes, ABgene 96-deep well and Axygen store plates.

10. TROUBLESHOOTING

1. If the resin is not pipette mixing correctly (eg. resin is not being drawn up into the pipette tip), pause the Winprep program and check that the support tile is clicked onto the MPII deck correctly. Alternatively, pipette and mix resin manually one more time from the corresponding column in the 12 channel plate.

2. If the Gripper is not placing the rack/plate properly on the deck, pause the program and manually adjust the rack/plate properly on the adapter support tile.

3. If the Gripper appears to be not gripping a plate correctly, or the pipetting alignment of a labware appears to be incorrect, the deck and / or labware can be re-calibrated. This is covered in the Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform SOP (QIS 23939)

11. VALIDATION

- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 9. Automation: Report on the Evaluation of DNA Extraction Chemistries." June 2007.

- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 11: Report on the Validation of the Automated Extraction Chemistry Kit using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Casework Platform." 2007.
- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 13: Report on the Verification of the Automated Extraction Chemistry Kit using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Casework Platform." 2007.
- Muharam I., Hlinka V., Gallagher, B., Lundie, G., Iannuzzi, C., Nurthen T., McNevin A., Ientile V. "Project 21: A Modified DNA IQ™ Method Consisting of Off-Deck Lysis to Allow Supernatant Retention for Presumptive Identification of α-Amylase" 2008
- Muharam I., Hlinka V., Gallagher, B., Lundie, G., Iannuzzi, C., Nurthen T., McNevin A., Ientile V. "Project 22: A Modified DNA IQ™ Method for Off-Deck Lysis Prior to Performing Automated DNA Extraction" 2008

12. QUALITY ASSURANCE/ACCEPTANCE CRITERIA

- Positive and negative (reagent blank) controls are included in each extraction batch as per Table 6. These controls are processed as normal samples through to completion.
- If any results are obtained from the reagent blank, either at quantification or during the CE QC check, then the possible source of the contamination is investigated. The samples extracted with this control are thoroughly checked and repeated if possible.

13. REFERENCES

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7. Mandrekar, P., V., Flanagan, L., & Tereba, A., Forensic Extraction and Isolation of DNA Form Hair, Tissue and Bone. Profiles in DNA, 2002: p. 11.
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14. STORAGE OF DOCUMENTS

All worksheets are stored in the Analytical area (Room 6117).

15. ASSOCIATED DOCUMENTS

QIS 17120 Operational Practices in the DNA Dedicated Laboratories
QIS 17171 Method for Chelex Extraction
QIS 17165 Receipt, Storage and Preparation of Chemicals, Reagents and Test Kits
QIS 23939 Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform
QIS 24255 Analytical Sample Storage
QIS 24256 Sequence Checking with the STORstar Instrument
QIS 24469 Batch functionality in AUSLAB
QIS 24919 DNA Analysis Workflow Procedure

16. AMENDMENT HISTORY

Version	Date	Author/s	Amendments
R0	23 Oct 2007	B. Gallagher, T. Nurthen, C. Iannuzzi, V. Hlinka, G. Lundie, I Muharam.	First Issue
R1	12 Dec 2007	M Harvey, C Iannuzzi, A McNevin	Reviewed and updated after initial training
R2	19 March 2008	M Harvey, B Andersen, C Iannuzzi, A McNevin	Addition of Off-deck Lysis procedure, Retention of fully automated method as Appendix, addition of reagent record tables into Appendix
R3	April 2008	QIS2 Migration Project	Headers and Footers changed to new CaSS format. Amended Business references from QHSS to FSS, QHPSS to CaSS and QHPS to Pathology Queensland
4	13 March 2009	QIS2 migration	Version incremented by one on migration to QIS2
5	03 June 2009	M Aguilera, B Micic, C Iannuzzi, A. Cheng, V. Hlinka, I. Muharam, G. Lundie, C. Weber	Major changes to reflect new procedure. Updated to reflect changes in procedure as an outcome of internal and external audits. Created ver.6.4 ODL in MPII Platforms. Minor changes in procedures using 4titude 4seal

			heat sealer to seal plates.
6	29 June 2009	A McNevin, K Lancaster	Removed references to retaining lysate and beads, fixed minor formatting errors. Created ver6.5 ODL in MPII Platforms. Substrates now to be retained in 2mL tube

17. APPENDIX

17.1. Manual method for extraction using DNA IQ™

17.1.1. Sampling and Sample Preparation

Refer to section 9 above.

17.1.2. QC samples

All extraction batches require two controls to be registered. The registration of control samples is covered in the DNA Analysis workflow procedure (QIS 24919)

17.1.3. Creating the Extraction Batch and Locating Samples

Refer to "DNA Analysis Workflow Procedure" (QIS 24919).

17.1.4. Procedure (No Retain Supernatant)

1. Turn on the Eppendorf Thermomixer and set the temperature to 37°C. Alternatively an appropriately calibrated hot block may be used.

2. Remove DTT 1M (small aliquot) and Prot K 20mg/mL (small aliquot) from freezer to thaw.

3. Label for each sample:
   - Original sample tube; 2mL SSI tube (if original sample is not in a 2mL tube); Spin basket or 2mL tube; and Nunc™ Bank-It™ storage tube.

   Note: Spin baskets are not required for the Negative Extraction control, tape lifts, nails and other non absorbent substrates. For these samples, excluding the Negative Extraction control, label a 2mL tube instead of a spin basket. Substrates will be retained into original 1.5mL or 2mL after being processed in a spin basket.

4. Have a second operator perform a sequence check of all tubes. This person must enter the sequence check in AUSLAB.

5. Using Table 7, prepare Extraction Buffer, Lysis Buffer & Resin solution. Ensure that the DNA IQ™ Resin solution is thoroughly vortexed prior to use.
   Note: Reagents need to be prepared fresh before each run and Lysis Buffer-DTT solution and Resin solution need to be prepared in the fume hood.

Table 7. Table of reagent volumes for DNA IQ Manual Extraction

Reagent (volume per sample)	Constituent	Volume per sample (µL)	Volume for 12 Samples (mL)	Volume for 24 Samples (mL)
Extraction Buffer (300µL/sample)	**TNE Buffer**	277.5	4.0	8.0
	Prot K (20mg/mL)	15.0	0.216	0.432
	Sarcosyl (40% w/v)	7.5	0.108	0.216
Lysis Buffer – DTT (726µL/sample)	**Lysis Buffer**	660	10.0	20.0
	DTT	6.6	0.1	0.2
Resin-Lysis Solution (50µL/sample)	**Lysis Buffer with DTT** (from above)	43	0.645	1.29
	DNA IQ RESIN	7	0.105	0.210
DNA IQ 1X Wash Buffer (300µL/sample)	See Reagent preparation		4.0	8.0
DNA IQ Elution Buffer (100µL/sample)	Use directly from Kit		1.4	2.8

Note: The volume of Lysis-DTT Buffer calculated includes the volume used in the Resin-Lysis solution preparation.

6. Add 300µL of Extraction Buffer. Ensure that large substrates including tape lifts are fully submerged. Vortex, then incubate at 37°C on the Thermomixer at 1000 rpm for 45 minutes. If no thermomixer available, incubate samples within a hotblock, vortex mix at the start and end of the incubation and at least one during the incubation.

7. Remove from the Thermomixer and increase the temperature on the Thermomixer to 65°C (for use in the Elution steps).

8. Transfer substrate to spin basket if required or transfer the lysate to an appropriately labelled 2.0mL tube. Retain original tube containing the substrate if no spin basket used.

9. Centrifuge spin basket at maximum speed (14,000rpm) for 2 minutes.

10. Retain the spin basket and transfer the flow through back into sample tube. Transfer the substrate into a labelled 2mL tube.
    Note: If original sample tube is not a 2mL tube, transfer flow through from spin basket and the supernatant from the original tube into a 2mL tube.

11. Add 550µL of Lysis-DTT Buffer solution.

12. Add 50µL of DNA IQ™ Resin-Lysis solution. Pipette mix the Resin-Lysis solution between each addition to keep the resin suspended in solution.

13. Vortex and place on the Multitube shaker set at 1200 rpm for 5 minutes.

14. Remove from the Multitube shaker, vortex and immediately place into the magnetic stand. Separation will occur instantly.
    Note: If resin does not form a distinct pellet on the side of the tube, or if the magnetic beads have re-suspended while in the stand, vortex the tube and quickly place back in the stand.

15. Carefully remove and discard the solution, ensuring that the resin is not disturbed. Remove from the magnetic stand.
    Note: If some resin is drawn up in the pipette tip, gently expel resin back into tube to allow re-separation.

16. Add 125µL of Lysis-DTT Buffer solution. Vortex and place into the magnetic stand.

17. Once separation has occurred, remove and discard the Lysis-DTT Buffer. Remove from the magnetic stand.

18. Add 100µL of 1X Wash Buffer, vortex and place into the magnetic stand. Once separation has occurred remove and discard the Wash Buffer. Remove from the magnetic stand.

19. Repeat the Wash Buffer step (step 18) two times for a total of three washes. Ensure that all of the solution has been removed after the last wash. Remove from the magnetic stand.

20. In a Biohazard hood, uncap the tubes and place the lids down onto a clean rediwipe. Air-dry the resin in the hood for 15 minutes and then recap tubes and remove from the Biohazard hood.
    Note: Do not dry for more than 20 minutes, as this may inhibit the elution of DNA.

21. Add 50µl of Elution Buffer by carefully pipetting the solution to the side of the tube, above the pellet. **Do not mix**.

22. Incubate in the Thermomixer at 65°C for 3 minutes and then continue to incubate for a further 3 minutes shaking at 1100 rpm. If no thermomixer available, incubate samples

within a hotblock, vortex mix at the start and end of 2nd 3 minute incubation. Remove samples.

23. Vortex and immediately place into the magnetic stand while hot to ensure maximum DNA yield during elution.

24. Carefully transfer the DNA extract to the corresponding labelled Nunc™ Bank-It™ tube.

25. Remove from the magnetic stand and repeat the Elution Buffer steps (step 21-24). The final volume after the double elution is approximately 95µL of DNA extract.

26. DNA extracts are stored in temporary storage in freezer 6117-2 (-20°C) located in the workflow area. Tubes containing the original substrate are to be stored in spin basket boxes in freezer 6117-5 located in the workflow area.

17.1.5. Procedure (Retain Supernatant)

1. Turn on the Eppendorf Thermomixer and set the temperature to 37°C. Alternatively an appropriately calibrated hot block may be used.

2. Remove DTT 1M (small aliquot) and Prot K 20mg/mL (small aliquot) from freezer to thaw.

3. Label for each sample: Original sample tube; 2mL SSI tube (if original sample is not in a 2mL tube); 1.5mL tube (for supernatant) these tubes should not be in contact with the substrate; Spin basket or 2mL tube; an extra 2mL tube for spin baskets; Nunc™ Bank-It™ storage tube.
   Note: Spin baskets are not required for the Negative Extraction control, tape lifts, nails and other non absorbent substrates. For these samples, excluding the Negative Extraction control, label a 2mL tube instead of a spin basket. Substrates will be retained into original 1.5mL or 2mL after being processed in a spin basket.

4. Have a second operator perform a sequence check of all tubes. This person must enter the sequence check in AUSLAB.

5. Using Table 8, prepare Lysis Buffer & Resin solution. Ensure that the DNA IQ™ Resin solution is thoroughly vortexed prior to use.
   Note: Reagents need to be prepared fresh before each run and Lysis Buffer-DTT solution and Resin solution need to be prepared in the fume hood.

Table 8. Table of reagent volumes for DNA IQ Manual Extraction

Reagent (volume per sample)	Constituent	Volume per sample (µL)	Volume for 12 Samples (mL)	Volume for 24 Samples (mL)
Lysis Buffer – DTT (726µL/sample)	**Lysis Buffer**	660	10.0	20.0
	DTT	6.6	0.1	0.2
Resin-Lysis Solution (50µL/sample)	**Lysis Buffer with DTT** (from above)	43	0.645	1.29
	DNA IQ RESIN	7	0.105	0.210
DNA IQ 1X Wash Buffer (300µL/sample)	See Reagent preparation		4.0	8.0
DNA IQ Elution Buffer (100µL/sample)	Use directly from Kit		1.4	2.8

Note: The volume of Lysis-DTT Buffer calculated includes the volume used in the Resin-Lysis solution preparation.

6. Add 450µL of TNE buffer and vortex.

7. Incubate at room temperature for 30 minutes.

8. Vortex, then centrifuge at maximum speed (14,000rpm) for 3 minutes.

9. Remove 150µL of supernatant and place into the respective 1.5mL tube labelled with "sup" (for further testing).

10. Add 14µL of 20mg/mL Proteinase K and 7µL 40% (w/v) Sarcosyl to the original sample tube containing substrate and TNE buffer, then vortex.

11. Vortex, then incubate at 37°C on the Thermomixer at 1000 rpm for 45 minutes. If no thermomixer available, incubate samples within a hotblock, vortex mix at the start and end of the incubation and at least one during the incubation.

12. Remove from the Thermomixer and increase the temperature on the Thermomixer to 65°C (for use in the Elution steps).

13. Transfer substrate to spin basket if required or transfer the lysate to an appropriately labelled 2.0mL tube. Retain original tube containing the substrate in if no spin basket used.

14. Centrifuge spin basket at maximum speed (14,000rpm) for 2 minutes.

15. Retain the spin basket and transfer the flow through back into sample tube.
Note: If original sample tube is not a 2mL tube, transfer flow through from spin basket and the supernatant from the original tube into a 2mL tube. Transfer the substrate into a labelled 2mL tube.

16. Add 550µL of Lysis-DTT Buffer solution.

17. Add 50µL of DNA IQ™ Resin-Lysis solution. Pipette mix the Resin-Lysis solution between each addition to keep the resin suspended in solution.

18. Vortex and place on the Multitube shaker set at 1200 rpm for 5 minutes.

19. Remove from the Multitube shaker, vortex and immediately place into the magnetic stand. Separation will occur instantly.
Note: If resin does not form a distinct pellet on the side of the tube, or if the magnetic beads have re-suspended while in the stand, vortex the tube and quickly place back in the stand.

20. Carefully remove and discard the solution, ensuring that the resin is not disturbed. Remove from the magnetic stand.
Note: If some resin is drawn up in the pipette tip, gently expel resin back into tube to allow re-separation.

21. Add 125µL of Lysis-DTT Buffer solution. Vortex and place into the magnetic stand.

22. Once separation has occurred, remove and discard the Lysis-DTT Buffer. Remove from the magnetic stand.

23. Add 100µL of 1X Wash Buffer, vortex and place into the magnetic stand. Once separation has occurred remove and discard the Wash Buffer. Remove from the magnetic stand.

24. Repeat the Wash Buffer step (step 23) two times for a total of three washes. Ensure that all of the solution has been removed after the last wash. Remove from the magnetic stand.

25. In a Biohazard hood, uncap the tubes and place the lids down onto a clean rediwipe. Air-dry the resin in the hood for 15 minutes and then recap tubes and remove from the Biohazard hood.
Note: Do not dry for more than 20 minutes, as this may inhibit the elution of DNA.

26. Add 50µl of Elution Buffer by carefully pipetting the solution to the side of the tube, above the pellet. **Do not mix**.

27. Incubate in the Thermomixer at 65°C for 3 minutes and then continue to incubate for a further 3 minutes shaking at 1100 rpm. If no thermomixer available, incubate samples within a hotblock, vortex mix at the start and end of 2nd 3 minute incubation. Remove samples.

28. Vortex and immediately place into the magnetic stand while hot to ensure maximum DNA yield during elution.

29. Carefully transfer the DNA extract to the corresponding labelled Nunc™ Bank-It™ tube.

30. Remove from the magnetic stand and repeat the Elution Buffer steps (step 26-29). The final volume after the double elution is approximately 95µL of DNA extract.

31. DNA extracts & retained supernatants ("sup" tubes) are stored in temporary storage in freezer 6117-2 (-20°C) located in the workflow area. Tubes containing the original substrate are to be stored in spin basket boxes in freezer 6117-5 located in the workflow area.

17.1.6. Sample storage

Refer to "DNA Analysis Workflow Procedure" (QIS 24919).

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DNA IQ™ Method of Extracting DNA from Casework and Reference Samples

1 PURPOSE AND SCOPE

This method describes the routine method for the extraction of DNA using the DNA IQ™ kit (Promega Corp., Madison, WI, USA). The automated method is the preferred procedure, utilising the MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms (PerkinElmer BioDiscovery, Downers Grove, IL, USA). The manual method has also been included. This method applies to all DNA Analysis staff members that are required to extract DNA from samples.

Reference samples and casework samples must be extracted separately. If casework and reference samples are to be extracted on the same instrument, the instrument (including all required labware) must be decontaminated between operations.

2 DEFINITIONS

DNA IQ™ Resin	Magnetic resin beads used to bind DNA
DTT	1,4 Dithiothreitol
EDTA	Ethylenediaminetetraacetatic acid
EP-A	Extraction Platform A
EP-B	Extraction Platform B
Extracts	Samples that have had a DNA extraction processes performed
Lysates	Samples that have had the off-deck lysis step performed, but have not yet completed the entire extraction process
MPII	MultiPROBE® II PLUS HT EX Platform
Paramagnetic	To become magnetic with the application of a magnetic force
Pro K	Proteinase K
Samples	Sample substrates (in tubes) awaiting DNA extraction
Sarcosyl	N-Lauroylsarcosine sodium
TNE	Tris, NaCl and EDTA buffer (10Mm Tris, 100Mm NaCl, 1Mm EDTA, Ph 8.0)

3 PRINCIPLE

3.1 OFF-DECK LYSIS

The Extraction Buffer used in the pre-lysis of cellular material contains Proteinase K (Pro K), TNE buffer (10Mm Tris, 100Mm NaCl, 1Mm EDTA, Ph 8.0) and Sarcosyl. TNE acts as a basic buffer with EDTA chelating ions in solution. Sarcosyl is a detergent that lyses open cell membranes. Proteinase K is added to digest cellular material, helping the DNA to be released. It also digests proteins that interfere with the DNA binding capacity of the resin. In addition, Proteinase K rapidly inactivates enzymatic activity that could potentially degrade DNA (e.g. nucleases).

Proteinase K (or Endoproteinase K) is a fungal enzyme from Engyodontium album (formerly Tritirachium album). It is a stable S8 family serine alkaline protease that has broad specificity and is inactivated by diisopropylfluorophosphate, phenyl-methane sulfonyl fluoride and Hg2+ ions. As a highly active endopeptidase, it cleaves peptide bonds adjacent to the carboxyl group of N-substituted (blocked alpha amino) hydrophobic, aliphatic and aromatic amino acids. Therefore, it actively hydrolyses proteins, glycoproteins, peptides and esters of amino acids. The smallest peptide that can be hydrolysed with Proteinase K is a tetrapeptide.

3.2 MANUAL DNA IQ™ KIT

The DNA IQ™ kit (Promega Corp., Madison, WI, USA) is designed to purify DNA from a number of different substrates and has been optimised for trace DNA samples. An in-house validation was performed using a modified version of the PerkinElmer automated protocol.

The in-house protocol includes:

- Off-deck lysis steps with the option to retain a portion of the supernatant for further testing;
- The use of 300µL Extraction Buffer containing TNE (10Mm Tris, 100Mm NaCl, 1Mm EDTA, Ph 8.0) and Proteinase K to lyse cellular material prior to performing the DNA IQ process;
- The use of tubes and spin baskets for off-deck lysis of samples prior to extraction on the MPII platform. At the conclusion of off-deck lysis, lysates are transferred to individual Nunc Bank-It™ tubes;
- Nunc Bank-It™ tubes (arranged in sequence using STORstar) containing lysates are presented to the MPII platform for automated transfer of lysates into a 96-deep well plate;
- DNA IQ™ Resin is added using the MPII platform, followed by addition of two volumes of DNA IQ™ Lysis Buffer;
- The 96-deep well plate containing DNA IQ™ Resin and Lysis Buffer is sealed using a heat sealed piercing film and is placed on a MixMate to mix the contents of each well. The plate is centrifuged and the film is then pierced using a 96 well half skirt PCR microplate and the plate is returned to the MPII platform;
- A double elution step is performed using two dispenses of DNA IQ™ Elution Buffer at 60µL, resulting in a final DNA extract volume of 100µL;
- DNA extracts are automatically transferred into Nunc Bank-It™ tubes for storage.

Cell lysis is performed using DNA IQ™ Lysis Buffer containing Dithiothreitol (DTT). 1,4 Dithiothreitol is a reducing agent used in extractions to break disulfide bonds of proteins. The Lysis Buffer used is a proprietary buffer containing chaotropic salts required for binding of DNA to the magnetic beads. According to the MSDS (Promega Corporation 2006), the Lysis Buffer contains 50-75% guanidinium thiocyanate, < 2.5% EDTA, < 2% 3-[(3-Choalamidopropryl)dimethylammonio] propanesulfonic and < 2% polyethylene glycol tert-octylphenyl ether.

The basis of the DNA IQ™ kit is a magnetic bead resin which contains novel paramagnetic particles covered with silica. The magnetic bead resin usually has a DNA binding capacity of 100ng but the addition of Proteinase K increases the binding capacity. Samples with small amounts of DNA are more efficient than samples with large amounts of DNA, and as a result a small sample size is critical to ensure efficient recovery of DNA.

The magnetic beads with silica have a negative charge at basic and near neutral Ph. The Lysis Buffer changes the Ph and salt concentration of the solution and the silica on the magnetic beads becomes positively charged, which is then able to bind the DNA.

Several washing steps are employed in the protocol to remove inhibitors. The first washing step is performed using Lysis Buffer. This wash ensures the DNA is bound to the magnetic bead resin and washes out inhibitors. The next three washing procedures incorporate the use of DNA IQ™ Wash Buffer. This buffer contains an alcohol/aqueous mixture which ensures the DNA is not eluted during washing by keeping the DNA dehydrated, and the aqueous phase washes out the inhibitor.

The DNA IQ™ Elution Buffer removes the DNA from the magnetic bead resin. The Elution Buffer changes the salt content. Heating the complex to 65ºC allows the DNA to be released from the magnetic beads. The Elution Buffer is a low ionic strength buffer that reduces the affinity of the DNA for the silica that covers the magnetic beads, by re-hydration of the phosphate backbone.

The DNA IQ™ kit isolates DNA greater than 80bp, smaller DNA is removed selectively to prevent PCR inhibition.

3.3 MULTIPROBE® II HT EX PLUS WITH GRIPPER™ INTEGRATION PLATFORM

Within DNA Analysis, routine DNA extractions are performed on casework or reference samples using two MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms (EP-A or EP-B) located in Room 3191.

Each platform uses a computer-controlled Cartesian XYZ liquid handling system designed for the efficient automation of sample preparation. Liquid transfer is performed by an 8-tip system with VersaTip® and VariSpan™ options. The VersaTip® option allows the use of both fixed and disposable tips (conductive and non-conductive). The VariSpan™ option permits variable probe spacing between each of the individual probes so that a wide range of labware such as micro plates, tubes, vials and reagent troughs can be accessed. Each sample probe is capable of independent motion in the Z direction due to independent Z drives.

The 8-tip system is also capable of multichannel liquid-level sensing by utilising Accusense™ technology. This technology works by each probe detecting a change in capacitance within the liquid. This capacitive mode of detection is also possible when using conductive disposable tips. Accusense™ also permits the detection of low or non-ionic polar solutions and solvents. Pipetting on the platforms is driven by 8 individually controlled syringes via the positive displacement of the system liquid (nanopure water) when transferring liquid.

The Gripper™ Integration allows for automated identification of labware via the scanning of barcodes and also allows for automated transfer of plates from one position on the deck to another. To increase deck space and capacity, the platforms include a left deck extension.

For automated DNA extraction using the DNA IQ™ kit, a platemap is utilised to provide the necessary information for correct volumes and locations for pipetting. It also contains information regarding batch and plate identification, sample positions, lab numbers of samples, and lists all the samples in the batch, providing the total number of samples. The program will fail to work without a platemap.

4 REAGENTS AND EQUIPMENT

4.1 REAGENTS

- DNA IQ™ System Kit (400 sample kit)
  - DNA IQ™ Resin
  - Lysis Buffer (LB)
  - 2x Wash Buffer (2Xwb)
  - Elution Buffer (EB)
- TNE (10Mm Tris, 100Mm NaCl, 1Mm EDTA, Ph 8.0)
- Proteinase K (Pro K) 20mg/Ml
- Dithiothreitol (DTT) 1M
- 5% TriGene
- 70% Ethanol
- 10% Bleach 7x Solution
- 1% Amphyl
- 0.2% Amphyl
- Isopropyl Alcohol
- AnalR 100% Ethanol
- 40% Sarcosyl
- Nanopure Water

These reagents are stored in locations as per Table 1.

Table 1. Reagent storage locations.

Reagent	Device	Storage Location
Pro K	Freezer	Room 3188
DTT	Freezer	Room 3188
40% Sarcosyl	Shelf	Room 3188
Isopropyl Alcohol	Shelf	Room 3188
AnalR 100 %Ethanol	Shelf	Room 3188
TNE Ph 8 Buffer	Shelf	Room 3188
DNA IQ™ Kit	Shelf	Room 3188
Amphyl (1% and 0.2%)	Shelf	Room 3191
Nanopure Water	Shelf	Room 3188
5% TriGene	Shelf	Room 3191
10% Bleach 7x Solution	Shelf	Room 3191

4.1.1 Extraction Buffer

Note: Prepare Extraction Buffer just prior to commencing the off-deck lysis and manual DNA IQ procedures

1. Determine the required volumes of reagents by using the appropriate appendix.
2. Remove the required amount of 20mg/mL Proteinase K from the freezer and thaw. Vortex and centrifuge before use.
3. Ensure that the 40% (w/v) Sarcosyl is completely dissolved and appears clear in the stock solution. If not dissolved, invert the container a few times and leave at room temperature.
4. Aliquot out the appropriate amount of TNE buffer required. It is best to not remove the buffer directly from the stock solution.
5. Add the appropriate volumes of 20mg/mL Proteinase K and 40% (w/v) Sarcosyl to the falcon tube containing TNE buffer, and invert gently to mix.
6. Label the tube with **"Extraction Buffer"**, your initials and the date.

4.1.2 Lysis Buffer with DTT Solution

Note: Lysis Buffer is supplied with the DNA IQ™ kit. The Lysis Buffer with DTT solution is prepared just prior to commencing the extraction procedure.

Warning: Lysis Buffer and DTT are toxic, use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling Lysis Buffer.

1. Determine the required volumes of reagents by using the appropriate appendix.
2. Remove the required amount of DTT from the freezer and thaw. Vortex and centrifuge before use.
3. In the biohazard cabinet, add the required volume of Lysis Buffer to a sterilised glass Schott bottle or 50mL falcon tube and then add the required volume of DTT.
4. Label the glass Schott bottle with **"Lysis Buffer + DTT"**, your initials and the date.

4.1.3 DNA IQ™ Resin

Note: DNA IQ™ Resin is supplied with the DNA IQ™ kit. The resin is prepared at the start of each run. Ensure the resin is properly mixed by *vortexing* prior to use.

1. Determine the required volumes of reagents by using the appropriate appendix.
2. Into a 10mL (or 5mL or 2mL) sterile tube, add the required volume of Lysis Buffer with DTT solution (from 4.1.2) followed by the required volume of DNA IQ™ Resin.
3. Mix by gentle inversion.
4. Label the tube with **"Resin"**, your initials and the date.

4.1.4 1x Wash Buffer

Note: 2x Wash Buffer is supplied with the DNA IQ™ kit. To prepare 1x Wash Buffer, add 35mL of *AnalR* Ethanol and 35mL of Isopropyl Alcohol to the 2x Wash Buffer bottle. The freshly made 1 x wash buffer reagent must be entered into Auslab:

- Log into Auslab
- 2 – Sample Processing
- 8 – Materials Processing
- 2 – Consumable Inventory
- Find the Wash Buffer in the list and enter
- Esc
- Highlight the lot number that is applicable
- Shift F8
- Add audit entry to state that the additional reagents were added.

1. Determine the required volumes of reagents by using the appropriate appendix.
2. Into a 50mL (or 10mL) tube, add the required volume of 1x Wash Buffer.
3. Label the tube with **"Wash Buffer"**, your initials and the date.

4.1.5 Elution Buffer

Note: Elution Buffer is supplied with the DNA IQ™ kit. The Elution Buffer can be used directly from the kit, which is stored in the clean room (3188), however it must be aliquoted into a new tube rather than using directly from the stock solution.

4.2 EQUIPMENT

4.2.1 Equipment and consumables required for Off Deck Lysis processes

Table 2. Equipment used and their locations

Equipment	Asset No.	Location
Vortex x 4	30435255 30435256 002123941 806021325	3189
Fridge	30433424	3189
Centrifuge x 4	30433323 30433324 10233209 30433322	3189
Hot Block x 4	30435115 30435113 30435114 30435112	3189
Mini Centrifuges x 4	30434993 30087075 30087057 041129	3189
Finnpipettes 100 µL – 1000 µL	N/A	3189

Table 3. Consumables used and their locations.

Consumables	Location
Racks	3189/3184
Spin baskets	3189/3184
1.5mL or 2mL tubes	3189/3184
Nunc Bank-it™ tubes	3189/3184
Nunc Bank-it™ caps	3189/3184
Sterile 50mL Falcon tubes for reagents	3189/3184
Sharps Bin	3189/3184
300µL ART tips	3189/3184
1000µL ART tips	3189/3184
Twirling Sticks	3189/3184

4.2.2 Equipment and consumables required for Manual DNA IQ™

Table 4. Equipment used and their locations

Equipment	Asset No.	Location
Vortex x 4	30435255 30435256 002123941 806021325	3189
Fridge	30433424	3189
Centrifuge x 4	30433323 30433324 10233209 30433322	3189
Hot Block x 4	30435115 30435113 30435114 30435112	3189
Mini Centrifuges x 4	30434993 30087075 30087057 041129	3189
Finnpipettes 100 µL – 1000 µL	N/A	3189

Table 5. Consumables used and their locations

Consumables	Location
Racks	3189
Spin baskets	3189
Nunc Bank-it™ tubes	3189
1.5mL or 2mL tubes	3189
Nunc Bank-it™ caps	3189
Sterile 50mL Falcon tubes for reagents	3188
10mL Sterile tubes	3188
5mL Sterile tubes	3188
Sharps Bin	3189
300µL ART tips	3189
1000µL ART tips	3189
Twirling Sticks	3189
Magnetic Stands	3189

Further consumables can be found in the Store Room (3184)

4.2.3 Equipment and consumables required for Automated DNA IQ™

Table 6. Equipment used and their locations.

Equipment	Asset No.	Location
STORstar (B system)	10238493	3190
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (EP-A)	10076438	3191
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (EP-B)	10076437	3191
DPC shaker (EP-A)	N/A	3191
DPC shaker (EP-B)	N/A	3191
Automated Temperature Controller, Heat Block tiles and heat block adapters (EP-A)	N/A	3191
Automated Temperature Controller, Heat Block tiles and heat block adapters (EP-B)	N/A	3191
Eppendorf 5804 centrifuge	10238416	3191
Vortex	30087015	3191
Fridge	30433424	3191
MixMate	30512822	3191
Decapper	None	3191
4titude 4seal Sealer	30512847	3191

Table 7. Consumables used and their locations

Consumables	Location
175µL Clear Non-Conductive Filter RoboRack tips – Pre-Sterilised	3191
MβP Pure 1000µL Tips – Pre-Sterilised	3191
Abgene 96-deep well plate	3191
Axygen 2mL deep well storage plate	3191
96 well Half Skirt PCR Microplate	3191
4titude Piercing Film	3191
12 Channel plate	3191
Nunc Bank-it™ tubes	3191
Nunc Bank-it™ caps	3191
Sterile 50mL Falcon tubes	3188
Sterile 10mL or 5mL tubes	3188
Autoclaved 100mL glass bottles	3191
Autoclaved 250mL glass bottles	3191
Aluminium sealing film	3191
300uL ART tips	3188
1000µL ART tips	3191

Further consumables can be found in the Store Room (3184)

5 SAFETY

As per the procedures in the QIS document "*Anti-Contamination procedure*" (QIS 22857), PPE is to be worn by all staff when performing this procedure. This includes the use of safety glasses.

The automated platforms, labware and associated equipment should be wiped with 5% TriGene™ followed by 70% Ethanol before and after use. In addition, clean the work area around the MPII instrument with 10% (v/v) Bleach 7x and 70% Ethanol.

While the MPII is running a procedure, refrain from placing any part of the body inside the cabinet. Either pause the program or use the emergency STOP button located at the front of the instrument before touching anything on the deck surface. Pressing the emergency STOP button may cause the program to pause or abort.

Warning: Tris base, EDTA, Sarcosyl and Lysis Buffer are irritants. DTT is a reducing agent that destroys disulfide bonds. Handle carefully and wear appropriate PPE.

Lysis Buffer contains guanidinium thiocyanate (GuSCN) which is toxic and can be harmful if inhaled, swallowed or comes in contact with skin. Left over Lysis Buffer-DTT is disposed of in a brown Winchester bottle in the ventilated cupboard in Room 3191. Never dispose down the sink. If spillage occurs, absorb with liquid-binding material (such as sand or kitty litter) and dispose in a biohazard bin. Do not combine bleach with Lysis Buffer and do not wipe areas of Lysis Buffer spillage with bleach, as the two reagents produce cyanide gas when combined. Handle carefully and wear appropriate PPE, including safety glasses, when handling Lysis Buffer. If Lysis Buffer is spilt onto PPE (eg. Gloves, gowns), discard the PPE and obtain new PPE.

6 SAMPLING AND SAMPLE PREPARATION

6.1 SAMPLE LOCATIONS

Samples waiting to be extracted are stored in freezers as described in Table 8.

Table 8. Sample storage locations.

Sample type	Storage Device	Storage Location
Urgent/High/Medium Priority Samples	Freezer	3190 or 6106*
Lysates in 1.0mL Nunc Bank-it™ tubes	Freezer	3190
Extracts in 1.0mL Nunc Bank-It™ tubes	Freezer	3194 A

* **Note:** Some Medium and Low Priority storage boxes are located in Block 6, within the Exhibit Room (6106).

6.2 QC SAMPLES

For all off-deck lysis batches one negative control and one positive control are required to be registered. In addition, 5 blanks are also to be registered. For manual DNA IQ and retain supernatant batches, one negative and one positive control are required to be registered.

Table 9. Extraction Quality Controls

QC Name	Batch Type	Description
Negative Control	Off-Deck Lysis,	Negative Extraction control
	Manual DNA IQ	
	Retain Supernatant	
Positive Control	Off-Deck Lysis	Positive Extraction control – dried blood swab from a known donor
	Manual DNA IQ	
	Retain Supernatant	
Blank Control x 5	Off-Deck Lysis	Negative Extraction control x 5

6.3 REGISTRATION OF QC SAMPLES

The registration of control samples is covered in QIS24919 DNA Analysis workflow procedure.

6.3.1 Create the DNA IQ™ Lysis, Manual DNA IQ™, Retain Supernatant batch or Automated Extraction

Creation of Lysis and retain supernatant batches is covered in QIS 24919 DNA Analysis Workflow Procedure.

6.3.2 Locating Samples

To locate samples refer to QIS 23959 Storage Guidelines for DNA Analysis.

6.4 ELECTRONIC WORKFLOW DIARY

An electronic workflow diary (I:\AAA Electronic Workflow Diary) is used for the recording of batches that are to be created and for scientists to delegate the work amongst themselves. Once batches are listed and created within the electronic workflow diary (by the operational officers), scientists are required to type in their initials next to the batch that they are to complete. This can also be used to record which scientist has nominated themselves for a particular task on each day and assists the analytical senior scientist with the recording of key performance indicators.

7 OFF-DECK LYSIS PROCEDURE

7.1 OFF-DECK LYSIS (NO RETAIN SUPERNATANT)

1. All reagents are to be made fresh within the clean room (3188) prior to commencing each process. For reagent volumes, please refer to the appropriate Appendix.

2. All manual extractions are to be performed within a biohazard cabinet in Room 3189

3. For each sample label:
   - Original sample tube
   - Spin basket as required
   - 1.0mL Nunc Bank-It™ tube
   - 1.5mL or 2.0mL tube

Note:
Samples that require a spin basket are: swabs, fabric, paper and cigarette butts.
Samples that do not require a spin basket are: tapelifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

4. If substrate is in a 0.5mL tube, transfer to a labelled 1.5mL or 2.0mL tube.

5. Have a second operator sequence check the tubes and enter the sequence check in AUSLAB.

6. Add 300µL of Extraction Buffer and vortex briefly. Ensure that substrates are immersed in the Extraction Buffer.

7. Incubate for 45 minutes at 37 degrees on a hotblock. It is best practice to vortex the samples intermittently throughout the duration of the incubation period. Record temperature on worksheet.

8. Remove from the hotblock. Transfer substrate to spin basket if required or transfer the lysate to an appropriately labelled 1.5mL or 2mL tube. Retain original tube containing the substrate if no spin basket is used.

9. Centrifuge spin baskets at 15800 g for 2 minutes.

10. Transfer the substrate in the spin basket to a new and appropriately labelled 1.5mL or 2mL tube and retain.Transfer the flow through back to original lysis tube.

11. Vortex lysate, then incubate on hotblock at 65°C for 10 minutes. Record temperature on worksheet.

12. Centrifuge at 15800 g for 1 minute.

13. Transfer 300µL of lysate to the corresponding Nunc Bank-It™ tube.

Note: If more than 300µL of lysate is present, retain the remaining lysate in the 1.5mL tube. In AUSLAB, write a specimen note for that sample (eg. extra lysate retained from sample XXXXXXXXX.). Store the retained 1.5mL lysate tube in appropriate box in freezer.

14. In AUSLAB, enter reagent and hotblock temperature details and complete the batch.

15. Store lysates in temporary storage boxes located within the extraction sorting room in the orford Freezer with equipment number 102384430 (Room 3190).

16. Store tubes containing substrates or extra lysate in Spin Basket boxes located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

7.2 OFF-DECK LYSIS (RETAIN SUPERNATANT)

1. All reagents are to be made fresh within the clean room (3188) prior to commencing each process. For reagent volumes, please refer to the appropriate Appendix.

2. All manual extractions are to be performed within a biohazard cabinet in Room 3189

3. For each sample label:
   - Original sample tube
   - Spin basket as required
   - 1.5mL tube (also labelled with "**sup**" to indicate supernatant)

- 1.0mL Nunc Bank-It™ tube
- 1.5mL or 2.0mL tube

Note: Samples that require a spin basket are: swabs, fabric, paper and cigarette butts. Samples that do not require spin baskets are: tapelifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

4. If substrate is in a 0.5mL tube, transfer to a labelled 1.5mL or 2.0mL tube.
5. Have a second operator sequence check the tubes and enter the sequence check in AUSLAB.
6. Add 450µL of TNE buffer and vortex.
7. Incubate at room temperature for 30 minutes.
8. Vortex, then centrifuge at 15800 g for 3 minutes.
9. Remove 150µL of supernatant and place into the respective 1.5mL tube labelled with "**sup**" (for further testing).
10. Add 14µL of 20mg/mL Proteinase K and 7µL 40% (w/v) Sarcosyl to the original sample tube containing substrate and TNE buffer, then vortex.
11. Incubate for 45 minutes at 37 degrees on the hotblock. If the batch is ≤24 samples, then the thermomixer can be used (incubate 45min at 37 degrees and 1000rpm). It is best practice to vortex the samples intermittently throughout the duration of the incubation period. Record temperature on worksheet.
12. Remove from the hotblock/thermomixer. Transfer substrate to spin basket if required or transfer the lysate to an appropriately labelled 1.5mL tube. Retain original tube containing the substrate in if no spin basket used.
13. Centrifuge spin basket at 15800 g for 2 minutes.
14. Transfer the substrate in the spin basket to a new and appropriately labelled 2mL tube to be retained. Transfer the flow through back to original lysis tube.
15. Vortex Lysate, then incubate on hotblock at 65°C for 10 minutes. Record temperature on worksheet.
16. Centrifuge at 15800 g for 1 minute.
17. Transfer 300 µL of lysate to the corresponding Nunc Bank-It™ tube.

    Note: If more than 300µL of lysate is present, retain the remaining lysate in the 1.5mL tube. In AUSLAB, write a specimen note for that sample (eg. extra lysate retained from sample XXXXXXXXX.). Store the retained 1.5mL lysate tube in appropriate box in freezer.
18. In AUSLAB, enter reagent and hotblock/Thermomixer temperature details and complete the batch.
19. Store the DNA extracts in temporary storage located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

20. Store tubes containing substrates in Spin Basket boxes located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

21. Store supernatants in the S/N Retention boxes located within the Extraction sorting room in the Orford Freezer 30512883 (Room 3190).

22. Ensure that the negative extraction control from the batch is also inserted onto the **'Saliva List'**:

    - Log into Auslab
    - 3 for Patient Enquiry
    - Type/Scan the lab number for the negative extraction control
    - From the 9Plex Page press Shift F12
    - A prompt will appear 'Enter List Name'
    - Type Saliva or use the F1 lookup list.
    - Press Enter

8 AUTOMATED EXTRACTION OF LYSED SAMPLES

8.1 BATCH CREATION

Creation of extraction batch is covered in QIS 24919 DNA Analysis Workflow Procedure.

8.2 SAMPLE LOCATION

To locate samples refer to QIS 23959 Storage Guidelines for DNA Analysis.

8.3 SEQUENCE CHECKING THE NUNC BANK-IT™ TUBES

The procedure for the automated checking of sample tubes is covered in QIS 24256 Procedure for the use of the STORstar unit for automated sequence checking.

8.4 MPII EXTRACTION PROCEDURE

Automated setup of DNA IQ extractions in 96-deep well format is performed using the MultiPROBE® II PLUS HT EX EP-A and EP-B platforms located in Room 3191.

Refer to QIS 23939 *Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform* for instructions on the use and maintenance of the MultiPROBE® II PLUS HT EX platforms.

8.5 PREPARATION OF REAGENTS FOR THE AUTOMATED EXTRACTION PROCESS

All reagents are to be made fresh within the clean room (3188) prior to commencing each process. For reagent volumes, please refer to the appropriate Appendix.

8.6 SUMMARY OF DNA IQ™ EXTRACTION VERSION 6.6_ODL (FOLLOWING OFF-DECK LYSIS PROCESS)

8.6.1 Transfer of lysates from Nunc Bank-It™ tubes into the Abgene 96-deep well plate

Lysates from the off-deck lysis protocol, contained in individual Nunc Bank-It™ tubes, are transferred automatically into an Abgene 96-deep well plate prior to commencing automated sample processing. Instead of a 96-deep well plate, the use of individual Nunc Bank-It™ tubes for storage of lysates removes the need for an adhesive film and therefore reduces the risk of well-to-well contamination from the action of peeling back an adhesive plate cover.

8.6.2 Automated addition of DNA IQ™ Resin and Lysis Buffer

DNA IQ™ Resin is added automatically into the Abgene 96-deep well plate on the platform. The program uses a waste mode dispensing step to add 53µL DNA IQ™ Resin-Lysis Buffer solution to each well in either half the plate or the whole plate. Because of this, batch sizes are restricted to either 48 or 96 samples in order to maintain efficiency and economy. Two volumes of Lysis Buffer are then added to promote binding of DNA to the paramagnetic resin.

8.6.3 Mixing using a MixMate to bind DNA to resin

Manual intervention is required to seal the Abgene 96-deep well plate with a 4titude Pierce Seal film using the 4titude sealer pre-heated at 175 °C. The plate is then transferred onto a MixMate instrument for mixing at 1100rpm for 5 minutes, followed by centrifugation on an Eppendorf 5804 centrifuge at 3000rpm for 2 minutes. The seal is then carefully pierced with a 96 well PCR microplate and the Abgene plate is returned to the Applied Biosystems magnet on the MPII platform.

8.6.4 Removing lysis reagents for storage

At this point, most of the DNA is bound to the paramagnetic resin. With the positioning of the Abgene plate on the ABI magnet, DNA IQ™ Resin becomes immobile at the bottom of the plate. The lysis reagents from each well are transferred automatically to a storage plate on the MPII platform without disturbing the DNA IQ™ Resin.

8.6.5 Washing of the resin-DNA complex

Washing steps are performed to remove any inhibitors in solution. The first wash uses 125µL Lysis Buffer with shaking at room temperature for 1 minute on the DPC shaker to ensure that the DNA is bound to the paramagnetic resin. The plate is moved to the ABI magnet and the supernatant is transferred into the storage plate. The next three washes incorporate 100µL of 1x Wash Buffer with shaking at room temperature for 1 minute on the DPC shaker. During each wash cycle, the Abgene 96-deep well plate is moved to the ABI magnet and the supernatant is discarded into the tip chute.

8.6.6 Removing any excess of 1x Wash Buffer

The samples are allowed to air dry at room temperature for 5 minutes in order to remove all traces of Ethanol from the Wash Buffer. The presence of Ethanol may potentially inhibit both the elution process and also downstream PCR.

8.6.7 Elution of DNA from the resin-DNA complex

A double elution method is employed in this procedure. At each elution step, 60µL of Elution Buffer is added to each sample, followed by incubation at 65°C for 6 minutes (3 minutes without shaking followed by 3 minutes shaking on the DPC shaker). The Abgene plate is moved to the ABI magnet and the eluted solution (supernatant containing eluted DNA) is transferred to fresh Nunc™ Bank-It™ tubes.

8.6.8 Flushing of liquid pathway

As a decontamination measure, the liquid pathway is washed with Amphyl and Nanopure water at the conclusion of the automated extraction process.

8.7 SETTING UP THE MPII PLATFORMS FOR AUTOMATED DNA IQ™ PROCESSING

1. All reagents are to be made fresh within the clean room (3188) prior to commencing each process. For reagent volumes, please refer to the appropriate Appendix.

The following steps are carried out in the automated extraction room (Room 3191).

2. Remove the Nunc Bank-It™ tubes containing lysates from the fridge to allow to come to room temperature before commencing the extraction procedure.

   Note: If the lysates are frozen, remove them from the freezer and thaw in Room 3191.

3. Restart or turn on the instrument PC.

4. Log onto the network using the **Robotics** login.

5. Open WinPrep® by double clicking icon on the computer desktop (Figure 1).

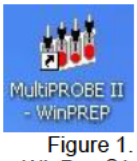

Figure 1.
The WinPrep® icon.

6. Log onto the WinPrep® software by entering your username and password, then press **"Enter"**.

7. Ensure that the daily/weekly start-up and maintenance has been performed before running any program. If WinPrep® has been closed or been idle for a long period of time initialise the MP II platform as described in QIS 23939.

8. Ensure the **System Liquid reservoir is FULL** and tubing is fully submerged in the system liquid before every run and perform a Flush/Wash. If visible air bubbles have appeared in tubing or in syringes between setting up the deck and executing the test, another flush wash will need to be performed before starting the MPII extraction run.

9. Open the Extraction setup MP II test file in WinPrep® by selecting:

   - **File**
   - **Open**, navigate to **C:\PACKARD\MULTIPROBE\BIN\QHSS PROTOCOLS**
   - Select "**DNA IQ Extraction_Ver 6.6_ODL.mpt**"
   - Click the "**Open**" button

10. Check the tree pane of the whole program for any bold fonts. See the Analytical Senior Scientist if bold fonts are present.

11. Copy the required plate map from the network folder **I:\EXTRACTION** into the local folder **C:\PACKARD\EXT PLATE MAPS.** Open the plate map using Excel and check that the plate map is complete, and make changes if necessary. If there are blanks (samples that have been removed), make all volumes to 0. If it is not a full batch, delete the rows of blank samples at the end of the platemap. Save all changes made to the platemap (as a .txt file).

12. Decontaminate the required labware with 5% TriGene followed by 70% Ethanol and place onto the instrument deck in the designated grid positions (as shown in the WinPrep® software). Additionally, ensure the DPC shaker is positioned properly.

13. Ensure that the DPC shaker and Heater Controller Box are switched on.
    For EP-A: Tile 1 at F22 (85^0C).
    For EP-B: Tile 2 at F22 (85^0C).
    Note: Press the start/stop button twice at the front of the DPC shaker to ensure that it displays zero on the screen.

14. Ensure the heat transfer tile is clicked into the plate adapter tile properly.
    Note: This is critical to ensure correct incubation temperatures.

15. To the Amphyl wash station in position **A10**, add 1% Amphyl to the far left hand side, 0.2% diluted Amphyl to the middle left and place an empty (waste) reagent trough in the middle right position of the station.

16. Add Wash Buffer to the far right hand side trough of the Amphyl wash station in position **A10**.

17. Add Lysis Buffer to the labelled 150mL reagent trough on the left hand side of the 2 trough holder in position **A13**.

18. Place the 12 channel plate into position **A16**. Add Elution Buffer to the plate by dividing the Elution Buffer between channels 11 and 12.

19. Add Resin to channel 1 of the 12 channel plate in position **A16**. It is important to add the resin in a uniform fashion to ensure equal distribution of resin along the channel.

20. **Nunc Bank-It™ lysate tubes:** The lysates should now be at room temperature. Ensure that the rack is labelled with the correct **AUSLAB batch ID** and that the label matches the batch ID on the worksheet and platemap. Additionally, randomly check some visible barcodes on the side of lysate tubes against the positions on the worksheet to ensure the correct batch of samples is to be processed.

21. Place the rack onto the MixMate to shake for 1 minute at 1000rpm.

22. Centrifuge the rack at 3000rpm for 2 minutes in the Eppendorf 5804 centrifuge and then place into position **C13**.

22. **Abgene 96-deep well plate:** With the plate in its correctt orientation label the **left hand side** of the plate with both the correct **AUSLAB batch ID** and **batch ID barcode**. With a marker, print the word "**FRONT-lysate**" on the front of the plate. Place the plate in its correct orientation in position **E13**.

23. **2mL 96-deep well storage plate:** With the plate in its correct orientation label the **left hand side** of the plate with both the correct **AUSLAB batch ID** and **batch ID barcode**. With a marker, print the word **"FRONT-store"** on the front of the plate. Place the plate in its correct orientation in position **E16**.

24. **Nunc Bank-It™ extract tubes:** Ensure that the rack is labelled with the correct **AUSLAB batch ID** on the **front** of the Nunc Bank-It™ tube rack. Additionally, randomly check some visible barcodes on the side of lysate tubes against the positions on the worksheet to ensure the correct batch of samples is to be processed. Place the rack in its correct orientation in position **G16**.

25. Add Nanopure water to the 160mL trough in the Flush/Wash station in position **G13**.

26. Ensure that all necessary labware have been positioned correctly as displayed within WinPrep®, then click "**EXECUTE TEST**". Record run information in the Run Log book.

27. The following message will appear (Figure 2 below):

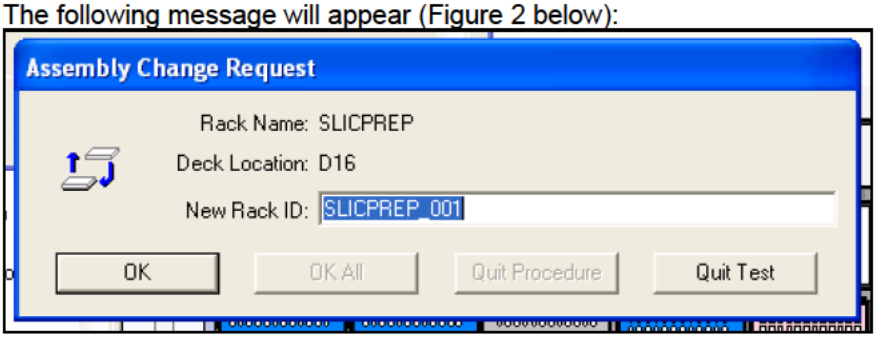

Figure 2. Scan batch ID request

Into **"New Rack ID:"** scan barcode off the worksheet. It is important this corresponds to the labelling of labware on the deck and the plate maps used.

28. Click "**Reset Tip Boxes**" and ensure that the number of tips displayed in this window match those as available on the deck platform. Fill new tips if necessary. Click "**Close**" to accept the tip count, and then click "**Next**".

29. Select the correct platemap by browsing to **C:\PACKARD\EXT PLATE MAPS**. Ensure that the platemap selected corresponds to the labelling of labware on the deck, the paperwork used and the **"New Rack ID"** entered.

30. For a full batch of 76 samples, ensure that all nodes are checked. Click "**Next**" to check all other node

31. For a batch of 48 samples or less, uncheck the node: **"Add resin to second half of plate"**. Click "**Next**" to check all other nodes.

32. Click "**Start**" to continue.

    - The MPII instrument will begin and user prompts will appear as a reminder to confirm the deck setup. **Always decap tubes from positions H1 to A1, H2 to A2 etc.**
    - Ensure all steps on the first prompt have been complete, Click **OK** to continue.
    - Ensure all steps on the second prompt have been complete, Click **OK** to continue.

33. The program will progress to transfer the lysates followed by automated addition of the Lysis-DTT buffer and the DNA IQ™ Resin solution. The next user prompt will appear. Follow the steps as outlined in the user prompt. Then click **OK** when ready.

    Note: Ensure that the plate is heated and sealed properly with the Pierce Seal on the 4titude Heat Sealer. Mix the plate on the mixmate for 5min at 1100rpm and centrifuge. Pierce the plate with a PCR Microplate and then discard. (A new plate is to be used each time). Ensure the pierced plate is returned to the magnet.

34. Once lysis steps have been completed, a user prompt will appear. Follow the directions as outlined in the user prompt. Then click **OK** when ready.

    Note 1: The Lysate Nunc tubes (including the rack) should be placed into a clipseal bag, sealed and discarded into a biohazard waste bin. The Store plate can also be removed, heat sealed and discarded into a biohazard waste bin.

    Note 2: When the supernatant storage plate is sealed, check the plate for the transfer of beads. To do this, place the plate onto the ABI magnet (if beads are present they will settle to the bottom of the plate) and then inspect the bottom of the plate. If no beads are present, discard the store plate. If, however, beads are present, refer to the Section 15, Troubleshooting.

35. Once the wash steps have been completed, a user prompt will appear. Follow the directions as outlined in the user prompt. Then click **OK** when ready.

36. **Note:** The Nunc extract tubes can be decapped on the bench in the MPII cabinet while the samples are drying and the heating tile is stabilising. At this point, the operator has approximately **12 minutes** to decap the tubes. If the operator has been interrupted and requires more time to decap the tubes, the program may be paused at this step.

37. A message will appear waiting for the heating tile to reach 85°C (for incubation at 65°C). **DO NOT PRESS CONTINUE** as the program will continue automatically when the temperature has been reached with sufficient stability.

38. A user prompt will appear. Follow the directions as outlined in the user prompt. Then click **OK** when ready.

39. Once the elution is completed, a user prompt appears. Follow the directions as outlined in the user prompt. Then click **OK** when ready.

40. The Lysate Plate is heat sealed and kept in temporary storage for one month.

41. Once all plates are removed from the deck and sealed. Click "**OK**" to proceed to the Amphyl wash step.

42. A final message will advise that the run has completed. Click "**OK**".

8.8 FINALISING THE MPII RUN

1. Transfer left over Resin solution from the 12 channel plate and the Lysis Buffer-DTT (wearing safety glasses) into the glass Lysis-DTT bottle previously used. Discard the 12 channel plate in the biohazard waste bin. Transfer the contents of glass bottle into the brown Winchester bottle located in the safety cupboard in room 3191.

2. Discard the contents of the Amphyl wash station into the sink and rinse the troughs with Nanopure water.

3. Remove all labware from the deck and clean with 5% TriGene™ followed by 70% Ethanol, and setup for the next run if necessary. In addition, clean the work area around the MPII instrument with 10% (v/v) Bleach 7x and 70% Ethanol.

4. Remove the tip chute and funnel, rinse with warm tap water to remove any residue inside the chute before cleaning with 5% TriGene and 70% Ethanol.

5. Import the platemap into Auslab and then delete it.

8.9 IMPORTING MPII LOG FILE INTO AUSLAB

1. Click on the Microsoft Access icon in the WinPrep® main menu to open the MultiPROBE log database.

2. Click **"Open"** when prompted.

3. Click on the relevant run Test ID in the Test Selection box. In the Report/Query/Action Selection dropdown menu, select **"Report: Test Summary (Sorted by Destination Rack ID)"**
4. In the Output Selection dropdown menu, select **"File"**. Save the output file as *.csv format to **C:\PACKARD\EXT PLATE MAPS\EXT LOGS** with the same name as the AUSLAB batch ID and click "**Apply**".

5. Open the log file and check for any errors that may have arisen during the extraction process. Compare the listed errors to any that were encountered during the run. Report any critical errors to the line manager.

6. Import the log file, entering the path, filename and extension (e.g. C:\Packard/ext plate maps/ext logs….) and press **[Enter]**. Delete the log file after importing.

7. For more details on importing files into AUSLAB refer to Batch functionality in AUSLAB SOP (QIS 24469).

8.10 IMPORTING EXTRACTION "RESULTS" INTO AUSLAB

1. Import the results file, entering the filename and .txt extension (e.g. CWIQEXT20100917_06.txt). For more details on importing files into AUSLAB refer to Batch functionality in AUSLAB SOP (QIS 24469).

2. The file will be imported into AUSLAB and appear in the Results Import Table.

3. Highlight entry and press **[Enter]**, for access to the Results Table.

4. Page down through the table and check that all sample results have been imported.

5. Press **[SF8]** ***Table Sort Order***, this sorts the table, sorting samples that have failed Autovalidation to the top. Samples that have failed are those that have processing comments present.

6. For samples that have failed, check the **Processing Comments,** by entering into the sample.

7. If processing comments state sample is to be sent to another batch type **other** than quant. Proceed with the following steps:
   a. Request the appropriate rework test code via the **[SF7]** results history table and the **[SF8]** request rework functions (e.g. samples requiring Microcon, NucleoSpin and pooling).
   b. Press **[Esc]** to exit back to the DNA results table. Do not toggle accept.
   c. Add the extraction batch ID into the 9PLEX or 9FTAR completed date fields in AUSLAB.

8. If processing comments do not state next step the sample will be processed as normal:
   a. Press **[Esc]** to exit back to the DNA results table.
   b. Highlight any entries to be changed and press **[SF7]** ***Toggle Accept.***

9. Press **[F7]** ***Complete Batch***, all of the samples with a ***Yes*** in the Accept column will be transferred to the outstanding Quant Casework or Quant Reference lists.

10. File the extraction worksheet into the relevant intray for scanning in room 3194A (Pre PCR-Sorting).

8.11 SAMPLE STORAGE

Refer to QIS 23959 Storage Guidelines for DNA Analysis for how to store the DNA extract Nunc™ Bank-It™ tubes and Abgene 96-deep well.

8.12 TROUBLESHOOTING WITH THE MPII

1. If the resin is not pipette mixing correctly (eg. Resin is not being drawn up into the pipette tip), pause the Winprep program and check that the support tile is clicked onto the MPII deck correctly. Alternatively, pipette and mix resin manually one more time from the corresponding column in the 12 channel plate.

2. If the Gripper is not placing the rack/plate properly on the deck, pause the program and manually adjust the rack/plate properly on the adapter support tile.

3. If the Gripper appears to be not gripping a plate correctly, or the pipetting alignment of a labware appears to be incorrect, the deck and / or labware can be re-calibrated. This is

covered in QIS 23939 Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform

9 MANUAL METHOD FOR EXTRACTION USING DNA IQ™

9.1 SAMPLING AND SAMPLE PREPARATION

Refer to section 8.0 above.

9.2 QC SAMPLES

All extraction batches require two controls to be registered. The registration of control samples is covered in QIS 24919 DNA Analysis Workflow Procedure.

9.3 CREATING THE EXTRACTION BATCH AND LOCATING SAMPLES

Refer to QIS 24919 DNA Analysis Workflow Procedure.

9.4 PROCEDURE FOR MANUAL DNA IQ™ (NO RETAIN SUPERNTANT)

1. All manual extractions are to be performed within a biohazard cabinet in Room 3189

2. All reagents are to be made fresh within the clean room (3188) prior to commencing each process. For reagent volumes, please refer to the appropriate Appendix.

Note: Lysis Buffer-DTT solution and Resin solution need to be prepared in the biohazard cabinet.

3. Turn on the Eppendorf Thermomixer and set the temperature to 37˚C.

Label for each sample:

- Original sample tube
- Spin basket if required
- 2mL tube and
- Nunc™ Bank-It™ storage tube.

Note: Samples that require a spin basket are: swabs, fabric, paper and cigarette butts. Samples that do not require a spin basket are: tapelifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

4. Have a second operator perform a sequence check of all tubes. This person must enter the sequence check in AUSLAB.

5. Add 300µL of Extraction Buffer. Ensure that large substrates including tape lifts are fully submerged.

6. Vortex, then incubate at 37°C on the Thermomixer at 1400 rpm for 45 minutes. Tapelifts MUST go onto a thermomixer.

7. Remove from the Thermomixer and increase the temperature on the Thermomixer to 65°C (for use in the Elution steps).

8. Transfer substrate to spin basket if required or transfer the lysate to an appropriately labelled 2.0mL tube. Retain original tube containing the substrate if no spin basket used.

9. Centrifuge spin basket at 15800 g for 2 minutes.

10. Transfer the substrate in the spin basket into a new and labelled 2mL tube to be retained.Transfer the flow through (lysate) back into the original sample tube.

    Note: If original sample tube is not a 2mL tube, transfer the lysate from spin basket and the supernatant from the original tube into a 2mL tube.

11. Add 550µL of Lysis-DTT Buffer solution.

12. Add 50µL of DNA IQ™ Resin-Lysis solution. Pipette mix the Resin-Lysis solution between each addition to keep the resin suspended in solution.

13. Vortex and place on the Multitube shaker set at 1200 rpm for 5 minutes.

14. Remove from the Multitube shaker, vortex and immediately place into the magnetic stand. Separation will occur instantly.

    Note: If resin does not form a distinct pellet on the side of the tube, or if the magnetic beads have re-suspended while in the stand, vortex the tube and quickly place back in the stand.

15. Carefully remove and discard the solution, ensuring that the resin is not disturbed. Remove from the magnetic stand.

    Note: If some resin is drawn up in the pipette tip, gently expel resin back into tube to allow re-separation.

16. Add 125µL of Lysis-DTT Buffer solution. Vortex and place into the magnetic stand.

17. Once separation has occurred, remove and discard the Lysis-DTT Buffer. Remove from the magnetic stand.

18. Add 100µL of 1X Wash Buffer, vortex and place into the magnetic stand. Once separation has occurred remove and discard the Wash Buffer. Remove from the magnetic stand.

19. Repeat the Wash Buffer step (step 17) two times for a total of three washes. Ensure that all of the solution has been removed after the last wash. Remove from the magnetic stand.

20. Uncap the tubes and place the lids down onto a clean rediwipe. Air-dry the resin in the hood for 15 minutes and then recap tubes.

    Note: Do not dry for more than 20 minutes, as this may inhibit the elution of DNA.

21. Add 50µl of Elution Buffer by carefully pipetting the solution to the side of the tube, above the pellet. **Do not mix**.

22. Incubate in the Thermomixer at 65°C for 3 minutes and then continue to incubate for a further 3 minutes shaking at 1100 rpm. If no thermomixer available, incubate samples within a hot block, vortex mix at the start and end of 2nd 3 minute incubation. Remove samples.

23. Vortex and immediately place into the magnetic stand while hot to ensure maximum DNA yield during elution.

24. Carefully transfer the DNA extract to the corresponding labelled Nunc™ Bank-It™ tube.

25. Remove from the magnetic stand and repeat the Elution Buffer steps (step 19-22). The final volume after the double elution is approximately 95µL of DNA extract.

26. Store the DNA extracts in temporary storage located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

27. Store tubes containing substrates in Spin Basket boxes located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

9.5 PROCEDURE FOR MANUAL DNA IQ™ (RETAIN SUPERNTANT)

1. All manual extractions are to be performed within a biohazard cabinet in Room 3189

2. All reagents are to be made fresh within the clean room (3188) prior to commencing each process. For reagent volumes, please refer to the appropriate Appendix.

3. Turn on the Eppendorf Thermomixer and set the temperature to 37ºC. Alternatively an appropriately calibrated hot block may be used.

4. Remove DTT 1M (small aliquot) and Prot K 20mg/mL (small aliquot) from freezer to thaw.

5. Label for each sample:

   - Original sample tube
   - 1.5mL tube (for supernatant) these tubes should not be in contact with the substrate
   - Spin basket if required
   - 2mL tube
   - Nunc™ Bank-It™ storage tube.

Note: Samples that require a spin basket are: swabs, fabric, paper and cigarette butts. Samples that do not require a spin basket are: tapelifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

6. Have a second operator perform a sequence check of all tubes. This person must enter the sequence check in AUSLAB.

7. Add 450µL of TNE buffer and vortex.

8. Incubate at room temperature for 30 minutes.

9. Vortex, then centrifuge at 15800 g for 3 minutes.

10. Remove 150µL of supernatant and place into the respective 1.5mL tube labelled with **"sup"** (for further testing).

11. Add 14µL of 20mg/mL Proteinase K and 7µL 40% (w/v) Sarcosyl to the original sample tube containing substrate and TNE buffer, then vortex.

12. Vortex, then incubate at 37°C on the Thermomixer at 1400 rpm for 45 minutes. If no thermomixer available, incubate samples within a hot block, vortex mix at the start and end of the incubation and at least one during the incubation.

13. Remove from the Thermomixer and increase the temperature on the Thermomixer to 65°C (for use in the Elution steps).

14. Transfer substrate to spin basket if required or transfer the lysate to an appropriately labelled 2.0mL tube. Retain original tube containing the substrate in if no spin basket used.

15. Centrifuge spin basket at 15800 g for 2 minutes.

16. Transfer the substrate in the spin basket into a new and labelled 2mL tube to be retained. Transfer the flow through (lysate) back into the original sample tube.

17. Add 550µL of Lysis-DTT Buffer solution.

18. Add 50µL of DNA IQ™ Resin-Lysis solution. Pipette mix the Resin-Lysis solution between each addition to keep the resin suspended in solution.

19. Vortex and place on the Multitube shaker set at 1200 rpm for 5 minutes.

20. Remove from the Multitube shaker, vortex and immediately place into the magnetic stand. Separation will occur instantly.

    Note: If resin does not form a distinct pellet on the side of the tube, or if the magnetic beads have re-suspended while in the stand, vortex the tube and quickly place back in the stand.

21. Carefully remove and discard the solution, ensuring that the resin is not disturbed. Remove from the magnetic stand.

    Note: If some resin is drawn up in the pipette tip, gently expel resin back into tube to allow re-separation.

22. Add 125µL of Lysis-DTT Buffer solution. Vortex and place into the magnetic stand.

23. Once separation has occurred, remove and discard the Lysis-DTT Buffer. Remove from the magnetic stand.

24. Add 100µL of 1X Wash Buffer, vortex and place into the magnetic stand. Once separation has occurred remove and discard the Wash Buffer. Remove from the magnetic stand.

25. Repeat the Wash Buffer step (step 23) two times for a total of three washes. Ensure that all of the solution has been removed after the last wash. Remove from the magnetic stand.

26. Uncap the tubes and place the lids down onto a clean rediwipe. Air-dry the resin in the hood for 15 minutes and then recap tubes

    Note: Do not dry for more than 20 minutes, as this may inhibit the elution of DNA.

27. Add 50µl of Elution Buffer by carefully pipetting the solution to the side of the tube, above the pellet. **Do not mix**.

28. Incubate in the Thermomixer at 65°C for 3 minutes and then continue to incubate for a further 3 minutes shaking at 1100 rpm. If no thermomixer available, incubate samples within a hot block, vortex mix at the start and end of 2nd 3 minute incubation. Remove samples.

29. Vortex and immediately place into the magnetic stand while hot to ensure maximum DNA yield during elution.

30. Carefully transfer the DNA extract to the corresponding labelled Nunc™ Bank-It™ tube.

31. Remove from the magnetic stand and repeat the Elution Buffer steps (step 26-29). The final volume after the double elution is approximately 95µL of DNA extract.

31. Store the DNA extracts in temporary storage located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

32. Store tubes containing substrates in Spin Basket boxes located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

33. Store supernatants in the S/N Retention boxes located within the Extraction sorting room in the Orford Freezer 30512883 (Room 3190).

34. Ensure that the negative extraction control from the batch is also inserted onto the **'Saliva List':**

- Log into Auslab
- 3 for Patient Enquiry
- Type/Scan the lab number for the negative extraction control
- From the 9Plex Page press Shift F12
- A prompt will appear 'Enter List Name'
- Type Saliva or use the F1 lookup list.
- Press Enter

9.6 SAMPLE STORAGE

Refer to QIS 24919 DNA Analysis Workflow Procedure

10 VALIDATION

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- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 11: Report on the Validation of the Automated Extraction Chemistry Kit using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Casework Platform." 2007.
- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 13: Report on the Verification of the Automated Extraction Chemistry Kit using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Casework Platform." 2007.
- Muharam I., Hlinka V., Gallagher, B., Lundie, G., Iannuzzi, C., Nurthen T., McNevin A., Ientile V. "Project 21: A Modified DNA IQ™ Method Consisting of Off-Deck Lysis to Allow Supernatant Retention for Presumptive Identification of α-Amylase" 2008
- Muharam I., Hlinka V., Gallagher, B., Lundie, G., Iannuzzi, C., Nurthen T., McNevin A., Ientile V. "Project 22: A Modified DNA IQ™ Method for Off-Deck Lysis Prior to Performing Automated DNA Extraction" 2008

11 QUALITY ASSURANCE/ACCEPTANCE CRITERIA

- Positive and negative (reagent blank) controls are included in each extraction batch. However, for off deck-lysis batches, an additional five negative (reagent blank) controls are included. These controls are processed as normal samples through to completion.
- If any results are obtained from the reagent blank, either at quantification or during the CE Q check, then the possible source of the contamination is investigated. The samples extracted with this control are thoroughly checked and repeated if possible.

12 REFERENCES

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13 STORAGE OF DOCUMENTS

The off-deck lysis worksheets are to be stored within the extraction sorting room (3190) in the nominated intray. It is essential that ALL off-deck lysis worksheets go within this intray so the operational officers are aware that batches are ready and waiting to be combined for extraction on the automated robots.

All worksheets, after auto extraction has been completed, are stored within the Pre-PCR sorting room (3194-A) in an allocated in-tray for scanning by the operational officers.

14 ASSOCIATED DOCUMENTS

QIS 17165 Receipt, Storage and Preparation of Chemicals, Reagents and Test Kits
QIS 17171 Method for Chelex Extraction
QIS 22857 Anti-Contamination procedure
QIS 23939 Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform
QIS 23959 Storage Guidelines for DNA Analysis
QIS 24256 Sequence Checking with the STORstar Instrument
QIS 24469 Batch functionality in AUSLAB
QIS 24919 DNA Analysis Workflow Procedure

15 AMENDMENT HISTORY

Version	Date	Author/s	Amendments
R0	23 Oct 2007	B. Gallagher, T. Nurthen, C. Iannuzzi, V. Hlinka, G. Lundie, I Muharam.	First Issue
R1	12 Dec 2007	M Harvey, C Iannuzzi, A McNevin	Reviewed and updated after initial training
R2	19 March 2008	M Harvey, B Andersen, C Iannuzzi, A McNevin	Addition of Off-deck Lysis procedure, Retention of fully automated method as Appendix, addition of reagent record tables into Appendix
R3	April 2008	QIS2 Migration Project	Headers and Footers changed to new CaSS format. Amended Business references from QHSS to FSS, QHPSS to CaSS and QHPS to Pathology Queensland
4	13 March 2009	QIS2 migration	Version incremented by one on migration to QIS2
5	03 June 2009	M Aguilera, B Micic, C Iannuzzi, A. Cheng, V. Hlinka, I. Muharam, G. Lundie, C. Weber	Major changes to reflect new procedure. Updated to reflect changes in procedure as an outcome of internal and external audits. Created ver.6.4 ODL in MPII Platforms. Minor changes in procedures using 4titude 4seal heat sealer to seal plates.
6	29 June 2009	A McNevin, K Lancaster	Removed references to retaining lysate and beads, fixed minor formatting errors. Created ver6.5 ODL in MPII Platforms. Substrates now to be retained in 2mL tube
7	30 September 2010	M.Cipollone, M.Mathieson	Major changes made RE: room numbers to reflect the move to Block 3. Re-formatted entire SOP. New Appendices added which outline all reagent volumes. All equipment and associated asset numbers have been included. Use of the electronic diary now included as an additional section.

			Preparation of reagents within the clean room (3188) now to be done prior to starting each process. Storage of worksheets updated. New software version 6.6 on automated extraction robots. S/N Retention Boxes now stored in Manual Ext Room. Associated Documents and hyperlinks updated. Consumables and Equipment table added for Manual DNA IQ.

16 APPENDICES

16.1 REAGENT VOLUMES FOR OFF DECK LYSIS PROCESSES (NO RETAIN SUPERNATANT)

Table 10 - Table of reagent volumes.

Reagent	Volume for 48 samples (mL)	Volume for 38 Samples (mL)
Extraction Buffer		
TNE buffer	16	12
Proteinase K (20mg/mL)	0.864	0.648
Sarcosyl (40%)	0.432	0.324
Lysis-DTT Buffer		
DNA IQ™ Lysis Buffer	N/A	N/A
DTT (1M)	N/A	N/A
DNA IQ™ Resin		

solution		
Lysis-DTT Buffer	N/A	N/A
DNA IQ™ Resin	N/A	N/A
DNA IQ™ 1x Wash Buffer	N/A	N/A
DNA IQ™ Elution Buffer	N/A	N/A

Note: Do not directly pipette from reagent stock bottles. Stock reagents should be decanted into working containers where appropriate.

16.2 REAGENT VOLUMES FOR OFF DECK LYSIS PROCESSES (RETAIN SUPERNATANT)

Table 11 - Table of reagent volumes.

Reagent	Volume for 48 samples (mL)	Volume for 38 Samples (mL)
TNE buffer	22	17
Proteinase K (20mg/mL)	0.672	0.504
Sarcosyl (40%)	0.336	0.252
Lysis-DTT Buffer		
DNA IQ™ Lysis Buffer	N/A	N/A
DTT (1M)	N/A	N/A
DNA IQ™ Resin solution		
Lysis-DTT Buffer	N/A	N/A
DNA IQ™ Resin	N/A	N/A
DNA IQ™ 1x Wash Buffer	N/A	N/A
DNA IQ™ Elution Buffer	N/A	N/A

Note: Do not directly pipette from reagent stock bottles. Stock reagents should be decanted into working containers where appropriate.

16.3 REAGENT VOLUMES FOR AUTOMATED DNA IQ™

Table 12 - Table of reagent volumes.

Reagent	Volume for 96 samples (mL)	Volume for 76 samples (mL)	Volume for 48 samples (mL)
Extraction Buffer (300µL/sample)			
TNE buffer	N/A	N/A	N/A
Proteinase K (20mg/mL)	N/A	N/A	N/A
Sarcosyl (40%)	N/A	N/A	N/A
Lysis-DTT Buffer			
DNA IQ™ Lysis Buffer	90	70	50
DTT (1M)	0.9	0.700	0.5
DNA IQ™ Resin solution			
Lysis-DTT Buffer	6.0	6.0	3
DNA IQ™ Resin	1.0	1.0	0.5

DNA IQ™ 1x Wash Buffer	35.0	30.0	18
DNA IQ™ Elution Buffer	14.0	12.0	8

Note: Do not directly pipette from reagent stock bottles. Stock reagents should be decanted into working containers where appropriate.

16.4 REAGENT VOLUMES FOR MANUAL DNA IQ™ (NO RETAIN SUPERNATANT)

Table 13 - Table of reagent volumes

Reagent	Volume for 12 samples (mL)	Volume for 24 Samples (mL)
TNE buffer	4.0	8.0
Proteinase K (20mg/mL)	0.216	0.432
Sarcosyl (40%)	0.108	0.216
Lysis-DTT Buffer		
DNA IQ™ Lysis Buffer	10	20
DTT (1M)	0.1	0.2
DNA IQ™ Resin solution		
Lysis-DTT Buffer	0.645	1.29
DNA IQ™ Resin	0.105	0.210
DNA IQ™ 1x Wash Buffer	4.0	8.0
DNA IQ™ Elution Buffer	1.4	2.8

Note: The volume of Lysis-DTT Buffer calculated includes the volume used in the Resin-Lysis solution preparation.

16.5 APPENDIX FIVE REAGENT VOLUMES FOR MANUAL DNA IQ™ (RETAIN SUPERNATANT)

Table 14 - Table of reagent volumes

Reagent	Volume per sample (uL)	Volume for 12 samples (mL)	Volume for 24 Samples (mL)
TNE buffer	450	5.4	10.8
Proteinase K (20mg/mL)	14	0.168	0.336
Sarcosyl (40%)	7	0.084	0.168
Lysis-DTT Buffer			
DNA IQ™ Lysis Buffer	660	10	20
DTT (1M)	6.6	0.1	0.2
DNA IQ™ Resin solution			
Lysis-DTT Buffer	43	0.645	1.29
DNA IQ™ Resin	7	0.105	0.210
DNA IQ™ 1x Wash Buffer	300	4.0	8.0
DNA IQ™ Elution Buffer	100	1.4	2.8

Note: The volume of Lysis-DTT Buffer calculated includes the volume used in the Resin-Lysis solution preparation.

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DNA IQ™ Method of Extracting DNA from Casework and Reference Samples

1 PURPOSE AND SCOPE

This document outlines the automated and manual procedures for extracting DNA from reference and casework samples using the DNA IQ™ kit (Promega Corp., Madison, WI, USA) within DNA Analysis Unit (DAU). The automated procedure within this document utilises the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (MPII) (PerkinElmer BioDiscovery, Downers Grove, IL, USA).

Reference samples and casework samples must be extracted on separate batches. If casework and reference batches are to be extracted on the same MPII, the instrument and all associated lab-ware must be decontaminated between operations.

This procedure applies to all DAU staff members who are required to extract DNA from samples using automated or manual DNA IQ™ methods.

2 DEFINITIONS

DTT	1,4 Dithiothreitol
EDTA	Ethylenediaminetetraacetatic acid
Extracts	Samples that have undergone DNA extraction
Lysates	Samples that have undergone off-deck lysis but not DNA extraction
MPII	MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform
Paramagnetic	Becomes magnetic with the application of a magnetic force
Pro K	Proteinase K
Samples	Sample substrates in tubes awaiting a DNA extraction process
Sarcosyl	N-Lauroylsarcosine
TNE	Tris, NaCl and EDTA buffer

3 PRINCIPLE

3.1 DNA IQ™ KIT

The DNA IQ™ system (Promega Corp., Madison, WI, USA) is designed to purify DNA from a number of different substrates and has been optimised for trace DNA samples. An in-house validation was performed using a modified version of the PerkinElmer automated protocol.

Cell lysis is performed using DNA IQ™ Lysis Buffer containing Dithiothreitol (DTT). 1,4 Dithiothreitol is a reducing agent used in extractions to break disulfide bonds of proteins. The Lysis Buffer used is a proprietary buffer containing chaotropic salts required for binding of DNA to the magnetic beads. According to the MSDS (Promega Corporation 2006), the Lysis Buffer contains 50-75% guanidinium thiocyanate, < 2.5% EDTA, < 2% 3-[(3-Choalamidopropryl)dimethylammonio] propanesulfonic and < 2% polyethylene glycol tert-octylphenyl ether.

The basis of the DNA IQ™ system is the magnetic bead resin which contains novel paramagnetic particles covered with silica. The resin has a negative charge at basic and near neutral pH. However, the addition of Lysis Buffer changes the pH and salt concentration of the solution, resulting in the silica on the magnetic beads becoming positively charged and then able to bind free DNA.

The magnetic bead resin has a DNA binding capacity of 100ng and binding is more efficient with samples containing small amounts of DNA rather than samples with large amounts of DNA. As a result sample size is critical to ensure efficient recovery of DNA.

Inhibitors are removed from the DNA through several washing steps. The first washing step is performed using Lysis Buffer to ensure the DNA is bound to the magnetic bead resin and to wash out inhibitors. The next three washing steps incorporate the use of DNA IQ™ Wash

Buffer. This buffer contains an alcohol/aqueous mixture which dehydrates the DNA to ensure it is not eluted during washing, while the aqueous phase washes out inhibitors.

The DNA IQ™ Elution Buffer changes the salt content which, in conjunction with heating to 65 ºC, allows the DNA to be released from the magnetic beads. The Elution Buffer is a low ionic strength buffer that reduces the affinity of the DNA for the silica that covers the magnetic beads, by re-hydration of the phosphate backbone. To prevent PCR inhibition caused by the presence of very small DNA fragments within extracts the DNA IQ™ System selectively isolates DNA fragments greater than 80bp.

3.2 OFF-DECK LYSIS PROCEDURE

Samples must undergo a pre-extraction Off-Deck Lysis step before processing with the DNA IQ™ reagents on the MPII instrument.

The Extraction Buffer used for the Off-Deck Lysis is designed to digest cellular material and release DNA. It contains TNE buffer (Tris, NaCl, EDTA, pH 8.0), Sarcosyl and Pro K.

TNE acts as a basic buffer with EDTA chelating ions in solution.

Sarcosyl is a detergent that releases cellular material from the substrate.

Pro K is an enzyme from the fungus *Engyodontium album* (formerly *Tritirachium album*). As a highly active endopeptidase, it cleaves peptide bonds adjacent to the carboxyl group of N-substituted (blocked alpha amino) hydrophobic, aliphatic and aromatic amino acids. Pro K is added to the extraction buffer to digest cellular material, helping the DNA to be released. It also digests proteins that interfere with the DNA binding capacity of the resin and rapidly inactivates enzymatic activity that could potentially degrade DNA (e.g. nucleases).

3.3 MULTIPROBE® II HT EX PLUS WITH GRIPPER™ INTEGRATION PLATFORM

Within DNA Analysis, DNA extractions are performed on casework or reference samples using two MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms (MPII-A or MPII-B) located in Room 3191.

Each platform uses a computer-controlled Cartesian XYZ liquid handling system designed for the efficient automation of sample preparation. Liquid transfer is performed by an 8-tip system with VersaTip® and VariSpan™ options. The VersaTip® option allows the use of both fixed and disposable tips (conductive and non-conductive). The VariSpan™ option permits variable probe spacing between each of the individual probes so that a wide range of lab-ware such as micro plates, tubes, vials and reagent troughs can be accessed. Each sample probe is capable of independent motion in the Z direction due to independent Z drives.

The 8-tip system is also capable of multichannel liquid-level sensing by utilising Accusense™ technology. This technology works by each probe detecting a change in capacitance within the liquid. This capacitive mode of detection is also possible when using conductive disposable tips. Accusense™ also permits the detection of low or non-ionic polar solutions and solvents. Pipetting on the platforms is driven by 8 individually controlled syringes via the positive displacement of the system liquid (nanopure water) when transferring liquid.

The Gripper™ Integration allows for automated identification of lab-ware via the scanning of barcodes and also allows for automated transfer of plates from one position on the deck to another. To increase deck space and capacity, the platforms include a left deck extension.

For automated DNA extraction using the DNA IQ™ kit, a plate map is utilised to provide the necessary information for correct volumes and locations for pipetting. It also contains

information regarding batch and plate identification, sample positions, lab numbers of samples, and lists all the samples in the batch, providing the total number of samples. The program will fail to work without a plate map.

4 REAGENTS AND EQUIPMENT

4.1 REAGENTS

Table 1 outlines all the reagents and storage locations required for Manual or Automated DNA IQ™ extraction.

Table 1 Reagents with storage room and location.

Reagent	Room	Location
Amphyl (0.2 and 1%)	3191	Sink
DNA IQ™ Elution Buffer	3188	In-use tray or shelf
DNA IQ™ Lysis Buffer	3188	In-use tray or shelf
DNA IQ™ Resin	3188	In-use tray or shelf
DNA IQ™ 2x Wash Buffer	3188	In-use tray or shelf
DTT (1M)	3188	Freezer
70 % v/v ethanol	3188, 3189, 3191	Sink or bench
100 % v/v ethanol	3188	Shelf
5 % v/v Hypo 10 bleach	3188, 3189, 3191	Sink or bench
Isopropyl alcohol	3188	Shelf
Nanopure water	3188, 3194	Sink
40 % w/v Sarcosyl	3188	In-use tray or shelf
Pro K (20 mg/mL)	3188	Freezer
TNE	3188	In-use tray or shelf
5 % v/v Trigene	3191	Sink

4.1.1 Extraction Buffer

Prepare Extraction Buffer just prior to commencing the Manual DNA IQ™ or Off-Deck Lysis procedures.

1. Use the correct table in the appendices (Section 16) for the required procedure to determine the reagent volumes.
2. Remove the appropriate aliquot/s of 20 mg/mL Pro K from the freezer and thaw. Vortex mix and briefly centrifuge before use.
3. Ensure that the 40 % w/v Sarcosyl is completely dissolved and appears clear in the stock solution. If not dissolved, invert the container a few times and leave at room temperature.
4. In the biohazard cabinet, aliquot the required amount of TNE buffer into a 50 mL tube. Do not use directly from the stock bottle, use a smaller working aliquot instead.
5. Add the appropriate volume of 20 mg/mL Pro K and 40% w/v Sarcosyl to the TNE buffer and invert gently to mix.
6. Label the tube with **"Extraction Buffer"**, your initials and the date.
7. Note down all reagent lot numbers.

4.1.2 Lysis Buffer with DTT Solution

Lysis Buffer is supplied with the DNA IQ™ kit. The Lysis Buffer with DTT solution is prepared just prior to commencing the Manual or Automated DNA IQ™ procedures.

Warning: Lysis Buffer and DTT are toxic, use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling Lysis Buffer.

1. Use the correct table in the appendices (Section 16) for the required procedure to determine the reagent volumes.

2. Remove the appropriate aliquot/s of DTT from the freezer and thaw. Vortex mix and centrifuge briefly before use.
3. In the biohazard cabinet, add the required volume of Lysis Buffer to a sterilised glass Schott bottle or 50mL tube.
4. Add the required volume of DTT to the Lysis Buffer and gently swirl to mix.
5. Label the container with **"Lysis Buffer + DTT"**, your initials and the date.
6. Note down all reagent lot numbers.

4.1.3 DNA IQ™ Resin

DNA IQ™ Resin is supplied with the DNA IQ™ kit. The resin solution is prepared using the Lysis Buffer with DTT solution (from section 4.1.2) just prior to commencing the Manual or Automated DNA IQ™ procedures.

Warning: Lysis Buffer and DTT are toxic, use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling Lysis Buffer.

Note: Ensure the resin is properly mixed by vortex mixing prior to use.

1. Use the correct table in the appendices (Section 16) for the required procedure to determine the reagent volumes.
2. In the biohazard cabinet, aliquot the required amount of Lysis Buffer with DTT solution (from 4.1.2) into a 10, 5 or 2 mL tube.
3. Thoroughly vortex mix the DNA IQ™ resin and immediately add the required volume to the Lysis Buffer with DTT solution.
4. Mix by gentle inversion.
5. Label the tube with **"Resin"**, your initials and the date.
6. Note down all reagent lot numbers.

4.1.4 1x Wash Buffer

Note: 2x Wash Buffer is supplied with the DNA IQ™ kit.

To prepare 1x Wash Buffer:

1. In a biohazard cabinet, add 35 mL of *AnalR* 100 % Ethanol to the 2x Wash Buffer bottle.
2. Add 35 mL of Isopropyl Alcohol to the 2x Wash Buffer bottle.
3. Update the 1x Wash Buffer reagent audit trail in AUSLAB:
   - From the main page press <2> "Sample Processing"
   - Press <8> "Materials Processing"
   - Press <2> "Consumable Inventory"
   - Highlight "DNA IQ Wash Buffer" in the list and press <enter>
   - Highlight the correct lot number and press <SF8> "Consumable Lot Audit"
   - Add audit entry that specifies the 1x Wash Buffer aliquot (e.g. A1) and the lot numbers of the ethanol and isopropyl alcohol used.

Note: 1x Wash Buffer can be prepared and stored at room temperature prior to use in an automated or manual DNA IQ™ procedure.

1. Use the correct table in the appendices (Section 16) for the required procedure to determine the reagent volume.
2. In the biohazard cabinet, aliquot the required amount of 1x Wash Buffer into a 50 or 10 mL tube.
3. Label the tube with **"Wash Buffer"**, your initials and the date.
4. Note down the reagent lot number.

4.1.5 Elution Buffer

Elution Buffer is supplied with the DNA IQ™ kit.

1. Use the correct table in the appendices (Section 16) for the required procedure to determine the reagent volume.
2. In the biohazard cabinet, aliquot the required amount of 1x Wash Buffer into a 50 or 10 mL tube.
3. Label the tube with **"Wash Buffer"**, your initials and the date.
4. Note down the reagent lot number.

4.2 EQUIPMENT

4.2.1 Equipment and consumables required for Manual DNA IQ™ and Off-Deck Lysis

Table 2 outlines the equipment and location required for Manual DNA IQ™ or Off-Deck Lysis procedures.

Table 2 Equipment with asset number and location for each procedure.

Equipment	Asset No.	Location	Procedure
Fridge	30433424	3189	Manual DNA IQ™; Off-deck Lysis
Freezers		3189 / 3190	Manual DNA IQ™; Off-deck Lysis
Biological safety cabinet class II x 5		3188 x 1 3189 x 4	Manual DNA IQ™; Off-deck Lysis
96 well tube racks	N/A	3189	Manual DNA IQ™; Off-deck Lysis
Vortex x 4	30435255 30435256 002123941 806021325	3189	Manual DNA IQ™; Off-deck Lysis
Hot block x 4	30435115 30435113 30435114 30435112	3189 / 3191	Manual DNA IQ™; Off-deck Lysis
Centrifuge x 4	30433323 30433324 10233209 30433322	3189	Manual DNA IQ™; Off-deck Lysis
Mini centrifuges x 4	30434993 30087075 30087057 041129	3189	Manual DNA IQ™; Off-deck Lysis
Finnpipettes 100 – 1000 µL	N/A	3189	Manual DNA IQ™; Off-deck Lysis
Finnpipette 20 – 200 µL	N/A	3189	Manual DNA IQ™
Thermo mixer x 2		3189	Manual DNA IQ™
Magnetic rack	N/A	3189	Manual DNA IQ™
Shaker		3191	Manual DNA IQ™

Table 3 outlines the consumables and location required for Manual DNA IQ™ or Off-Deck Lysis procedures.

Table 3 Consumables and location for each procedure.

Consumables	Location	Procedure
5 mL, 10 mL and 50mL sterile tubes	3188 / 3189	Manual DNA IQ™; Off-deck Lysis
1000 µL ART or One-touch tips	3188 / 3189	Manual DNA IQ™; Off-deck Lysis
1.5mL or 2mL tubes	3188 / 3189	Manual DNA IQ™; Off-deck Lysis
Spin baskets and 2 mL collection tubes	3189	Manual DNA IQ™; Off-deck Lysis
Inoculation sticks	3189	Manual DNA IQ™; Off-deck Lysis
300 µL ART or 200 µL One-touch tips	3188 / 3189	Manual DNA IQ™

Nunc Bank-it™ tubes	3189	Manual DNA IQ™; Off-deck Lysis
Nunc Bank-it™ caps	3189	Off-deck lysis
Sharps bin	3188 / 3189	Manual DNA IQ™; Off-deck Lysis

Further consumables can be found in the Store Room (3184).

4.2.2 Equipment and consumables required for Automated DNA IQ™

Table 4 outlines the equipment with asset number and location required for Automated DNA IQ™.

Table 4 Equipment with asset number and location for Automated DNA IQ™.

Equipment	Asset No.	Location
STORstar (B)	10238493	3190
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (MPII-A)	10076438	3191
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (MPII-B)	10076437	3191
DPC shaker x 2	N/A	3191
Automated temperature controller, heat block tiles and heat block adapters x 2	N/A	3191
Milli-Q Integral 3 (A10) water purification system		3194
Eppendorf 5804 centrifuge	10238416	3191
Vortex	30087015	3191
Fridge	30433424	3191
MixMate	30512822	3191
Capit-All automated decapper	None	3191
4titude 4seal sealer	30512847	3191

Table 5 outlines the consumables and location required for Automated DNA IQ™.

Table 5 Consumables and location for Automated DNA IQ™.

Consumables	Location
175µL Clear Non-Conductive Filter RoboRack tips – Pre-Sterilised	3191
MβP Pure 1000µL Tips – Pre-Sterilised	3191
Abgene 96-deep well plate	3191
Axygen 2mL deep well storage plate	3191
96 well Half Skirt PCR Microplate	3191
4titude Piercing Film	3191
12 Channel plate	3191
Nunc Bank-it™ tubes	3191
Nunc Bank-it™ caps	3191
Sterile 50mL Falcon tubes	3188
Sterile 10mL or 5mL tubes	3188
Autoclaved 100mL glass bottles	3191
Autoclaved 250mL glass bottles	3191
Aluminium sealing film	3191
300uL ART tips	3188
1000µL ART tips	3191

Further consumables can be found in the Store Room (3184).

5 SAFETY

As per the standard laboratory procedure QIS 22857 Anti-Contamination Procedure, PPE is to be worn by all staff when performing this procedure. This includes the use of safety glasses.

The MPII instruments, labware, biohazard cabinets and centrifuges should be wiped with **5 % v/v Trigene only**, followed by 70 % v/v ethanol. Work benches and non-metallic equipment should be wiped with 5 % v/v Hypo 10 bleach, followed by 70 % v/v ethanol.

Warning: Do not place any part of the body inside the cabinet while the MPII is running a procedure.

If it is necessary to access the deck, pause the program using the computer or the emergency STOP button located on the front of the instrument.

Warning: Tris base, EDTA, Sarcosyl and Lysis Buffer are irritants. DTT is a reducing agent that destroys disulfide bonds. Handle carefully and wear appropriate PPE.

Lysis Buffer contains guanidinium thiocyanate (GuSCN) which is toxic and can be harmful if inhaled, swallowed or comes in contact with skin. Left over Lysis Buffer-DTT is disposed of in a brown Winchester bottle in the ventilated cupboard in Room 3191. Never dispose down the sink. If spillage occurs, absorb with liquid-binding material (such as sand or kitty litter) and dispose in a biohazard bin.

Do not combine bleach with Lysis Buffer and do not wipe areas of Lysis Buffer spillage with bleach, as the two reagents produce cyanide gas when combined. Handle carefully and wear appropriate PPE, including safety glasses, when handling Lysis Buffer. If Lysis Buffer is spilt onto PPE (eg. Gloves, gowns), remove and discard the contaminated item/s in a biohazard bin.

6 SAMPLE PREPARATION

6.1 SAMPLE LOCATIONS

Samples waiting to be extracted are stored in freezers as detailed in Table 6.

Table 6 Sample storage locations.

Sample type	Storage Device	Storage Location
Urgent/High/Medium Priority Samples	Freezer	3190 or 6106*
Lysates in 1.0mL Nunc Bank-it™ tubes	Freezer	3190
Extracts in 1.0mL Nunc Bank-It™ tubes	Freezer	3194 A

* Some Medium and Low Priority storage boxes are located in Block 6, within the Exhibit Room (6106).

6.2 QC SAMPLES

One negative reagent control and one positive blood control (from a known donor) must be registered for all extraction batches. Additionally, off-deck lysis batches require five blank controls as detailed in Table 7.

Table 7 Extraction Quality Controls.

Batch Type	Control
Off-deck lysis	Positive control (x1)
	Negative control (x1)
	Blank control (x5)
Manual DNA IQ™	Positive control (x1)

	Negative control (x1)
Retain supernatant DNA IQ™	Positive control (x1)
	Negative control (x1)

6.3 REGISTRATION OF QC SAMPLES

Register control samples according to the standard laboratory procedure QIS 24919 DNA Analysis Workflow Procedure.

6.4 CREATION OF EXTRACTION BATCHES

Create extraction batches according to the standard laboratory procedure QIS 24919 DNA Analysis Workflow Procedure.

6.5 LOCATING SAMPLES

Locate samples according to the standard laboratory procedure QIS 23959 Storage Guidelines for DNA Analysis.

6.6 SEQUENCE CHECKING AUTOMATED EXTRACTION BATCHES

Sequence check lysates in Nunc Bank-It tubes for automated extraction batches according to the standard laboratory procedure QIS 24256 Procedure for the use of the STORstar unit for automated sequence checking.

6.7 ELECTRONIC WORKFLOW DIARY

Within the electronic workflow diary I:\AAA Electronic Workflow Diary record details of the batch sample retrieval and processing.

7 MANUAL METHOD FOR EXTRACTION USING DNA IQ™

7.1 PROCEDURE FOR MANUAL DNA IQ™ (NO RETAIN SUPERNTANT)

Note: All manual extractions are to be performed within a biohazard cabinet in room 3189.

1. Prepare fresh reagents within the biohazard hood of the clean room (3188) prior to commencing the procedure. Refer to Section 4.1 for preparation of reagents. Use Table 8 in Appendix 12.1 for reagent volumes.

2. Turn on the Eppendorf Thermomixer and set to 37 °C and 1400 rpm.

3. Label for each sample:
   - Original sample tube;
   - Spin basket (if required);
   - 2mL tube; and
   - Nunc™ Bank-It™ tube.

Note: A spin basket is required for swabs, fabric, paper and cigarette butts. Samples that do not require a spin basket are tape-lifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

4. Have a second operator perform a sequence check of all tubes. This person must enter the sequence check in AUSLAB.

5. Add 300 µL of Extraction Buffer to each sample. Ensure that large substrates including tape-lifts are fully submerged.

6. Vortex and incubate at 37 °C on the Thermomixer at 1400 rpm for 45 minutes.

7. Remove from the Thermomixer and increase the temperature on the Thermomixer to 65 °C for use in the elution steps.

8. Transfer the substrate to spin basket (if required) or transfer the lysate to an appropriately labelled 2.0 mL tube. Retain the original tube containing the substrate if no spin basket used.

9. Centrifuge the spin basket at 15800 xg for 2 minutes.

10. Transfer the lysate from the original sample tube and the flow-through from the collection tube of the spin basket to an appropriately labelled 2 mL tube. Transfer the substrate from the spin basket back into the original sample tube.

    Note: If original sample tube is an appropriate 2 mL tube, transfer the substrate into the new 2 mL tube and add the flow-through from the spin basket to the lysate within the original sample tube.

11. Add 550 µL of Lysis Buffer-DTT solution to the lysate.

12. Add 50 µL of DNA IQ™ Resin-Lysis Buffer-DTT solution to the lysate. Pipette mix or vortex the solution frequently to keep a homogenous suspension of resin within the solution.

13. Vortex and place on the Multitube shaker set at 1200 rpm for 5 minutes.

14. Remove from the Multitube shaker, vortex and immediately place into the magnetic stand. Separation will occur instantly.

Note: If resin does not form a distinct pellet on the side of the tube, or if the magnetic beads have re-suspended while in the stand, vortex the tube and quickly place back in the stand.

15. Carefully remove and discard the liquid, ensuring that the resin is not disturbed. Remove from the magnetic stand.

Note: If some resin is drawn up in the pipette tip, gently expel resin back into tube to allow re-separation.

16. Add 125 µL of Lysis Buffer-DTT solution. Vortex and place into the magnetic stand.

17. Once separation has occurred, remove and discard the liquid. Remove from the magnetic stand.

18. Add 100 µL of 1X Wash Buffer, vortex and place into the magnetic stand. Once separation has occurred remove and discard the liquid. Remove from the magnetic stand.

19. Repeat step 18 two times for a total of three Wash Buffer washes. Ensure that all of the liquid has been removed after the last wash. Remove from the magnetic stand.

20. Uncap the tubes and place the lids down onto a clean rediwipe within the biohazard cabinet. Allow the resin to air-dry in the hood for 15 minutes and then recap the tubes.

Note: Do not allow the resin to dry for more than 20 minutes as this may inhibit DNA elution.

21. Add 50 µl of Elution Buffer by carefully pipetting the solution to the side of the tube, above the pellet. **Do not mix**.

22. Incubate in the Thermomixer at 65°C for 3 minutes and then continue to incubate for a further 3 minutes shaking at 1100 rpm. If no thermomixer is available, incubate samples within a hot block and vortex mix at the start and end of the second 3 minute incubation. Remove samples.

23. Vortex and immediately place into the magnetic stand while hot to ensure maximum DNA yield during elution.

24. Carefully transfer the DNA extract to the corresponding labelled Nunc™ Bank-It™ tube.

25. Remove from the magnetic stand and repeat steps 21-23 once. The final volume after the second elution is approximately 95 µL of DNA extract.

26. Store the DNA extracts in temporary storage located within the Pre-PCR sorting room 3194-A in the upright freezer with equipment number 30512883.

27. Store tubes containing substrates in Spin Basket boxes located within the Pre-PCR sorting room 3194-A in the upright freezer with equipment number 30512883.

7.2 PROCEDURE FOR MANUAL DNA IQ™ (RETAIN SUPERNTANT)

Note: All manual extractions are to be performed within a biohazard cabinet in room 3189.

1. Prepare fresh reagents within the biohazard hood of the clean room (3188) prior to commencing the procedure. Refer to Section 4.1 for preparation of reagents. Use Table 9 in Appendix 12.2 for reagent volumes.

2. Turn on the Eppendorf Thermomixer and set to 37 ˚C and 1400 rpm.

3. Label for each sample:
   - Original sample tube;
   - 1.5 or 2 mL tube labelled 'SUP' for supernatant;
   - Spin basket (if required);
   - 2 mL tube; and
   - Nunc™ Bank-It™ tube.

Note: A spin basket is required for swabs, fabric, paper and cigarette butts. Samples that do not require a spin basket are tape-lifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

4. Have a second operator perform a sequence check of all tubes. This person must enter the sequence check in AUSLAB.

5. Add 450µL of TNE buffer to the sample and vortex.

6. Incubate at room temperature for 30 minutes.

7. Vortex, then centrifuge at 15800 xg for 3 minutes.

8. Remove 150 µL of supernatant and place into the respective 1.5 mL tube labelled with 'SUP' (for further testing).

9. Add 14µL of 20mg/mL Proteinase K and 7µL 40% (w/v) Sarcosyl to the original sample tube containing substrate and TNE buffer, then vortex.

10. Vortex, then incubate at 37°C on the Thermomixer at 1400 rpm for 45 minutes. If no thermomixer available, incubate samples within a hot block, vortex mix at the start and end of the incubation and at least one during the incubation.

11. Remove from the Thermomixer and increase the temperature on the Thermomixer to 65°C (for use in the Elution steps).

12. Transfer substrate to spin basket if required or transfer the lysate to an appropriately labelled 2.0mL tube. Retain original tube containing the substrate in if no spin basket used.

13. Centrifuge spin basket at 15800 g for 2 minutes.

14. Transfer the substrate in the spin basket into a new and labelled 2mL tube to be retained. Transfer the flow through (lysate) back into the original sample tube.

15. Add 550µL of Lysis-DTT Buffer solution.

16. Add 50µL of DNA IQ™ Resin-Lysis solution. Pipette mix the Resin-Lysis solution between each addition to keep the resin suspended in solution.

17. Vortex and place on the Multitube shaker set at 1200 rpm for 5 minutes.

18. Remove from the Multitube shaker, vortex and immediately place into the magnetic stand. Separation will occur instantly.

    Note: If resin does not form a distinct pellet on the side of the tube, or if the magnetic beads have re-suspended while in the stand, vortex the tube and quickly place back in the stand.

19. Carefully remove and discard the solution, ensuring that the resin is not disturbed. Remove from the magnetic stand.

    Note: If some resin is drawn up in the pipette tip, gently expel resin back into tube to allow re-separation.

20. Add 125µL of Lysis-DTT Buffer solution. Vortex and place into the magnetic stand.

21. Once separation has occurred, remove and discard the Lysis-DTT Buffer. Remove from the magnetic stand.

22. Add 100µL of 1X Wash Buffer, vortex and place into the magnetic stand. Once separation has occurred remove and discard the Wash Buffer. Remove from the magnetic stand.

23. Repeat the Wash Buffer step (step 23) two times for a total of three washes. Ensure that all of the solution has been removed after the last wash. Remove from the magnetic stand.

24. Uncap the tubes and place the lids down onto a clean rediwipe. Air-dry the resin in the hood for 15 minutes and then recap tubes

    Note: Do not dry for more than 20 minutes, as this may inhibit the elution of DNA.

25. Add 50µl of Elution Buffer by carefully pipetting the solution to the side of the tube, above the pellet. **Do not mix**.

26. Incubate in the Thermomixer at 65°C for 3 minutes and then continue to incubate for a further 3 minutes shaking at 1100 rpm. If no thermomixer available, incubate samples within a hot block, vortex mix at the start and end of 2nd 3 minute incubation. Remove samples.

27. Vortex and immediately place into the magnetic stand while hot to ensure maximum DNA yield during elution.

28. Carefully transfer the DNA extract to the corresponding labelled Nunc™ Bank-It™ tube.

29. Remove from the magnetic stand and repeat the Elution Buffer steps (step 26-29). The final volume after the double elution is approximately 95µL of DNA extract.

30. Store the DNA extracts in temporary storage located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

31. Store tubes containing substrates in Spin Basket boxes located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

32. Store supernatants in the S/N Retention boxes located within the Extraction sorting room in the Orford Freezer 30512883 (Room 3190).

33. Ensure that the negative extraction control from the batch is also inserted onto the **'Saliva List':**

    - Log into Auslab
    - 3 for Patient Enquiry
    - Type/Scan the lab number for the negative extraction control
    - From the 9Plex Page press Shift F12
    - A prompt will appear 'Enter List Name'
    - Type Saliva or use the F1 lookup list.
    - Press Enter

8 OFF-DECK LYSIS PROCEDURE

1. All reagents are to be made fresh within the clean room (3188) prior to commencing each process. Use Table 10 in Appendix 12.3 for reagent volumes.

2. All manual extractions are to be performed within a biohazard cabinet in Room 3189

3. For each sample label:

    - Original sample tube
    - Spin basket as required
    - 1.0mL Nunc Bank-It™ tube
    - 1.5mL or 2.0mL tube

    Note: Samples that require a spin basket are: swabs, fabric, paper and cigarette butts. Samples that do not require a spin basket are: tapelifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

4. If substrate is in a 0.5mL tube, transfer to a labelled 1.5mL or 2.0mL tube.

5. Have a second operator sequence check the tubes and enter the sequence check in AUSLAB.

6. Add 300µL of Extraction Buffer and vortex briefly. Ensure that substrates are immersed in the Extraction Buffer.

7. Incubate for 45 minutes at 37 degrees on a hotblock. It is best practice to vortex the samples intermittently throughout the duration of the incubation period. Record temperature on worksheet.

8. Remove from the hotblock. Transfer substrate to spin basket if required or transfer the lysate to an appropriately labelled 1.5mL or 2mL tube. Retain original tube containing the substrate if no spin basket is used.

9. Centrifuge spin baskets at 15800 g for 2 minutes.

10. Transfer the substrate in the spin basket to a new and appropriately labelled 1.5mL or 2mL tube and retain.Transfer the flow through back to original lysis tube.

11. Vortex lysate, then incubate on hotblock at 65°C for 10 minutes. Record temperature on worksheet.

12. Centrifuge at 15800 g for 1 minute.

13. Transfer 300µL of lysate to the corresponding Nunc Bank-It™ tube.

Note: If more than 300µL of lysate is present, retain the remaining lysate in the 1.5mL tube. In AUSLAB, write a specimen note for that sample (eg. Extra lysate retained from sample XXXXXXXXX.). Store the retained 1.5mL lysate tube in appropriate box in freezer.

14. In AUSLAB, enter reagent and hotblock temperature details and complete the batch.

15. Store lysates in temporary storage boxes located within the extraction sorting room in the orford Freezer with equipment number 102384430 (Room 3190).

16. Store tubes containing substrates or extra lysate in Spin Basket boxes located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

9 AUTOMATED EXTRACTION OF LYSED SAMPLES

9.1 MPII EXTRACTION PROCEDURE

Automated setup of DNA IQ extractions in 96-deep well format is performed using the MultiPROBE® II PLUS HT EX EP-A and EP-B platforms located in Room 3191.

Refer to QIS 23939 *Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform* for instructions on the use and maintenance of the MultiPROBE® II PLUS HT EX platforms.

9.2 PREPARATION OF REAGENTS FOR THE AUTOMATED EXTRACTION PROCESS

All reagents are to be made fresh within the clean room (3188) prior to commencing each process. Use Table 11 in Appendix 12.4 for reagent volumes.

9.3 SUMMARY OF DNA IQ™ EXTRACTION VERSION 6.6_ODL

9.3.1 Transfer of lysates from Nunc Bank-It™ tubes into the Abgene 96-deep well plate

Lysates from the off-deck lysis protocol, contained in individual Nunc Bank-It™ tubes, are transferred automatically into an Abgene 96-deep well plate prior to commencing automated sample processing. Instead of a 96-deep well plate, the use of individual Nunc Bank-It™ tubes for storage of lysates removes the need for an adhesive film and therefore reduces the risk of well-to-well contamination from the action of peeling back an adhesive plate cover.

9.3.2 Automated addition of DNA IQ™ Resin and Lysis Buffer

DNA IQ™ Resin is added automatically into the Abgene 96-deep well plate on the platform. The program uses a waste mode dispensing step to add 53µL DNA IQ™ Resin-Lysis Buffer solution to each well in either half the plate or the whole plate. Because of this, batch sizes are restricted to either 48 or 96 samples in order to maintain efficiency and economy. Two volumes of Lysis Buffer are then added to promote binding of DNA to the paramagnetic resin.

9.3.3 Mixing using a MixMate to bind DNA to resin

Manual intervention is required to seal the Abgene 96-deep well plate with a 4titude Pierce Seal film using the 4titude sealer pre-heated at 175 °C. The plate is then transferred onto a MixMate instrument for mixing at 1100rpm for 5 minutes, followed by centrifugation on an Eppendorf 5804 centrifuge at 3000rpm for 2 minutes. The seal is then carefully pierced with a 96 well PCR microplate and the Abgene plate is returned to the Applied Biosystems magnet on the MPII platform.

9.3.4 Removing lysis reagents for storage

At this point, most of the DNA is bound to the paramagnetic resin. With the positioning of the Abgene plate on the ABI magnet, DNA IQ™ Resin becomes immobile at the bottom of the plate. The lysis reagents from each well are transferred automatically to a storage plate on the MPII platform without disturbing the DNA IQ™ Resin.

9.3.5 Washing of the resin-DNA complex

Washing steps are performed to remove any inhibitors in solution. The first wash uses 125µL Lysis Buffer with shaking at room temperature for 1 minute on the DPC shaker to ensure that the DNA is bound to the paramagnetic resin. The plate is moved to the ABI magnet and the supernatant is transferred into the storage plate. The next three washes incorporate 100µL of 1x Wash Buffer with shaking at room temperature for 1 minute on the DPC shaker. During each wash cycle, the Abgene 96-deep well plate is moved to the ABI magnet and the supernatant is discarded into the tip chute.

9.3.6 Removing any excess of 1x Wash Buffer

The samples are allowed to air dry at room temperature for 5 minutes in order to remove all traces of Ethanol from the Wash Buffer. The presence of Ethanol may potentially inhibit both the elution process and also downstream PCR.

9.3.7 Elution of DNA from the resin-DNA complex

A double elution method is employed in this procedure. At each elution step, 60µL of Elution Buffer is added to each sample, followed by incubation at 65°C for 6 minutes (3 minutes without shaking followed by 3 minutes shaking on the DPC shaker). The Abgene plate is moved to the ABI magnet and the eluted solution (supernatant containing eluted DNA) is transferred to fresh Nunc™ Bank-It™ tubes.

9.3.8 Flushing of liquid pathway

As a decontamination measure, the liquid pathway is washed with Amphyl and Nanopure water at the conclusion of the automated extraction process.

9.4 SETTING UP THE MPII PLATFORMS FOR AUTOMATED DNA IQ™ PROCESSING

The following steps are carried out in the automated extraction room (Room 3191).

1. Remove the Nunc Bank-It™ tubes containing lysates from the fridge to allow to come to room temperature before commencing the extraction procedure.

   Note: If the lysates are frozen, remove them from the freezer and thaw in Room 3191.

2. Restart or turn on the instrument PC.

3. Log onto the network using the **Robotics** login.

4. Open WinPrep® by double clicking icon on the computer desktop (Figure 1).

Figure 1.
The WinPrep® icon.

5. Log onto the WinPrep® software by entering your username and password, then press **"Enter"**.

6. Ensure that the daily/weekly start-up and maintenance has been performed before running any program. If WinPrep® has been closed or been idle for a long period of time initialise the MP II platform as described in QIS 23939.

7. Ensure the **System Liquid reservoir is FULL** and tubing is fully submerged in the system liquid before every run and perform a Flush/Wash. If visible air bubbles have appeared in tubing or in syringes between setting up the deck and executing the test, another flush wash will need to be performed before starting the MPII extraction run.

8. Open the Extraction setup MP II test file in WinPrep® by selecting:

   - **File**
   - **Open**, navigate to **C:\PACKARD\MULTIPROBE\BIN\QHSS PROTOCOLS**
   - Select "**DNA IQ Extraction_Ver 6.6_ODL.mpt**"
   - Click the "**Open**" button

9. Check the tree pane of the whole program for any bold fonts. See the Analytical Senior Scientist if bold fonts are present.

10. Copy the required plate map from the network folder **I:\EXTRACTION** into the local folder **C:\PACKARD\EXT PLATE MAPS.** Open the plate map using Excel and check that the plate map is complete, and make changes if necessary. If there are blanks (samples that have been removed), make all volumes to 0. If it is not a full batch, delete the rows of blank samples at the end of the platemap. Save all changes made to the platemap (as a .txt file).

11. Decontaminate the required labware with 5% TriGene followed by 70% Ethanol and place onto the instrument deck in the designated grid positions (as shown in the WinPrep® software). Additionally, ensure the DPC shaker is positioned properly.

12. Ensure that the DPC shaker and Heater Controller Box are switched on.
    For EP-A: Tile 1 at F22 (85^0C).
    For EP-B: Tile 2 at F22 (85^0C).

Note: Press the start/stop button twice at the front of the DPC shaker to ensure that it displays zero on the screen.

13. Ensure the heat transfer tile is clicked into the plate adapter tile properly.
Note: This is critical to ensure correct incubation temperatures.

14. To the Amphyl wash station in position **A10**, add 1% Amphyl to the far left hand side, 0.2% diluted Amphyl to the middle left and place an empty (waste) reagent trough in the middle right position of the station.

15. Add Wash Buffer to the far right hand side trough of the Amphyl wash station in position **A10**.

16. Add Lysis Buffer to the labelled 150mL reagent trough on the left hand side of the 2 trough holder in position **A13**.

17. Place the 12 channel plate into position **A16**. Add Elution Buffer to the plate by dividing the Elution Buffer between channels 11 and 12.

18. Add Resin to channel 1 of the 12 channel plate in position **A16**. It is important to add the resin in a uniform fashion to ensure equal distribution of resin along the channel.

19. **Nunc Bank-It™ lysate tubes:** The lysates should now be at room temperature. Ensure that the rack is labelled with the correct **AUSLAB batch ID** and that the label matches the batch ID on the worksheet and platemap. Additionally, randomly check some visible barcodes on the side of lysate tubes against the positions on the worksheet to ensure the correct batch of samples is to be processed.

20. Place the rack onto the MixMate to shake for 1 minute at 1000rpm.

21. Centrifuge the rack at 3000rpm for 2 minutes in the Eppendorf 5804 centrifuge and then place into position **C13**.

22. **Abgene 96-deep well plate:** With the plate in its correct orientation label the **left hand side** of the plate with both the correct **AUSLAB batch ID** and **batch ID barcode**. With a marker, print the word **"FRONT-lysate"** on the front of the plate. Place the plate in its correct orientation in position **E13**.

23. **2mL 96-deep well storage plate:** With the plate in its correct orientation label the **left hand side** of the plate with both the correct **AUSLAB batch ID** and **batch ID barcode**. With a marker, print the word **"FRONT-store"** on the front of the plate. Place the plate in its correct orientation in position **E16**.

24. **Nunc Bank-It™ extract tubes:** Ensure that the rack is labelled with the correct **AUSLAB batch ID** on the **front** of the Nunc Bank-It™ tube rack. Additionally, randomly check some visible barcodes on the side of lysate tubes against the positions on the worksheet to ensure the correct batch of samples is to be processed. Place the rack in its correct orientation in position **G16**.

25. Add Nanopure water to the 160mL trough in the Flush/Wash station in position **G13**.

26. Ensure that all necessary labware have been positioned correctly as displayed within WinPrep®, then click "**EXECUTE TEST**". Record run information in the Run Log book.

27. The following message will appear (Figure 2 below):

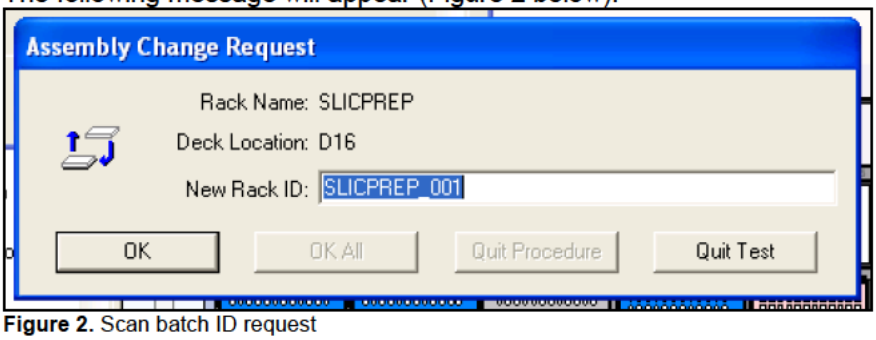

Figure 2. Scan batch ID request

Into **"New Rack ID:"** scan barcode off the worksheet. It is important this corresponds to the labelling of labware on the deck and the plate maps used.

28. Click "**Reset Tip Boxes**" and ensure that the number of tips displayed in this window match those as available on the deck platform. Fill new tips if necessary. Click "**Close**" to accept the tip count, and then click "**Next**".

29. Select the correct platemap by browsing to **C:\PACKARD\EXT PLATE MAPS**. Ensure that the platemap selected corresponds to the labelling of labware on the deck, the paperwork used and the **"New Rack ID"** entered.

30. For a full batch of 76 samples, ensure that all nodes are checked. Click "**Next**" to check all other nodes.

31. For a batch of 48 samples or less, uncheck the node: **"Add resin to second half of plate"**. Click "**Next**" to check all other nodes.

32. Click "**Start**" to continue.

    - The MPII instrument will begin and user prompts will appear as a reminder to confirm the deck setup. **Always decap tubes from positions H1 to A1, H2 to A2 etc.**
    - Ensure all steps on the first prompt have been complete, Click **OK** to continue.
    - Ensure all steps on the second prompt have been complete, Click **OK** to continue.

33. The program will progress to transfer the lysates followed by automated addition of the Lysis-DTT buffer and the DNA IQ™ Resin solution. The next user prompt will appear. Follow the steps as outlined in the user prompt. Then click **OK** when ready.

    Note: Ensure that the plate is heated and sealed properly with the Pierce Seal on the 4titude Heat Sealer. Mix the plate on the mixmate for 5min at 1100rpm and centrifuge. Pierce the plate with a PCR Microplate and then discard. (A new plate is to be used each time). Ensure the pierced plate is returned to the magnet.

34. Once lysis steps have been completed, a user prompt will appear. Follow the directions as outlined in the user prompt. Then click **OK** when ready.

    Note 1: The Lysate Nunc tubes (including the rack) should be placed into a clipseal bag, sealed and discarded into a biohazard waste bin. The Store plate can also be removed, heat sealed and discarded into a biohazard waste bin.

Note 2: When the supernatant storage plate is sealed, check the plate for the transfer of beads. To do this, place the plate onto the ABI magnet (if beads are present they will settle to the bottom of the plate) and then inspect the bottom of the plate. If no beads are present, discard the store plate. If, however, beads are present, refer to the Section 15, Troubleshooting.

35. Once the wash steps have been completed, a user prompt will appear. Follow the directions as outlined in the user prompt. Then click **OK** when ready.

36. **Note:** The Nunc extract tubes can be decapped on the bench in the MPII cabinet while the samples are drying and the heating tile is stabilising. At this point, the operator has approximately **12 minutes** to decap the tubes. If the operator has been interrupted and requires more time to decap the tubes, the program may be paused at this step.

37. A message will appear waiting for the heating tile to reach 85°C (for incubation at 65°C). **DO NOT PRESS CONTINUE** as the program will continue automatically when the temperature has been reached with sufficient stability.

38. A user prompt will appear. Follow the directions as outlined in the user prompt. Then click **OK** when ready.

39. Once the elution is completed, a user prompt appears. Follow the directions as outlined in the user prompt. Then click **OK** when ready.

40. The Lysate Plate is heat sealed and kept in temporary storage for one month.

41. Once all plates are removed from the deck and sealed. Click "**OK**" to proceed to the Amphyl wash step.

42. A final message will advise that the run has completed. Click "**OK**".

9.5 FINALISING THE MPII RUN

1. Transfer left over Resin solution from the 12 channel plate and the Lysis Buffer-DTT (wearing safety glasses) into the glass Lysis-DTT bottle previously used. Discard the 12 channel plate in the biohazard waste bin. Transfer the contents of glass bottle into the brown Winchester bottle located in the safety cupboard in room 3191.

2. Discard the contents of the Amphyl wash station into the sink and rinse the troughs with Nanopure water.

3. Remove all labware from the deck and clean with 5% TriGene™ followed by 70% Ethanol, and setup for the next run if necessary. In addition, clean the work area around the MPII instrument with 10% (v/v) Bleach 7x and 70% Ethanol.

4. Remove the tip chute and funnel, rinse with warm tap water to remove any residue inside the chute before cleaning with 5% TriGene and 70% Ethanol.

5. Import the platemap into Auslab and then delete it.

9.6 IMPORTING MPII LOG FILE INTO AUSLAB

1. Click on the Microsoft Access icon in the WinPrep® main menu to open the MultiPROBE log database.

2. Click **"Open"** when prompted.

3. Click on the relevant run Test ID in the Test Selection box. In the Report/Query/Action Selection dropdown menu, select **"Report: Test Summary (Sorted by Destination Rack ID)"**

4. In the Output Selection dropdown menu, select **"File"**. Save the output file as *.csv format to **C:\PACKARD\EXT PLATE MAPS\EXT LOGS** with the same name as the AUSLAB batch ID and click "**Apply**".

5. Open the log file and check for any errors that may have arisen during the extraction process. Compare the listed errors to any that were encountered during the run. Report any critical errors to the line manager.

6. Import the log file, entering the path, filename and extension (e.g. C:\Packard/ext plate maps/ext logs….) and press **[Enter]**. Delete the log file after importing.

7. For more details on importing files into AUSLAB refer to Batch functionality in AUSLAB SOP (QIS 24469).

9.7 IMPORTING EXTRACTION "RESULTS" INTO AUSLAB

1. Import the results file, entering the filename and .txt extension (e.g. CWIQEXT20100917_06.txt). For more details on importing files into AUSLAB refer to Batch functionality in AUSLAB SOP (QIS 24469).

2. The file will be imported into AUSLAB and appear in the Results Import Table.

3. Highlight entry and press **[Enter]**, for access to the Results Table.

4. Page down through the table and check that all sample results have been imported.

5. Press **[SF8]** ***Table Sort Order***, this sorts the table, sorting samples that have failed Autovalidation to the top. Samples that have failed are those that have processing comments present.

6. For samples that have failed, check the **Processing Comments,** by entering into the sample.

7. If processing comments state sample is to be sent to another batch type **other** than quant. Proceed with the following steps:
   a. Request the appropriate rework test code via the **[SF7]** results history table and the **[SF8]** request rework functions (e.g. samples requiring Microcon, NucleoSpin and pooling).
   b. Press **[Esc]** to exit back to the DNA results table. Do not toggle accept.
   c. Add the extraction batch ID into the 9PLEX or 9FTAR completed date fields in AUSLAB.

8. If processing comments do not state next step the sample will be processed as normal:
   a. Press **[Esc]** to exit back to the DNA results table.
   b. Highlight any entries to be changed and press **[SF7]** ***Toggle Accept.***

9. Press **[F7]** ***Complete Batch***, all of the samples with a ***Yes*** in the Accept column will be transferred to the outstanding Quant Casework or Quant Reference lists.

10. File the extraction worksheet into the relevant intray for scanning in room 3194A (Pre PCR-Sorting).

10 SAMPLE STORAGE

Refer to QIS 23959 Storage Guidelines for DNA Analysis for how to store the DNA extract Nunc™ Bank-It™ tubes and Abgene 96-deep well.

11 TROUBLESHOOTING WITH THE MPII

1. If the resin is not pipette mixing correctly (eg. Resin is not being drawn up into the pipette tip), pause the Winprep program and check that the support tile is clicked onto the MPII deck correctly. Alternatively, pipette and mix resin manually one more time from the corresponding column in the 12 channel plate.

2. If the Gripper is not placing the rack/plate properly on the deck, pause the program and manually adjust the rack/plate properly on the adapter support tile.

3. If the Gripper appears to be not gripping a plate correctly, or the pipetting alignment of a labware appears to be incorrect, the deck and / or labware can be re-calibrated. This is covered in QIS 23939 Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform

11.1 SAMPLE STORAGE

Refer to QIS 24919 DNA Analysis Workflow Procedure

11.2 VALIDATION

- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 9. Automation: Report on the Evaluation of DNA Extraction Chemistries." June 2007.
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11.3 QUALITY ASSURANCE/ACCEPTANCE CRITERIA

- Positive and negative (reagent blank) controls are included in each extraction batch. However, for off deck-lysis batches, an additional five negative (reagent blank) controls are included. These controls are processed as normal samples through to completion.
- If any results are obtained from the reagent blank, either at quantification or during the CE Q check, then the possible source of the contamination is investigated. The samples extracted with this control are thoroughly checked and repeated if possible.

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11.5 STORAGE OF DOCUMENTS

The off-deck lysis worksheets are to be stored within the extraction sorting room (3190) in the nominated intray. It is essential that ALL off-deck lysis worksheets go within this intray so the operational officers are aware that batches are ready and waiting to be combined for extraction on the automated robots.

All worksheets, after auto extraction has been completed, are stored within the Pre-PCR sorting room (3194-A) in an allocated in-tray for scanning by the operational officers.

11.6 ASSOCIATED DOCUMENTS

QIS 17165 Receipt, Storage and Preparation of Chemicals, Reagents and Test Kits
QIS 17171 Method for Chelex Extraction
QIS 22857 Anti-Contamination procedure
QIS 23939 Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform
QIS 23959 Storage Guidelines for DNA Analysis
QIS 24256 Sequence Checking with the STORstar Instrument

QIS 24469 Batch functionality in AUSLAB
QIS 24919 DNA Analysis Workflow Procedure

11.7 AMENDMENT HISTORY

Version	Date	Author/s	Amendments
R0	23 Oct 2007	B. Gallagher, T. Nurthen, C. Iannuzzi, V. Hlinka, G. Lundie, I Muharam.	First Issue
R1	12 Dec 2007	M Harvey, C Iannuzzi, A McNevin	Reviewed and updated after initial training
R2	19 March 2008	M Harvey, B Andersen, C Iannuzzi, A McNevin	Addition of Off-deck Lysis procedure, Retention of fully automated method as Appendix, addition of reagent record tables into Appendix
R3	April 2008	QIS2 Migration Project	Headers and Footers changed to new CaSS format. Amended Business references from QHSS to FSS, QHPSS to CaSS and QHPS to Pathology Queensland
4	13 March 2009	QIS2 migration	Version incremented by one on migration to QIS2
5	03 June 2009	M Aguilera, B Micic, C Iannuzzi, A. Cheng, V. Hlinka, I. Muharam, G. Lundie, C. Weber	Major changes to reflect new procedure. Updated to reflect changes in procedure as an outcome of internal and external audits. Created ver.6.4 ODL in MPII Platforms. Minor changes in procedures using 4titude 4seal heat sealer to seal plates.
6	29 June 2009	A McNevin, K Lancaster	Removed references to retaining lysate and beads, fixed minor formatting errors. Created ver6.5 ODL in MPII Platforms. Substrates now to be retained in 2mL tube
7	30 September 2010	M.Cipollone, M.Mathieson	Major changes made RE: room numbers to reflect the move to Block 3. Re-formatted entire SOP. New Appendices added which outline all reagent volumes. All equipment and associated asset numbers have been included. Use of the electronic diary now included as an additional section. Preparation of reagents within the clean room (3188) now to be done prior to starting each process. Storage of worksheets updated. New software version 6.6 on automated extraction robots. S/N Retention Boxes now stored in Manual Ext Room. Associated

			Documents and hyperlinks updated. Consumables and Equipment table added for Manual DNA IQ.
8	24 May 2012	A. Speirs M. Cipollone	Major revision of document. Revision of sections 1-3 to improve clarity. Updated Tables 1 to 7. Removed redundant sections on locating samples and creating batches. Removed procedure for retain supernatant Off-Deck Lysis and thus associated Appendix. Table of Contents amended to reflect all changes. Moved the Manual DNA IQ procedures before the Off-Deck Lysis procedure. Re-formatted spacing and alignments of headings. Re-numbered Tables in Appendices. Updated sections to include which relevant Appendix to use in regards to reagents. Minor grammatical and spelling errors fixed.

12 APPENDICES

12.1 REAGENT VOLUMES FOR MANUAL DNA IQ™ (NO RETAIN SUPERNATANT)

Table 8 - Table of reagent volumes

Reagent	Volume for 12 samples (mL)	Volume for 24 Samples (mL)
TNE buffer	4.0	8.0
Proteinase K (20mg/mL)	0.216	0.432
Sarcosyl (40%)	0.108	0.216
Lysis-DTT Buffer		
DNA IQ™ Lysis Buffer	10	20
DTT (1M)	0.1	0.2
DNA IQ™ Resin solution		
Lysis-DTT Buffer	0.645	1.29
DNA IQ™ Resin	0.105	0.210
DNA IQ™ 1x Wash Buffer	4.0	8.0
DNA IQ™ Elution Buffer	1.4	2.8

Note: The volume of Lysis-DTT Buffer calculated includes the volume used in the Resin-Lysis solution preparation.

12.2 REAGENT VOLUMES FOR MANUAL DNA IQ™ (RETAIN SUPERNATANT)

Table 9 - Table of reagent volumes

Reagent	Volume per sample (uL)	Volume for 12 samples (mL)	Volume for 24 Samples (mL)
TNE buffer	450	5.4	10.8
Proteinase K (20mg/mL)	14	0.168	0.336
Sarcosyl (40%)	7	0.084	0.168
Lysis-DTT Buffer			
DNA IQ™ Lysis Buffer	660	10	20
DTT (1M)	6.6	0.1	0.2
DNA IQ™ Resin solution			
Lysis-DTT Buffer	43	0.645	1.29
DNA IQ™ Resin	7	0.105	0.210
DNA IQ™ 1x Wash Buffer	300	4.0	8.0
DNA IQ™ Elution Buffer	100	1.4	2.8

Note: The volume of Lysis-DTT Buffer calculated includes the volume used in the Resin-Lysis solution preparation.

12.3 REAGENT VOLUMES FOR THE OFF DECK LYSIS PROCEDURE

Table 10 - Table of reagent volumes.

Reagent	Volume for 48 samples (mL)	Volume for 39 Samples (mL)
Extraction Buffer		
TNE buffer	16	12
Proteinase K (20mg/mL)	0.864	0.648
Sarcosyl (40%)	0.432	0.324
Lysis-DTT Buffer		
DNA IQ™ Lysis Buffer	N/A	N/A
DTT (1M)	N/A	N/A
DNA IQ™ Resin solution		
Lysis-DTT Buffer	N/A	N/A
DNA IQ™ Resin	N/A	N/A
DNA IQ™ 1x Wash Buffer	N/A	N/A
DNA IQ™ Elution Buffer	N/A	N/A

Note: Do not directly pipette from reagent stock bottles. Stock reagents should be decanted into working containers where appropriate.

12.4 REAGENT VOLUMES FOR AUTOMATED DNA IQ™

Table 11 - Table of reagent volumes.

Reagent	Volume for 96 samples (mL)	Volume for 76 samples (mL)	Volume for 48 samples (mL)
Extraction Buffer (300µL/sample)			
TNE buffer	N/A	N/A	N/A
Proteinase K (20mg/mL)	N/A	N/A	N/A
Sarcosyl (40%)	N/A	N/A	N/A
Lysis-DTT Buffer			
DNA IQ™ Lysis Buffer	90	70	50
DTT (1M)	0.9	0.700	0.5
DNA IQ™ Resin solution			
Lysis-DTT Buffer	6.0	6.0	3
DNA IQ™ Resin	1.0	1.0	0.5
DNA IQ™ 1x Wash Buffer	35.0	30.0	18
DNA IQ™ Elution Buffer	14.0	12.0	8

Note: Do not directly pipette from reagent stock bottles. Stock reagents should be decanted into working containers where appropriate.

Forensic and Scientific Services
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DNA IQ™ Method of Extracting DNA from Casework and Reference Samples

1 PURPOSE AND SCOPE

This document outlines the automated and manual procedures for extracting DNA from reference and casework samples using the DNA IQ™ kit (Promega Corp., Madison, WI, USA) within Forensic DNA Analysis Unit. The automated procedure within this document utilises the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (MPII) (PerkinElmer BioDiscovery, Downers Grove, IL, USA).

Reference samples and casework samples must be extracted on separate batches. If casework and reference batches are to be extracted on the same MPII, the instrument and all associated lab-ware must be decontaminated between operations.

This procedure applies to all staff members who are required to extract DNA from samples using automated or manual DNA IQ™ methods

2 DEFINITIONS

DTT	1,4 Dithiothreitol
EDTA	Ethylenediaminetetraacetatic acid
Extracts	Samples that have undergone DNA extraction
Lysates	Samples that have undergone off-deck lysis but not DNA extraction
MPII	MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform
Paramagnetic	Becomes magnetic with the application of a magnetic force
Pro K	Proteinase K
Samples	Sample substrates in tubes awaiting a DNA extraction process
Sarcosyl	N-Lauroylsarcosine
TNE	Tris, NaCl and EDTA buffer

3 PRINCIPLE

3.1 DNA IQ™ KIT

The DNA IQ™ system (Promega Corp., Madison, WI, USA) is designed to extract and purify DNA from a number of different substrates and has been optimised for trace DNA samples. An in-house validation was performed using a modified version of the PerkinElmer automated protocol.

Cell lysis is performed using DNA IQ™ Lysis Buffer containing Dithiothreitol (DTT). 1,4 Dithiothreitol is a reducing agent used in extractions to break disulfide bonds of proteins. The Lysis Buffer used is a proprietary buffer containing chaotropic salts required for binding of DNA to the magnetic beads. According to the MSDS (Promega Corporation 2006), the Lysis Buffer contains 50-75% guanidinium thiocyanate, < 2.5% EDTA, < 2% 3-[(3-Choalamidopropryl)dimethylammonio] propanesulfonic and < 2% polyethylene glycol tert-octylphenyl ether.

The foundation of the DNA IQ™ system is the magnetic bead resin which contains novel paramagnetic particles covered with silica. The resin has a negative charge at basic and

near neutral pH. However, the addition of Lysis Buffer changes the pH and salt concentration of the solution, resulting in the silica on the magnetic beads becoming positively charged and able to bind free DNA.

The magnetic bead resin has a DNA binding capacity of 100ng and binding is more efficient with samples containing small amounts of DNA rather than samples with large amounts of DNA. As a result, small sample size is necessary to ensure efficient recovery of DNA.

Inhibitors are removed from the DNA through several washing steps. The first washing step is performed using Lysis Buffer to ensure the DNA is bound to the magnetic bead resin and to wash out inhibitors. The next three washing steps incorporate the use of DNA IQ™ Wash Buffer. This buffer contains an alcohol/aqueous mixture which dehydrates the DNA to ensure it is not eluted during washing, while the aqueous phase washes out inhibitors.

The DNA IQ™ Elution Buffer changes the salt content which, in conjunction with heating to 65 ºC, allows the DNA to be released from the magnetic beads. The Elution Buffer is a low ionic strength buffer that reduces the affinity of the DNA for the silica that covers the magnetic beads by re-hydration of the phosphate backbone. To prevent PCR inhibition caused by the presence of very small DNA fragments within extracts the DNA IQ™ System selectively isolates DNA fragments greater than 80bp.

3.2 OFF-DECK LYSIS PROCEDURE

Samples must undergo a pre-extraction Off-Deck Lysis step before processing with the DNA IQ™ reagents on the MPII instrument.

The Extraction Buffer used for the Off-Deck Lysis is designed to digest cellular material and release DNA. It contains TNE buffer (Tris, NaCl, EDTA, pH 8.0), Sarcosyl and Pro K.

TNE acts as a basic buffer with EDTA chelating ions in solution.

Sarcosyl is a detergent that releases cellular material from the substrate.

Pro K is an enzyme from the fungus *Engyodontium album* (formerly *Tritirachium album*). As a highly active endopeptidase, it cleaves peptide bonds adjacent to the carboxyl group of N-substituted (blocked alpha amino) hydrophobic, aliphatic and aromatic amino acids. Pro K is added to the extraction buffer to digest cellular material, helping the DNA to be released. It also digests proteins that interfere with the DNA binding capacity of the resin and rapidly inactivates enzymatic activity that could potentially degrade DNA (e.g. nucleases).

3.3 MULTIPROBE® II HT EX PLUS WITH GRIPPER™ INTEGRATION PLATFORM

Within Forensic DNA Analysis, DNA extractions are performed on casework or reference samples using two MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms (MPII-A or MPII-B) located in Room 3191.

Each platform uses a computer-controlled Cartesian XYZ liquid handling system designed for the efficient automation of sample preparation. Liquid transfer is performed by an 8-tip system with VersaTip® and VariSpan™ options. The VersaTip® option allows the use of both fixed and disposable tips (conductive and non-conductive). The VariSpan™ option permits variable probe spacing between each of the individual probes so that a wide range of lab-ware such as micro plates, tubes, vials and reagent troughs can be accessed. Each sample probe is capable of independent motion in the Z direction due to independent Z drives.

The 8-tip system is also capable of multichannel liquid-level sensing by utilising Accusense™ technology. This technology works by each probe detecting a change in capacitance when in contact with the liquid. This capacitive mode of detection is also

possible when using conductive disposable tips. Accusense™ also permits the detection of low or non-ionic polar solutions and solvents. Pipetting on the platforms is driven by 8 individually controlled syringes via positive displacement of the system liquid (nanopure water) when transferring liquid.

The Gripper™ Integration allows for automated identification of lab-ware via the scanning of barcodes and also allows for automated transfer of plates from one position on the deck to another. To increase deck space and capacity, the platforms are equipped with a left deck extension.

For automated DNA extraction using the DNA IQ™ kit, a plate map is utilised to provide the necessary information for correct volumes and locations for pipetting. It also contains information regarding batch and plate identification, sample positions, lab numbers of samples, and lists all the samples in the batch, providing the total number of samples. The program will fail to work without a plate map.

4 REAGENTS AND EQUIPMENT

4.1 REAGENTS

Table 1 outlines all the reagents and storage locations required for Manual or Automated DNA IQ™ extraction.

Table 1 Reagents with storage room and location

Reagent	Room	Location
Amphyl (0.2 and 1%)	3191	Sink
DNA IQ™ Elution Buffer	3188	In-use tray or shelf
DNA IQ™ Lysis Buffer	3188	In-use tray or shelf
DNA IQ™ Resin	3188	In-use tray or shelf
DNA IQ™ 2x Wash Buffer	3188	In-use tray or shelf
DTT (1M)	3188	Freezer
70 % v/v ethanol	3188, 3189, 3191	Sink or bench
100 % v/v ethanol	3188	Shelf
5 % v/v Hypo 10 bleach	3188, 3189, 3191	Sink or bench
Isopropyl alcohol	3188	Shelf
Nanopure water	3188, 3194	Sink
40 % w/v Sarcosyl	3188	In-use tray or shelf
Pro K (20 mg/mL)	3188	Freezer
TNE	3188	In-use tray or shelf
5 % v/v Trigene	3191	Sink

4.1.1 Extraction Buffer

Prepare Extraction Buffer just prior to commencing the Manual DNA IQ™ or Off-Deck Lysis procedures.

1. Use the correct table in the appendices (Section 18) for the required procedure to determine the reagent volumes.
2. Remove the appropriate aliquot/s of 20 mg/mL Pro K from the freezer and thaw. Vortex mix and briefly centrifuge before use.

3. Ensure that the 40 % w/v Sarcosyl is completely dissolved and appears clear in the stock solution. If not dissolved, invert the container a few times and leave at room temperature.
4. In the biohazard cabinet, aliquot the required amount of TNE buffer into a 50 mL tube. Do not use directly from the stock bottle, use a smaller working aliquot instead.
5. Add the appropriate volume of 20 mg/mL Pro K and 40% w/v Sarcosyl to the TNE buffer and invert gently to mix.
6. Label the tube with **"Extraction Buffer"**, your initials and the date.
7. Note down all reagent lot numbers.

4.1.2 Lysis Buffer with DTT Solution

Lysis Buffer is supplied with the DNA IQ™ kit. The Lysis Buffer with DTT solution is prepared just prior to commencing the Manual or Automated DNA IQ™ procedures.

Warning: Lysis Buffer and DTT are toxic, use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling Lysis Buffer.

1. Use the correct table in the appendices (Section 18) for the required procedure to determine the reagent volumes.
2. Remove the appropriate aliquot/s of DTT from the freezer and thaw. Vortex mix and centrifuge briefly before use.
3. In the biohazard cabinet, add the required volume of Lysis Buffer to a sterilised glass Schott bottle or 50mL tube.
4. Add the required volume of DTT to the Lysis Buffer and gently swirl to mix.
5. Label the container with **"Lysis Buffer + DTT"**, your initials and the date.
6. Note down all reagent lot numbers.

4.1.3 DNA IQ™ Resin

DNA IQ™ Resin is supplied with the DNA IQ™ kit. The resin solution is prepared using the Lysis Buffer with DTT solution (from section 4.1.2) just prior to commencing the Manual or Automated DNA IQ™ procedures.

Warning: Lysis Buffer and DTT are toxic, use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling Lysis Buffer.

Note: Ensure the resin is properly mixed by vortex mixing prior to use.

1. Use the correct table in the appendices (Section 18) for the required procedure to determine the reagent volumes.
2. In the biohazard cabinet, aliquot the required amount of Lysis Buffer with DTT solution (from 4.1.2) into a 10, 5 or 2 mL tube.
3. Thoroughly vortex mix the DNA IQ™ resin and immediately add the required volume to the Lysis Buffer with DTT solution.
4. Mix by gentle inversion.
5. Label the tube with **"Resin"**, your initials and the date.
6. Note down all reagent lot numbers.

4.1.4 1x Wash Buffer

Note: 2x Wash Buffer is supplied with the DNA IQ™ kit.

To prepare 1x Wash Buffer:
1. In a biohazard cabinet, add 35 mL of *AnalR* 100 % Ethanol to the 2x Wash Buffer bottle.
2. Add 35 mL of Isopropyl Alcohol to the 2x Wash Buffer bottle.
3. Update the 1x Wash Buffer reagent audit trail in AUSLAB:

- From the main page press <2> "Sample Processing"
- Press <8> "Materials Processing"
- Press <2> "Consumable Inventory"
- Highlight "DNA IQ Wash Buffer" in the list and press <enter>
- Highlight the correct lot number and press <SF8> "Consumable Lot Audit"
- Add audit entry that specifies the 1x Wash Buffer aliquot (e.g. A1) and the lot numbers of the ethanol and isopropyl alcohol used.

Note: 1x Wash Buffer can be prepared and stored at room temperature prior to use in an automated or manual DNA IQ™ procedure.

1. Use the correct table in the appendices (Section 16) for the required procedure to determine the reagent volume.
2. In the biohazard cabinet, aliquot the required amount of 1x Wash Buffer into a 50 or 10 mL tube.
3. Label the tube with **"Wash Buffer"**, your initials and the date.
4. Note down the reagent lot number.

4.1.5 Elution Buffer

Elution Buffer is supplied with the DNA IQ™ kit.

1. Use the correct table in the appendices (Section 18) for the required procedure to determine the reagent volume.
2. In the biohazard cabinet, aliquot the required amount of 1x Wash Buffer into a 50 or 10 mL tube.
3. Label the tube with **"Wash Buffer"**, your initials and the date.
4. Note down the reagent lot number.

4.2 EQUIPMENT

4.2.1 Equipment and consumables required for Manual DNA IQ™ and Off-Deck Lysis

Table 2 outlines the equipment and location required for Manual DNA IQ™ or Off-Deck Lysis procedures.

Table 2 Equipment with asset number and location for each procedure

Equipment	Asset No.	Location	Procedure
Fridge	30433424	3189	Manual DNA IQ™; Off-deck Lysis
Freezers		3189 / 3190	Manual DNA IQ™; Off-deck Lysis
Biological safety cabinet class II x 5		3188 x 1 3189 x 3	Manual DNA IQ™; Off-deck Lysis
PCR cabinet		3189	Manual DNA IQ™; Off-deck Lysis
96 well tube racks	N/A	3189	Manual DNA IQ™; Off-deck Lysis
Vortex x 4	30435255 30435256 002123941 806021325	3189	Manual DNA IQ™; Off-deck Lysis
Hot block x 4	30435115 30435113 30435114 30435112	3189 / 3191	Manual DNA IQ™; Off-deck Lysis
Centrifuge x 4	30433323 30433324 10233209 30433322	3189	Manual DNA IQ™; Off-deck Lysis

Mini centrifuges x 4	30434993 30087075 30087057 041129	3189	Manual DNA IQ™; Off-deck Lysis
Finnpipettes 100 – 1000 µL	N/A	3189	Manual DNA IQ™; Off-deck Lysis
Finnpipette 20 – 200 µL	N/A	3189	Manual DNA IQ™
Thermo mixer x 2		3189	Manual DNA IQ™
Magnetic rack	N/A	3189	Manual DNA IQ™
Shaker		3191	Manual DNA IQ™

Table 3 outlines the consumables and location required for Manual DNA IQ™ or Off-Deck Lysis procedures.

Table 3 Consumables and location for each procedure

Consumables	Location	Procedure
5 mL, 10 mL and 50mL sterile tubes	3188 / 3189	Manual DNA IQ™; Off-deck Lysis
1000 µL ART or One-touch tips	3188 / 3189	Manual DNA IQ™; Off-deck Lysis
1.5mL or 2mL tubes	3188 / 3189	Manual DNA IQ™; Off-deck Lysis
Spin baskets and 2 mL collection tubes	3189	Manual DNA IQ™; Off-deck Lysis
Inoculation sticks	3189	Manual DNA IQ™; Off-deck Lysis
300 µL ART or 200 µL One-touch tips	3188 / 3189	Manual DNA IQ™
Nunc Bank-it™ tubes	3189	Manual DNA IQ™; Off-deck Lysis
Nunc Bank-it™ caps	3189	Off-deck lysis
Sharps bin	3188 / 3189	Manual DNA IQ™; Off-deck Lysis

Further consumables can be found in the Store Room (3184).

4.2.2 Equipment and consumables required for Automated DNA IQ™

Table 4 outlines the equipment with asset number and location required for Automated DNA IQ™.

Table 4 Equipment with asset number and location for Automated DNA IQ™

Equipment	Asset No.	Location
STORstar (B)	10238493	3190
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (MPII-A)	10076438	3191
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (MPII-B)	10076437	3191
DPC shaker x 2	N/A	3191
Automated temperature controller, heat block tiles and heat block adapters x 2	N/A	3191
Milli-Q Integral 3 (A10) water purification system		3194
Eppendorf 5804 centrifuge	10238416	3191
Vortex	30087015	3191
Fridge	30433424	3191
MixMate	30512822	3191
Capit-All automated decapper	None	3191
4titude 4seal sealer	30512847	3191

Table 5 outlines the consumables and location required for Automated DNA IQ™.

Table 5 Consumables and location for Automated DNA IQ™

Consumables	Location

175µL Clear Non-Conductive Filter RoboRack tips – Pre-Sterilised	3191
MβP Pure 1000µL Tips – Pre-Sterilised	3191
Abgene 96-deep well plate	3191
Axygen 2mL deep well storage plate	3191
96 well Half Skirt PCR Microplate	3191
4titude Piercing Film	3191
12 Channel plate	3191
Nunc Bank-it™ tubes	3191
Nunc Bank-it™ caps	3191
Sterile 50mL Falcon tubes	3188
Sterile 10mL or 5mL tubes	3188
Autoclaved 100mL glass bottles	3191
Autoclaved 250mL glass bottles	3191
Aluminium sealing film	3191
300uL ART tips	3188
1000µL ART tips	3191

Further consumables can be found in the Store Room (3184).

5 SAFETY

As per the standard laboratory procedure QIS 22857 Anti-Contamination Procedure, PPE is to be worn by all staff when performing this procedure. This includes the use of safety glasses.

The MPII instruments, labware, biohazard cabinets and centrifuges should be wiped with **5% v/v Trigene only**, followed by 70 % v/v ethanol.
Work benches and non-metallic equipment should be wiped with 5 % v/v Hypo 10 bleach, followed by 70 % v/v ethanol.

Warning: Do not place any part of the body inside the cabinet while the MPII is running a procedure.

If it is necessary to access the deck, pause the program using the computer software or the emergency STOP button located on the front of the instrument.

Warning: Tris base, EDTA, Sarcosyl and Lysis Buffer are irritants. DTT is a reducing agent that destroys disulfide bonds. Handle carefully and wear appropriate PPE.

Lysis Buffer contains guanidinium thiocyanate (GuSCN) which is toxic and can be harmful if inhaled, swallowed or comes in contact with skin. Left over Lysis Buffer-DTT is to be disposed of in a brown Winchester bottle in the ventilated cupboard in Room 3191. Never dispose down the sink. If spillage occurs, absorb with liquid-binding material (such as sand or kitty litter) and dispose in a biohazard bin.

Do not combine bleach with Lysis Buffer and do not wipe areas of Lysis Buffer spillage with bleach, as the two reagents produce cyanide gas when combined. Handle carefully and wear appropriate PPE, including safety glasses, when handling Lysis Buffer. If Lysis Buffer is spilt onto PPE (eg. Gloves, gowns), remove and discard the contaminated item/s in a biohazard bin.

6 SAMPLING AND SAMPLE PREPARATION

6.1 SAMPLE LOCATIONS

Samples waiting to be extracted are stored in freezers as detailed in Table 6.

Table 6 Sample storage locations

Sample type	Storage Device	Storage Location
Urgent/High/Medium Priority Samples	Freezer	3190 or 6106*
Lysates in 1.0mL Nunc Bank-it™ tubes	Freezer	3190
Extracts in 1.0mL Nunc Bank-It™ tubes	Freezer	3194 A

* Some Medium and Low Priority storage boxes are located in Block 6, within the Exhibit Room (6106).

6.2 QC SAMPLES

One negative reagent control and one positive blood control (from a known donor) must be registered for all extraction batches. Additionally, off-deck lysis batches require five blank controls as detailed in Table 7.

Table 7 Extraction Quality Controls

Batch Type	Control
Off-deck lysis	Positive control (x1)
	Negative control (x1)
	Blank control (x5)
Manual DNA IQ™	Positive control (x1)
	Negative control (x1)
Retain supernatant DNA IQ™	Positive control (x1)
	Negative control (x1)

6.3 REGISTRATION OF QC SAMPLES

Register control samples according to the standard laboratory procedure QIS 24919 Forensic DNA Analysis Workflow Procedure. CQ samples for off-deck lysis and Auto extractions are to be registered with a 9PLEX test code. QC samples for a DNA IQ Maxwell extraction are to be registered using the XPLEX test code.

6.4 CREATION OF EXTRACTION BATCHES

Create extraction batches according to the standard laboratory procedure QIS 24919 Forensic DNA Analysis Workflow Procedure. All samples that are registered using a 9PLEX test code will be extracted using the automated DNA IQ method with the exception of tapelifts that will be processed using the DNA IQ Maxwell procedure.

6.5 LOCATING SAMPLES

Locate samples according to the standard laboratory procedure QIS 23959 Storage Guidelines for Forensic DNA Analysis.

6.6 SEQUENCE CHECKING AUTOMATED EXTRACTION BATCHES

Sequence check lysates in Nunc Bank-It tubes for automated extraction batches according to the standard laboratory procedure QIS 24256 Procedure for the use of the STORstar unit for automated sequence checking.

6.7 ELECTRONIC WORKFLOW DIARY

Within the electronic workflow diary I:\AAA Electronic Workflow Diary record details of the batch sample creation and processing.

7 MANUAL METHODS FOR EXTRACTION USING DNA IQ™

7.1 MANUAL DNA IQ™ (NO RETAIN SUPERNTANT)

Note: All manual extractions are to be performed within a biohazard cabinet in room 3189.

1. Prepare fresh reagents within the biohazard hood of the clean room (3188) prior to commencing the procedure. Refer to Section 4.1 for preparation of reagents. Use Table 8 in Appendix 12.1 for reagent volumes.

2. Turn on the Eppendorf Thermomixer and set to 37 °C and 1400 rpm.

3. Label for each sample:
   - Original sample tube;
   - Spin basket (if required);
   - 2mL tube; and
   - Nunc™ Bank-It™ tube.

Note: A spin basket is required for swabs, fabric, paper and cigarette butts. Samples that do not require a spin basket are tape-lifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

4. Have a second operator perform a sequence check of all tubes. Record the sequence check in AUSLAB.

5. Add 300 µL of Extraction Buffer to each sample. Ensure that large substrates including tape-lifts are fully submerged.

6. Vortex and incubate at 37 °C on the Thermomixer at 1400 rpm for 45 minutes.

7. Remove from the Thermomixer and increase the temperature on the Thermomixer to 65 °C for use in the elution steps.

8. Transfer the substrate to spin basket (if required) or transfer the lysate to an appropriately labelled 2.0 mL tube. Retain the original tube containing the substrate if no spin basket is used.

9. Centrifuge the spin basket at 15800 xg for 2 minutes.

10. Transfer the lysate from the original sample tube and the flow-through from the collection tube of the spin basket to an appropriately labelled 2 mL tube. Transfer the substrate from the spin basket back into the original sample tube.

    Note: If original sample tube is an appropriate 2 mL tube, transfer the substrate into the new 2 mL tube and add the flow-through from the spin basket to the lysate still in the original sample tube.

11. Add 550 µL of Lysis Buffer-DTT solution to the lysate.

12. Add 50 µL of DNA IQ™ Resin-Lysis Buffer-DTT solution to the lysate. Pipette mix or vortex the solution frequently to keep a homogenous suspension of resin within the solution.

13. Vortex and place on the Multitube shaker set at 1200 rpm for 5 minutes.

14. Remove from the Multitube shaker, vortex and immediately place into the magnetic stand. Separation will occur instantly.

    Note: If resin does not form a distinct pellet on the side of the tube, or if the magnetic beads have re-suspended while in the stand, vortex the tube and quickly place back in the stand.

15. Carefully remove and discard the liquid, ensuring that the resin is not disturbed. Remove from the magnetic stand.

    Note: If some resin is drawn up in the pipette tip, gently expel resin back into tube to allow re-separation.

16. Add 125 µL of Lysis Buffer-DTT solution. Vortex and place into the magnetic stand.

17. Once separation has occurred, remove and discard the liquid. Remove the tube from the magnetic stand.

18. Add 100 µL of 1X Wash Buffer, vortex and place into the magnetic stand. Once separation has occurred remove and discard the liquid. Remove the tube from the magnetic stand.

19. Repeat step 18 twice for a total of three Wash Buffer washes. Ensure that all of the liquid has been removed after the last wash. Remove the tube from the magnetic stand.

20. Uncap the tubes and place the lids down onto a clean rediwipe within the biohazard cabinet. Allow the resin to air-dry in the hood for 15 minutes and then recap the tubes.

    Note: Do not allow the resin to dry for more than 20 minutes as this may inhibit DNA elution.

21. Add 50 µl of Elution Buffer by carefully pipetting the solution to the side of the tube, above the pellet. **Do not mix**.

22. Incubate in the Thermomixer at 65°C for 3 minutes and then continue to incubate for a further 3 minutes shaking at 1100 rpm. If no thermomixer is available, incubate samples within a hot block and vortex mix at the start and end of the second 3 minute incubation. Remove samples.

23. Vortex and immediately place into the magnetic stand while hot to ensure maximum DNA yield during elution.

24. Carefully transfer the DNA extract to the corresponding labelled Nunc™ Bank-It™ tube.

25. Remove from the magnetic stand and repeat steps 21-23 once. The final volume after the second elution is approximately 95 µL of DNA extract.

26. Store the DNA extracts in temporary storage located within the Pre-PCR sorting room 3194-A in the upright freezer with equipment number 30512883.

27. Store tubes containing substrates in Spin Basket boxes located within the Pre-PCR sorting room 3194-A in the upright freezer with equipment number 30512883.

7.2 PROCEDURE FOR MANUAL DNA IQ™ (RETAIN SUPERNTANT)

Note: All manual extractions are to be performed within a biohazard cabinet in room 3189.

1. Prepare fresh reagents within the biohazard hood of the clean room (3188) prior to commencing the procedure. Refer to Section 4.1 for preparation of reagents. Use Table 9 in the Appendix for reagent volumes.

2. Turn on the Eppendorf Thermomixer and set to 37 ˚C and 1400 rpm.

3. Label for each sample:
   - Original sample tube;
   - 1.5 or 2 mL tube labelled 'SUP' for supernatant;
   - Spin basket (if required);
   - 2 mL tube; and
   - Nunc™ Bank-It™ tube.

   Note: A spin basket is required for swabs, fabric, paper and cigarette butts. Samples that do not require a spin basket are tape-lifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

4. Have a second operator perform a sequence check of all tubes. This person must enter the sequence check in AUSLAB.

5. Add 450µL of TNE buffer to the sample and vortex.

6. Incubate at room temperature for 30 minutes.

7. Vortex, then centrifuge at 15800 xg for 3 minutes.

8. Remove 150 µL of supernatant and place into the respective 1.5 mL tube labelled with 'SUP' (for further testing).

9. Add 14µL of 20mg/mL Proteinase K and 7µL 40% (w/v) Sarcosyl to the original sample tube containing substrate and TNE buffer, then vortex.

10. Vortex, then incubate at 37°C on the Thermomixer at 1400 rpm for 45 minutes. If no thermomixer available, incubate samples within a hot block, vortex mix at the start and end of the incubation and at least one during the incubation.

11. Remove from the Thermomixer and increase the temperature on the Thermomixer to 65°C (for use in the Elution steps).

12. Transfer substrate to spin basket if required or transfer the lysate to an appropriately labelled 2.0mL tube. Retain original tube containing the substrate in if no spin basket used.

13. Centrifuge spin basket at 15800 g for 2 minutes.

14. Transfer the substrate in the spin basket into a new and labelled 2mL tube to be retained. Transfer the flow through (lysate) back into the original sample tube.

15. Add 550µL of Lysis-DTT Buffer solution.

16. Add 50µL of DNA IQ™ Resin-Lysis solution. Pipette mix the Resin-Lysis solution between each addition to keep the resin suspended in solution.

17. Vortex and place on the Multitube shaker set at 1200 rpm for 5 minutes.

18. Remove from the Multitube shaker, vortex and immediately place into the magnetic stand. Separation will occur instantly.

    Note: If resin does not form a distinct pellet on the side of the tube, or if the magnetic beads have re-suspended while in the stand, vortex the tube and quickly place back in the stand.

19. Carefully remove and discard the solution, ensuring that the resin is not disturbed. Remove from the magnetic stand.

    Note: If some resin is drawn up in the pipette tip, gently expel resin back into tube to allow re-separation.

20. Add 125µL of Lysis-DTT Buffer solution. Vortex and place into the magnetic stand.

21. Once separation has occurred, remove and discard the Lysis-DTT Buffer. Remove from the magnetic stand.

22. Add 100µL of 1X Wash Buffer, vortex and place into the magnetic stand. Once separation has occurred remove and discard the Wash Buffer. Remove from the magnetic stand.

23. Repeat the Wash Buffer step (step 23) two times for a total of three washes. Ensure that all of the solution has been removed after the last wash. Remove from the magnetic stand.

24. Uncap the tubes and place the lids down onto a clean rediwipe. Air-dry the resin in the hood for 15 minutes and then recap tubes

    Note: Do not dry for more than 20 minutes, as this may inhibit the elution of DNA.

25. Add 50µl of Elution Buffer by carefully pipetting the solution to the side of the tube, above the pellet. **Do not mix**.

26. Incubate in the Thermomixer at 65°C for 3 minutes and then continue to incubate for a further 3 minutes shaking at 1100 rpm. If no thermomixer available, incubate samples within a hot block, vortex mix at the start and end of 2nd 3 minute incubation. Remove samples.

27. Vortex and immediately place into the magnetic stand while hot to ensure maximum DNA yield during elution.

28. Carefully transfer the DNA extract to the corresponding labelled Nunc™ Bank-It™ tube.

29. Remove from the magnetic stand and repeat the Elution Buffer steps (step 26-29). The final volume after the double elution is approximately 95µL of DNA extract.

30. Store the DNA extracts in temporary storage located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

31. Store tubes containing substrates in Spin Basket boxes located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

32. Store supernatants in the S/N Retention boxes located within the Extraction sorting room in the Orford Freezer 30512883 (Room 3190).

33. Ensure that the negative extraction control from the batch is also inserted onto the **'Saliva List':**

    - Log into Auslab
    - 3 for Patient Enquiry
    - Type/Scan the lab number for the negative extraction control
    - From the 9Plex page or the XPLEX Page press Shift F12
    - A prompt will appear 'Enter List Name'
    - Type Saliva or use the F1 lookup list.
    - Press Enter

8 OFF-DECK LYSIS PROCEDURE

1. All reagents are to be made fresh within the clean room (3188) prior to commencing each process. Use Table 10 in Appendix 12.3 for reagent volumes.

2. All manual extractions are to be performed within a biohazard cabinet in Room 3189

3. For each sample label:
    - Original sample tube
    - Spin basket as required
    - 1.0mL Nunc Bank-It™ tube
    - 1.5mL or 2.0mL tube

    Note: Samples that require a spin basket are: swabs, fabric, paper and cigarette butts. Samples that do not require a spin basket are: tapelifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

4. If substrate is in a 0.5mL tube, transfer to a labelled 1.5mL or 2.0mL tube.

5. Have a second operator sequence check the tubes and enter the sequence check in AUSLAB.

6. Add 300µL of Extraction Buffer and vortex briefly. Ensure that substrates are immersed in the Extraction Buffer.

7. Incubate for 45 minutes at 37 degrees on a hotblock. It is best practice to vortex the samples intermittently throughout the duration of the incubation period. Record temperature on worksheet.

8. Remove from the hotblock. Transfer substrate to spin basket if required or transfer the lysate to an appropriately labelled 1.5mL or 2mL tube. Retain original tube containing the substrate if no spin basket is used.

9. Centrifuge spin baskets at 15800 g for 2 minutes.

10. Transfer the substrate in the spin basket to a new and appropriately labelled 1.5mL or 2mL tube and retain.Transfer the flow through back to original lysis tube.

11. Vortex lysate, then incubate on hotblock at 65°C for 10 minutes. Record temperature on worksheet.

12. Centrifuge at 15800 g for 1 minute.

13. Transfer 300µL of lysate to the corresponding Nunc Bank-It™ tube.

Note: If more than 300µL of lysate is present, retain the remaining lysate in the 1.5mL tube. In AUSLAB, write a specimen note for that sample (eg. Extra lysate retained from sample XXXXXXXXX.). Store the retained 1.5mL lysate tube in appropriate box in freezer.

14. In AUSLAB, enter reagent and hotblock temperature details and complete the batch.

15. Store lysates in temporary storage boxes located within the extraction sorting room in the orford Freezer with equipment number 102384430 (Room 3190).

16. Store tubes containing substrates or extra lysate in Spin Basket boxes located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

9 AUTOMATED EXTRACTION OF LYSED SAMPLES

9.1 MPII Extraction PROCEDURE

Automated setup of DNA IQ extractions in 96-deep well format is performed using the MultiPROBE® II PLUS HT EX EP-A and EP-B platforms located in Room 3191.

Refer to QIS 23939 Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform for instructions on the use and maintenance of the MultiPROBE® II PLUS HT EX platforms.

9.2 PREPARATION OF REAGENTS FOR THE AUTOMATED EXTRACTION PROCESS

All reagents are to be made fresh within the clean room (3188) prior to commencing each process. Use Table 11 in the Appendix for reagent volumes.

9.3 SUMMARY OF DNA IQ™ EXTRACTION VERSION 6.6_ODL

9.3.1 Transfer of lysates from Nunc Bank-It™ tubes into the Abgene 96-deep well plate

Lysates from the off-deck lysis protocol, contained in individual Nunc Bank-It™ tubes, are transferred automatically into an Abgene 96-deep well plate prior to commencing automated sample processing. Instead of a 96-deep well plate, the use of individual Nunc Bank-It™ tubes for storage of lysates removes the need for an adhesive film and therefore reduces the risk of well-to-well contamination from the action of peeling back an adhesive plate cover.

9.3.2 Automated addition of DNA IQ™ Resin and Lysis Buffer

DNA IQ™ Resin is added automatically into the Abgene 96-deep well plate on the platform. The program uses a waste mode dispensing step to add 53µL DNA IQ™ Resin-Lysis Buffer solution to each well in either half the plate or the whole plate. Because of this, batch sizes are restricted to either 48 or 96 samples in order to maintain efficiency and economy. Two volumes of Lysis Buffer are then added to promote binding of DNA to the paramagnetic resin.

9.3.3 Mixing using a MixMate to bind DNA to resin

Manual intervention is required to seal the Abgene 96-deep well plate with a 4titude Pierce Seal film using the 4titude sealer pre-heated at 175 °C. The plate is then transferred onto a MixMate instrument for mixing at 1100rpm for 5 minutes, followed by centrifugation on an Eppendorf 5804 centrifuge at 3000rpm for 2 minutes. The seal is then carefully pierced with a 96 well PCR microplate and the Abgene plate is returned to the Applied Biosystems magnet on the MPII platform.

9.3.4 Removing lysis reagents for storage

At this point, most of the DNA is bound to the paramagnetic resin. With the positioning of the Abgene plate on the ABI magnet, DNA IQ™ Resin becomes immobile at the bottom of the plate. The lysis reagents from each well are transferred automatically to a storage plate on the MPII platform without disturbing the DNA IQ™ Resin.

9.3.5 Washing of the resin-DNA complex

Washing steps are performed to remove any inhibitors in solution. The first wash uses 125µL Lysis Buffer with shaking at room temperature for 1 minute on the DPC shaker to ensure that the DNA is bound to the paramagnetic resin. The plate is moved to the ABI magnet and the supernatant is transferred into the storage plate. The next three washes incorporate 100µL of 1x Wash Buffer with shaking at room temperature for 1 minute on the DPC shaker. During each wash cycle, the Abgene 96-deep well plate is moved to the ABI magnet and the supernatant is discarded into the tip chute.

9.3.6 Removing any excess of 1x Wash Buffer

The samples are allowed to air dry at room temperature for 5 minutes in order to remove all traces of Ethanol from the Wash Buffer. The presence of Ethanol may potentially inhibit both the elution process and also downstream PCR.

9.3.7 Elution of DNA from the resin-DNA complex

A double elution method is employed in this procedure. At each elution step, 60µL of Elution Buffer is added to each sample, followed by incubation at 65°C for 6 minutes (3 minutes without shaking followed by 3 minutes shaking on the DPC shaker). The Abgene plate is moved to the ABI magnet and the eluted solution (supernatant containing eluted DNA) is transferred to fresh Nunc™ Bank-It™ tubes.

9.3.8 Flushing of liquid pathway

As a decontamination measure, the liquid pathway is washed with Amphyl and Nanopure water at the conclusion of the automated extraction process.

9.4 SETTING UP THE MPII PLATFORMS FOR AUTOMATED DNA IQ™ PROCESSING

The following steps are carried out in the automated extraction room (Room 3191).

1. Remove the Nunc Bank-It™ tubes containing lysates from the fridge to allow to come to room temperature before commencing the extraction procedure.

   Note: If the lysates are frozen, remove them from the freezer and thaw in Room 3191.

2. Restart or turn on the instrument PC.
3. Log onto the network using the **Robotics** login.
4. Open WinPrep® by double clicking icon on the computer desktop (Figure 1).

Figure 1.
The WinPrep® icon.

5. Log onto the WinPrep® software by entering your username and password, then press **"Enter"**.

6. Ensure that the daily/weekly start-up and maintenance has been performed before running any program. If WinPrep® has been closed or been idle for a long period of time initialise the MP II platform as described in QIS 23939.

7. Ensure the **System Liquid reservoir is FULL** and tubing is fully submerged in the system liquid before every run and perform a Flush/Wash. If visible air bubbles have appeared in tubing or in syringes between setting up the deck and executing the test, another flush wash will need to be performed before starting the MPII extraction run.

8. Open the Extraction setup MP II test file in WinPrep® by selecting:

   - **File**
   - **Open**, navigate to **C:\PACKARD\MULTIPROBE\BIN\QHSS PROTOCOLS**
   - Select "**DNA IQ Extraction_Ver 6.6_ODL.mpt**"
   - Click the "**Open**" button

9. Check the tree pane of the whole program for any bold fonts. See the Analytical Senior Scientist if bold fonts are present.

10. Copy the required plate map from the network folder **I:\EXTRACTION** into the local folder **C:\PACKARD\EXT PLATE MAPS.** Open the plate map using Excel and check that the plate map is complete, and make changes if necessary. If there are blanks (samples that have been removed), make all volumes to 0. If it is not a full batch, delete the rows of blank samples at the end of the platemap. Save all changes made to the platemap (as a .txt file).

11. Decontaminate the required labware with 5% TriGene followed by 70% Ethanol and place onto the instrument deck in the designated grid positions (as shown in the WinPrep® software). Additionally, ensure the DPC shaker is positioned properly.

12. Ensure that the DPC shaker and Heater Controller Box are switched on.
    For EP-A: Tile 1 at F22 (850C).
    For EP-B: Tile 2 at F22 (850C).

    Note: Press the start/stop button twice at the front of the DPC shaker to ensure that it displays zero on the screen.

13. Ensure the heat transfer tile is clicked into the plate adapter tile properly.

    Note: This is critical to ensure correct incubation temperatures.

14. To the Amphyl wash station in position **A10**, add 1% Amphyl to the far left hand side, 0.2% diluted Amphyl to the middle left and place an empty (waste) reagent trough in the middle right position of the station.

15. Add Wash Buffer to the far right hand side trough of the Amphyl wash station in position **A10**.

16. Add Lysis Buffer to the labelled 150mL reagent trough on the left hand side of the 2 trough holder in position **A13**.

17. Place the 12 channel plate into position **A16**. Add Elution Buffer to the plate by dividing the Elution Buffer between channels 11 and 12.

18. Add Resin to channel 1 of the 12 channel plate in position **A16**. It is important to add the resin in a uniform fashion to ensure equal distribution of resin along the channel.

19. **Nunc Bank-It™ lysate tubes:** The lysates should now be at room temperature. Ensure that the rack is labelled with the correct **AUSLAB batch ID** and that the label matches the batch ID on the worksheet and platemap. Additionally, randomly check some visible barcodes on the side of lysate tubes against the positions on the worksheet to ensure the correct batch of samples is to be processed.

20. Place the rack onto the MixMate to shake for 1 minute at 1000rpm.

21. Centrifuge the rack at 3000rpm for 2 minutes in the Eppendorf 5804 centrifuge and then place into position **C13**.

22. **Abgene 96-deep well plate:** With the plate in its correct orientation label the **left hand side** of the plate with both the correct **AUSLAB batch ID** and **batch ID barcode**. With a marker, print the word "**FRONT-lysate**" on the front of the plate. Place the plate in its correct orientation in position **E13**.

23. **2mL 96-deep well storage plate:** With the plate in its correct orientation label the **left hand side** of the plate with both the correct **AUSLAB batch ID** and **batch ID barcode**. With a marker, print the word **"FRONT-store"** on the front of the plate. Place the plate in its correct orientation in position **E16**.

24. **Nunc Bank-It™ extract tubes:** Ensure that the rack is labelled with the correct **AUSLAB batch ID** on the **front** of the Nunc Bank-It™ tube rack. Additionally, randomly check some visible barcodes on the side of lysate tubes against the positions on the worksheet to ensure the correct batch of samples is to be processed. Place the rack in its correct orientation in position **G16**.

25. Add Nanopure water to the 160mL trough in the Flush/Wash station in position **G13**.

26. Ensure that all necessary labware have been positioned correctly as displayed within WinPrep®, then click "**EXECUTE TEST**". Record run information in the Run Log book.

27. The following message will appear (Figure 2 below):

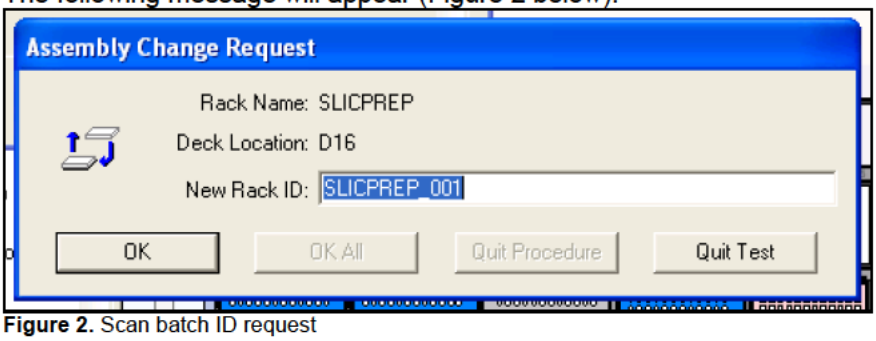

Figure 2. Scan batch ID request

Into **"New Rack ID:"** scan barcode off the worksheet. It is important this corresponds to the labelling of labware on the deck and the plate maps used.

28. Click "**Reset Tip Boxes**" and ensure that the number of tips displayed in this window match those as available on the deck platform. Fill new tips if necessary. Click "**Close**" to accept the tip count, and then click "**Next**".

29. Select the correct platemap by browsing to **C:\PACKARD\EXT PLATE MAPS**. Ensure that the platemap selected corresponds to the labelling of labware on the deck, the paperwork used and the **"New Rack ID"** entered.

30. For a full batch of 76 samples, ensure that all nodes are checked. Click "**Next**" to check all other nodes.

31. For a batch of 48 samples or less, uncheck the node: **"Add resin to second half of plate"**. Click "**Next**" to check all other nodes.

32. Click "**Start**" to continue.
    - The MPII instrument will begin and user prompts will appear as a reminder to confirm the deck setup. **Always decap tubes from positions H1 to A1, H2 to A2 etc.**
    - Ensure all steps on the first prompt have been complete, Click **OK** to continue.
    - Ensure all steps on the second prompt have been complete, Click **OK** to continue.

33. The program will progress to transfer the lysates followed by automated addition of the Lysis-DTT buffer and the DNA IQ™ Resin solution. The next user prompt will appear. Follow the steps as outlined in the user prompt. Then click **OK** when ready.

    Note: Ensure that the plate is heated and sealed properly with the Pierce Seal on the 4titude Heat Sealer. Mix the plate on the mixmate for 5min at 1100rpm and centrifuge. Pierce the plate with a PCR Microplate and then discard. (A new plate is to be used each time). Ensure the pierced plate is returned to the magnet.

34. Once lysis steps have been completed, a user prompt will appear. Follow the directions as outlined in the user prompt. Then click **OK** when ready.

    Note 1: The Lysate Nunc tubes (including the rack) should be placed into a clipseal bag, sealed and discarded into a biohazard waste bin. The Store plate can also be removed, heat sealed and discarded into a biohazard waste bin.

    Note 2: When the supernatant storage plate is sealed, check the plate for the transfer of beads. To do this, place the plate onto the ABI magnet (if beads are present they will settle to the bottom of the plate) and then inspect the bottom of the plate. If no beads are present, discard the store plate. If, however, beads are present, refer to the Section 15, Troubleshooting.

35. Once the wash steps have been completed, a user prompt will appear. Follow the directions as outlined in the user prompt. Then click **OK** when ready.

    Note: The Nunc extract tubes can be decapped on the bench in the MPII cabinet while the samples are drying and the heating tile is stabilising. At this point, the operator has approximately **12 minutes** to decap the tubes. If the operator has been interrupted and requires more time to decap the tubes, the program may be paused at this step.

36. A message will appear waiting for the heating tile to reach 85°C (for incubation at 65°C). **DO NOT PRESS CONTINUE** as the program will continue automatically when the temperature has been reached with sufficient stability.

37. A user prompt will appear. Follow the directions as outlined in the user prompt. Then click **OK** when ready.

38. Once the elution is completed, a user prompt appears. Follow the directions as outlined in the user prompt. Then click **OK** when ready.

39. The Lysate Plate is heat sealed and kept in temporary storage for one month.

40. Once all plates are removed from the deck and sealed. Click "**OK**" to proceed to the Amphyl wash step.

41. A final message will advise that the run has completed. Click "**OK**".

9.5 FINALISING THE MPII RUN

1. Transfer left over Resin solution from the 12 channel plate and the Lysis Buffer-DTT (wearing safety glasses) into the glass Lysis-DTT bottle previously used. Discard the 12 channel plate in the biohazard waste bin. Transfer the contents of glass bottle into the brown Winchester bottle located in the safety cupboard in room 3191.

2. Discard the contents of the Amphyl wash station into the sink and rinse the troughs with Nanopure water.

3. Remove all labware from the deck and clean with 5% TriGene™ followed by 70% Ethanol, and setup for the next run if necessary. In addition, clean the work area around the MPII instrument with 10% (v/v) Bleach 7x and 70% Ethanol.

4. Remove the tip chute and funnel, rinse with warm tap water to remove any residue inside the chute before cleaning with 5% TriGene and 70% Ethanol.

5. Import the platemap into Auslab and then delete it.

9.6 IMPORTING MPII LOG FILE INTO AUSLAB

1. Click on the Microsoft Access icon in the WinPrep® main menu to open the MultiPROBE log database.

2. Click **"Open"** when prompted.

3. Click on the relevant run Test ID in the Test Selection box. In the Report/Query/Action Selection dropdown menu, select **"Report: Test Summary (Sorted by Destination Rack ID)"**

4. In the Output Selection dropdown menu, select **"File"**. Save the output file as *.csv format to **C:\PACKARD\EXT PLATE MAPS\EXT LOGS** with the same name as the AUSLAB batch ID and click "**Apply**".

5. Open the log file and check for any errors that may have arisen during the extraction process. Compare the listed errors to any that were encountered during the run. Report any critical errors to the line manager.

6. Import the log file, entering the path, filename and extension (e.g. C:\Packard\ext plate maps\ext logs….) and press **[Enter]**. Delete the log file after importing.

7. For more details on importing files into AUSLAB refer to Batch functionality in AUSLAB SOP (QIS 24469).

9.7 IMPORTING EXTRACTION "RESULTS" INTO AUSLAB

1. Import the results file, entering the filename and .txt extension (e.g. CWIQEXT20100917_06.txt). For more details on importing files into AUSLAB refer to Batch functionality in AUSLAB SOP (QIS 24469).

2. The file will be imported into AUSLAB and appear in the Results Import Table.

3. Highlight entry and press **[Enter]**, for access to the Results Table.

4. Page down through the table and check that all sample results have been imported.

5. Press **[SF8]** ***Table Sort Order***, this sorts the table, sorting samples that have failed Autovalidation to the top. Samples that have failed are those that have processing comments present.

6. For samples that have failed, check the **Processing Comments,** by entering into the sample.

7. If processing comments state sample is to be sent to another batch type **other** than quant. Proceed with the following steps:
   a. Request the appropriate rework test code via the **[SF7]** results history table and the **[SF8]** request rework functions (e.g. samples requiring Microcon, NucleoSpin and pooling).
   b. Press **[Esc]** to exit back to the DNA results table. Do not toggle accept.
   c. Add the extraction batch ID ONLY into the 9PLEX or 9FTAR completed date fields in AUSLAB.

8. If processing comments do not state next step the sample will be processed as normal:
   a. Press **[Esc]** to exit back to the DNA results table.
   b. Highlight any entries to be changed and press **[SF7]** ***Toggle Accept.***

9. Press **[F7]** ***Complete Batch***, all of the samples with a ***Yes*** in the Accept column will be transferred to the outstanding Quant Casework or Quant Reference lists.

10. File the extraction worksheet into the relevant intray for scanning in room 3194A (Pre PCR-Sorting).

10 SAMPLE STORAGE

Refer to QIS 23959 Storage Guidelines for Forensic DNA Analysis for how to store the DNA extract Nunc™ Bank-It™ tubes and Abgene 96-deep well. Refer to QIS 24919 Forensic DNA Analysis Workflow Procedure for storage of extracts.

11 TROUBLESHOOTING WITH THE MPII

1. If the resin is not pipette mixing correctly (eg. Resin is not being drawn up into the pipette tip), pause the Winprep program and check that the support tile is clicked onto

the MPII deck correctly. Alternatively, pipette and mix resin manually one more time from the corresponding column in the 12 channel plate.

2. If the Gripper is not placing the rack/plate properly on the deck, pause the program and manually adjust the rack/plate properly on the adapter support tile.

3. If the Gripper appears to be not gripping a plate correctly, or the pipetting alignment of a labware appears to be incorrect, the deck and / or labware can be re-calibrated. This is covered in QIS 23939 Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform

12 QUALITY ASSURANCE/ACCEPTANCE CRITERIA

- Positive and negative (reagent blank) controls are included in each extraction batch. However, for off deck-lysis batches, an additional five negative (reagent blank) controls are included. These controls are processed as normal samples through to completion.
- If any results are obtained from the reagent blank, either at quantification or during the CEQ check, then the possible source of the contamination is investigated. The samples extracted with this control are thoroughly checked and repeated if possible

13 VALIDATION

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- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 13: Report on the Verification of the Automated Extraction Chemistry Kit using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Casework Platform." 2007.
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- Muharam I., Hlinka V., Gallagher, B., Lundie, G., Iannuzzi, C., Nurthen T., McNevin A., Ientile V. "Project 22: A Modified DNA IQ™ Method for Off-Deck Lysis Prior to Performing Automated DNA Extraction" 2008

14 STORAGE OF DOCUMENTS

The off-deck lysis worksheets are to be stored within the extraction sorting room (3190) in the nominated intray. It is essential that ALL off-deck lysis worksheets go within this intray so the operational officers are aware that batches are ready and waiting to be combined for extraction on the automated robots.

All worksheets, after auto extraction has been completed, are stored within the Pre-PCR sorting room (3194-A) in an allocated in-tray for scanning by the operational officers.

15 REFERENCES

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- Vandenberg, N., van Oorchot., R. A. H., & Mitchell, R. J., An evaluation of selected DNA extraction strategies for short tandem repeat typing. Electrophoresis, 1997. 18: p. 1624-

16 ASSOCIATED DOCUMENTS

QIS 17165 Receipt, Storage and Preparation of Chemicals, Reagents and Test Kits
QIS 17171 Method for Chelex Extraction
QIS 22857 Anti-Contamination procedure
QIS 23939 Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform
QIS 23959 Storage Guidelines for Forensic DNA Analysis
QIS 24256 Sequence Checking with the STORstar Instrument
QIS 24469 Batch functionality in AUSLAB
QIS 24919 Forensic DNA Analysis Workflow Procedure

17 AMENDMENT HISTORY

Version	Date	Author/s	Amendments
R0	23 Oct 2007	B. Gallagher, T. Nurthen, C. Iannuzzi, V. Hlinka, G. Lundie, I Muharam.	First Issue
R1	12 Dec 2007	M Harvey, C Iannuzzi, A McNevin	Reviewed and updated after initial training
R2	19 March 2008	M Harvey, B Andersen, C Iannuzzi, A McNevin	Addition of Off-deck Lysis procedure, Retention of fully automated method as Appendix, addition of reagent record tables into Appendix
R3	April 2008	QIS2 Migration Project	Headers and Footers changed to new CaSS format. Amended Business references from QHSS to FSS, QHPSS to CaSS and QHPS to Pathology Queensland
4	13 March 2009	QIS2 migration	Version incremented by one on migration to QIS2
5	03 June 2009	M Aguilera, B Micic, C Iannuzzi, A. Cheng, V. Hlinka, I. Muharam, G. Lundie, C. Weber	Major changes to reflect new procedure. Updated to reflect changes in procedure as an outcome of internal and external audits. Created ver.6.4 ODL in MPII Platforms. Minor changes in procedures using 4titude 4seal heat sealer to seal plates.
6	29 June 2009	A McNevin, K Lancaster	Removed references to retaining lysate and beads, fixed minor formatting errors. Created ver6.5 ODL in MPII Platforms. Substrates now to be retained in 2mL tube
7	30 September 2010	M.Cipollone, M.Mathieson	Major changes made RE: room numbers to reflect the move to Block 3. Re-formatted entire SOP. New Appendices added which outline all reagent volumes. All equipment and associated asset numbers have been included. Use of the electronic diary now included as an additional section. Preparation of reagents within the clean room (3188) now to be done prior to starting each process. Storage of worksheets updated. New software version 6.6 on automated extraction robots. S/N Retention Boxes now stored in Manual Ext Room. Associated Documents and hyperlinks updated. Consumables and Equipment table added for Manual DNA IQ.
8	24 May 2012	A. Speirs M. Cipollone	Major revision of document. Revision of sections 1-3 to improve clarity. Updated Tables 1 to 7. Removed redundant sections on locating

Version	Date	Author/s	Amendments
			samples and creating batches. Removed procedure for retain supernatant Off-Deck Lysis and thus associated Appendix. Table of Contents amended to reflect all changes. Moved the Manual DNA IQ procedures before the Off-Deck Lysis procedure. Re-formatted spacing and alignments of headings. Re-numbered Tables in Appendices. Updated sections to include which relevant Appendix to use in regards to reagents. Minor grammatical and spelling errors fixed.
9	4 Dec 2013	B. Andersen, M. Cipollone	Updated Section 7 of the Manual DNA IQ Retain Supernatant procedure to reflect differences in list inserting between P+ and PP21 samples. Updated Section 6 to clarify 9PLEX and XPLEX test codes. New template applied and minor formatting and wording changes.

18 APPENDICES

18.1 REAGENT VOLUMES FOR MANUAL DNA IQ™ (NO RETAIN SUPERNATANT)

Table 8 - Reagent volumes for manual method

Reagent	Volume for 12 samples (mL)	Volume for 24 Samples (mL)
TNE buffer	4.0	8.0
Proteinase K (20mg/mL)	0.216	0.432
Sarcosyl (40%)	0.108	0.216
Lysis-DTT Buffer		
DNA IQ™ Lysis Buffer	10	20
DTT (1M)	0.1	0.2
DNA IQ™ Resin solution		
Lysis-DTT Buffer	0.645	1.29
DNA IQ™ Resin	0.105	0.210
DNA IQ™ 1x Wash Buffer	4.0	8.0
DNA IQ™ Elution Buffer	1.4	2.8

Note: The volume of Lysis-DTT Buffer calculated includes the volume used in the Resin-Lysis solution preparation.

18.2 REAGENT VOLUMES FOR MANUAL DNA IQ™ (RETAIN SUPERNATANT)

Table 9 - Reagent volumes for manual method (retain supernatant)

Reagent	Volume per sample (uL)	Volume for 12 samples (mL)	Volume for 24 Samples (mL)
TNE buffer	450	5.4	10.8
Proteinase K (20mg/mL)	14	0.168	0.336
Sarcosyl (40%)	7	0.084	0.168
Lysis-DTT Buffer			
DNA IQ™ Lysis Buffer	660	10	20
DTT (1M)	6.6	0.1	0.2
DNA IQ™ Resin solution			
Lysis-DTT Buffer	43	0.645	1.29
DNA IQ™ Resin	7	0.105	0.210
DNA IQ™ 1x Wash Buffer	300	4.0	8.0
DNA IQ™ Elution Buffer	100	1.4	2.8

Note: The volume of Lysis-DTT Buffer calculated includes the volume used in the Resin-Lysis solution preparation.

18.3 REAGENT VOLUMES FOR THE OFF DECK LYSIS PROCEDURE

Table 10 - Reagent volumes for off-deck procedure

Reagent	Volume for 48 samples (mL)	Volume for 39 Samples (mL)
Extraction Buffer		
TNE buffer	16	12
Proteinase K (20mg/mL)	0.864	0.648
Sarcosyl (40%)	0.432	0.324
Lysis-DTT Buffer		
DNA IQ™ Lysis Buffer	N/A	N/A
DTT (1M)	N/A	N/A
DNA IQ™ Resin solution		
Lysis-DTT Buffer	N/A	N/A
DNA IQ™ Resin	N/A	N/A
DNA IQ™ 1x Wash Buffer	N/A	N/A
DNA IQ™ Elution Buffer	N/A	N/A

Note: Do not directly pipette from reagent stock bottles. Stock reagents should be decanted into working containers where appropriate.

18.4 REAGENT VOLUMES FOR AUTOMATED DNA IQ™

Table 11 - Reagent volumes for automated procedure

Reagent	Volume for 96 samples (mL)	Volume for 76 samples (mL)	Volume for 48 samples (mL)
Extraction Buffer (300µL/sample)			
TNE buffer	N/A	N/A	N/A
Proteinase K (20mg/mL)	N/A	N/A	N/A
Sarcosyl (40%)	N/A	N/A	N/A
Lysis-DTT Buffer			
DNA IQ™ Lysis Buffer	90	70	50
DTT (1M)	0.9	0.700	0.5
DNA IQ™ Resin solution			
Lysis-DTT Buffer	6.0	6.0	3
DNA IQ™ Resin	1.0	1.0	0.5
DNA IQ™ 1x Wash Buffer	35.0	30.0	18
DNA IQ™ Elution Buffer	14.0	12.0	8

Note: Do not directly pipette from reagent stock bottles. Stock reagents should be decanted into working containers where appropriate.

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DNA IQ™ Method of Extracting DNA from Casework and Reference Samples

1 PURPOSE AND SCOPE

This document outlines the automated and manual procedures for extracting DNA from reference and casework samples using the DNA IQ™ kit (Promega Corp., Madison, WI, USA) within Forensic DNA Analysis Unit. The automated procedure within this document utilises the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (MPII) (PerkinElmer BioDiscovery, Downers Grove, IL, USA).

Reference samples and casework samples must be extracted on separate batches. If casework and reference batches are to be extracted on the same MPII, the instrument and all associated lab-ware must be decontaminated between operations.

This procedure applies to all staff members who are required to extract DNA from samples using automated or manual DNA IQ™ methods

2 DEFINITIONS

DTT	1,4 Dithiothreitol
EDTA	Ethylenediaminetetraacetatic acid
Extracts	Samples that have undergone DNA extraction
Lysates	Samples that have undergone off-deck lysis but not DNA extraction
MPII	MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform
Paramagnetic	Becomes magnetic with the application of a magnetic force
Pro K	Proteinase K
Samples	Sample substrates in tubes awaiting a DNA extraction process
Sarcosyl	N-Lauroylsarcosine
TNE	Tris, NaCl and EDTA buffer

3 PRINCIPLE

3.1 DNA IQ™ KIT

The DNA IQ™ system (Promega Corp., Madison, WI, USA) is designed to extract and purify DNA from a number of different substrates and has been optimised for trace DNA samples. An in-house validation was performed using a modified version of the PerkinElmer automated protocol.

Cell lysis is performed using DNA IQ™ Lysis Buffer containing Dithiothreitol (DTT). 1,4 Dithiothreitol is a reducing agent used in extractions to break disulfide bonds of proteins. The Lysis Buffer used is a proprietary buffer containing chaotropic salts required for binding of DNA to the magnetic beads. According to the MSDS (Promega Corporation 2006), the Lysis Buffer contains 50-75% guanidinium thiocyanate, < 2.5% EDTA, < 2% 3-[(3-Choalamidopropryl)dimethylammonio] propanesulfonic and < 2% polyethylene glycol tert-octylphenyl ether.

The foundation of the DNA IQ™ system is the magnetic bead resin which contains novel paramagnetic particles covered with silica. The resin has a negative charge at basic and near neutral pH. However, the addition of Lysis Buffer changes the pH and salt concentration of the solution, resulting in the silica on the magnetic beads becoming positively charged and able to bind free DNA.

The magnetic bead resin has a DNA binding capacity of 100ng and binding is more efficient with samples containing small amounts of DNA rather than samples with large amounts of DNA. As a result, small sample size is necessary to ensure efficient recovery of DNA.

Inhibitors are removed from the DNA through several washing steps. The first washing step is performed using Lysis Buffer to ensure the DNA is bound to the magnetic bead resin and to wash out inhibitors. The next three washing steps incorporate the use of DNA IQ™ Wash Buffer. This buffer contains an alcohol/aqueous mixture which dehydrates the DNA to ensure it is not eluted during washing, while the aqueous phase washes out inhibitors.

The DNA IQ™ Elution Buffer changes the salt content which, in conjunction with heating to 65 ºC, allows the DNA to be released from the magnetic beads. The Elution Buffer is a low ionic strength buffer that reduces the affinity of the DNA for the silica that covers the magnetic beads by re-hydration of the phosphate backbone. To prevent PCR inhibition caused by the presence of very small DNA fragments within extracts the DNA IQ™ System selectively isolates DNA fragments greater than 80bp.

3.2 OFF-DECK LYSIS PROCEDURE

Samples must undergo a pre-extraction Off-Deck Lysis step before processing with the DNA IQ™ reagents on the MPII instrument.

The Extraction Buffer used for the Off-Deck Lysis is designed to digest cellular material and release DNA. It contains TNE buffer (Tris, NaCl, EDTA, pH 8.0), Sarcosyl and Pro K.

TNE acts as a basic buffer with EDTA chelating ions in solution.

Sarcosyl is a detergent that releases cellular material from the substrate.

Pro K is an enzyme from the fungus *Engyodontium album* (formerly *Tritirachium album*). As a highly active endopeptidase, it cleaves peptide bonds adjacent to the carboxyl group of N-substituted (blocked alpha amino) hydrophobic, aliphatic and aromatic amino acids. Pro K is added to the extraction buffer to digest cellular material, helping the DNA to be released. It also digests proteins that interfere with the DNA binding capacity of the resin and rapidly inactivates enzymatic activity that could potentially degrade DNA (e.g. nucleases).

3.3 MULTIPROBE® II HT EX PLUS WITH GRIPPER™ INTEGRATION PLATFORM

Within Forensic DNA Analysis, DNA extractions are performed on casework or reference samples using two MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms (MPII Ext-A or MPII Ext-B) located in Room 3191.

Each platform uses a computer-controlled Cartesian XYZ liquid handling system designed for the efficient automation of sample preparation. Liquid transfer is performed by an 8-tip system with VersaTip® and VariSpan™ options. The VersaTip® option allows the use of both fixed and disposable tips (conductive and non-conductive). The VariSpan™ option permits variable probe spacing between each of the individual probes so that a wide range of lab-ware such as micro plates, tubes, vials and reagent troughs can be accessed. Each sample probe is capable of independent motion in the Z direction due to independent Z drives.

The 8-tip system is also capable of multichannel liquid-level sensing by utilising Accusense™ technology. This technology works by each probe detecting a change in capacitance when in contact with the liquid. This capacitive mode of detection is also possible when using conductive disposable tips. Accusense™ also permits the detection of low or non-ionic polar solutions and solvents. Pipetting on the platforms is driven by 8 individually controlled syringes via positive displacement of the system liquid (nanopure water) when transferring liquid.

For automated DNA extraction using the DNA IQ™ kit, a plate map is utilised to provide the necessary information for correct volumes, and locations for pipetting. It also contains information regarding batch and plate identification, sample positions, lab numbers of samples, and lists all the samples in the batch, providing the total number of samples. The program will fail to work without a plate map.

4 REAGENTS AND EQUIPEMENT

4.1 REAGENTS

Table 1 outlines all the reagents and storage locations required for Manual or Automated DNA IQ™ extraction.

Table 1 Reagents with storage room and location

Reagent	Room	Location
Amphyl (0.2 and 1%)	3191	Sink
DNA IQ™ Elution Buffer	3188	In-use tray or shelf
DNA IQ™ Lysis Buffer	3188	In-use tray or shelf
DNA IQ™ Resin	3188	In-use tray or shelf
DNA IQ™ 2x Wash Buffer	3188	In-use tray or shelf
DTT (1M)	3188	Freezer
70 % v/v ethanol	3188, 3189, 3191	Sink or bench
AnalR 100 % v/v Ethanol	3188	Shelf
0.5 % v/v CleanTech Bleach	3188, 3189, 3191	Sink or bench
2- Propanol	3188	Shelf
Nanopure water	3188, 3194	Sink
40 % w/v Sarcosyl	3188	In-use tray or shelf
Pro K (20 mg/mL)	3188	Freezer
TNE	3188	In-use tray or shelf
5 % v/v Trigene Advance	3191, 3188	Sink

4.1.1 Extraction Buffer

Prepare Extraction Buffer just prior to commencing the Manual DNA IQ™ or Off-Deck Lysis procedures.

Warning: The TNE, Pro K and Sarcosyl contained in the Extraction buffer may cause irritation or damage to eyes if contact occurs, may cause irritation to skin and respiratory system, or sensitisation if inhaled. Please use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling.

1. Use the correct table in the appendices (Section 18) for the required procedure to determine the reagent volumes.
2. Remove the appropriate aliquot/s of 20 mg/mL Pro K from the freezer and thaw. Vortex mix and briefly centrifuge before use.
3. Ensure that the 40 % w/v Sarcosyl is completely dissolved and appears clear in the stock solution. If not dissolved, invert the container a few times and leave at room temperature.
4. In the biohazard cabinet, aliquot the required amount of TNE buffer into a 50 mL tube. Do not use directly from the stock bottle, use a smaller working aliquot instead.
5. Add the appropriate volume of 20 mg/mL Pro K and 40% w/v Sarcosyl to the TNE buffer and invert gently to mix.
6. Label the tube with **"Extraction Buffer"**, your initials and the date.

7. Note down all reagent lot numbers.

4.1.2 Lysis Buffer with DTT Solution

Lysis Buffer is supplied with the DNA IQ™ kit. The Lysis Buffer with DTT solution is prepared just prior to commencing the Manual or Automated DNA IQ™ procedures.

Warning: Lysis Buffer is harmful if swallowed or inhaled, and causes severe skin burns and eye damage. DTT is also harmful if swallowed and irritating to eyes, respiratory system and skin. DTT is a reducing agent that destroys disulfide bonds. Please use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling.

1. Use the correct table in the appendices (Section 18) for the required procedure to determine the reagent volumes.
2. Remove the appropriate aliquot/s of DTT from the freezer and thaw. Vortex mix and centrifuge briefly before use.
3. In the biohazard cabinet, add the required volume of Lysis Buffer to a sterilised glass Schott bottle or 50mL tube.
4. Add the required volume of DTT to the Lysis Buffer and gently swirl to mix.
5. Label the container with **"Lysis Buffer + DTT"**, your initials and the date.
6. Note down all reagent lot numbers.

4.1.3 DNA IQ™ Resin

DNA IQ™ Resin is supplied with the DNA IQ™ kit. The resin solution is prepared using the Lysis Buffer with DTT solution (from section 4.1.2) just prior to commencing the Manual or Automated DNA IQ™ procedures.

Warning: Resin may cause an allergic skin reaction. Lysis Buffer is harmful if swallowed or inhaled, and causes severe skin burns and eye damage. DTT is also harmful if swallowed and irritating to eyes, respiratory system and skin. DTT is a reducing agent that destroys disulfide bonds. Please use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling.

Note: Ensure the resin is properly mixed by vortex mixing prior to use.

1. Use the correct table in the appendices (Section 18) for the required procedure to determine the reagent volumes.
2. In the biohazard cabinet, aliquot the required amount of Lysis Buffer with DTT solution (from 4.1.2) into a 10, 5 or 2 mL tube.
3. Thoroughly vortex mix the DNA IQ™ resin and immediately add the required volume to the Lysis Buffer with DTT solution.
4. Mix by gentle inversion.
5. Label the tube with **"Resin"**, your initials and the date.
6. Note down all reagent lot numbers.

4.1.4 1x Wash Buffer

Note: 2x Wash Buffer is supplied with the DNA IQ™ kit.

Warning: The 2 x Wash Buffer, 2-Propanol and Ethanol is flammable and hazardous. It may cause serious eye irritation, drowsiness or dizziness if vapours are inhaled. Please use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling. It is recommended that this reagent is not to be prepared / handled by pregnant staff.

To prepare 1x Wash Buffer:

1. In a biohazard cabinet, add 35 mL of *AnalR* 100 % Ethanol to the 2x Wash Buffer bottle.
2. Add 35 mL of 2- Propanol to the 2x Wash Buffer bottle.
3. Update the information on the bottle to indicate that it now contains 1x Wash Buffer and when and by whom it was prepared by. Update the 1x Wash Buffer reagent audit trail in AUSLAB by:

    - From the main page press <2> "Sample Processing"
    - Press <8> "Materials Processing"
    - Press <2> "Consumable Inventory"
    - Highlight "DNA IQ Wash Buffer" in the list and press <enter>
    - Highlight the correct lot number and press <SF8> "Consumable Lot Audit"
    - Add audit entry that specifies the 1x Wash Buffer aliquot (e.g. A1) and the lot numbers of the ethanol and isopropyl alcohol used.

Note: 1x Wash Buffer can be prepared and stored at room temperature prior to use in an automated or manual DNA IQ™ procedure.

1. Use the correct table in the appendices (Section 18) for the required procedure to determine the reagent volume.
2. In the biohazard cabinet, aliquot the required amount of 1x Wash Buffer into a 50 or 10 mL tube.
3. Label the tube with **"Wash Buffer"**, your initials and the date.
4. Note down the reagent lot number.

4.1.5 Elution Buffer

Elution Buffer is supplied with the DNA IQ™ kit.

1. Use the correct table in the appendices (Section 18) for the required procedure to determine the reagent volume.
2. In the biohazard cabinet, aliquot the required amount of 1x Wash Buffer into a 50 or 10 mL tube.
3. Label the tube with **"Wash Buffer"**, your initials and the date.
4. Note down the reagent lot number.

4.2 EQUIPMENT

4.2.1 Equipment and consumables required for Manual DNA IQ™ and Off-Deck Lysis

Table 2 outlines the equipment and location required for Manual DNA IQ™ or Off-Deck Lysis procedures.

Table 2 Equipment with asset number and location for each procedure

Equipment	Location	Procedure
Fridge	3189	Manual DNA IQ™; Off-deck Lysis
Freezers	3189 / 3190	Manual DNA IQ™; Off-deck Lysis
Biological safety cabinet class II x **4**	3188 x 1 3189 x 3	Manual DNA IQ™; Off-deck Lysis
PCR cabinet x 1	3189	Manual DNA IQ™; Off-deck Lysis
96 well tube racks	3189	Manual DNA IQ™; Off-deck Lysis
Vortex x 4	3189	Manual DNA IQ™; Off-deck Lysis
Hot block x 4	3189 / 3191 / 3194	Manual DNA IQ™; Off-deck Lysis
Centrifuge x 4	3189/3194	Manual DNA IQ™; Off-deck Lysis
Mini/Micro centrifuges x 4	3189	Manual DNA IQ™; Off-deck Lysis
Finnpipettes 100 – 1000 µL	3189	Manual DNA IQ™; Off-deck Lysis
Finnpipette 20 – 200 µL	3189	Manual DNA IQ™
Thermo mixer x 2	3189	Manual DNA IQ™
Magnetic rack	3189	Manual DNA IQ™
Multitube Shaker	3191	Manual DNA IQ™

Table 3 outlines the consumables and location required for Manual DNA IQ™ or Off-Deck Lysis procedures.

Table 3 Consumables and location for each procedure

Consumables	Location	Procedure
5 mL, 10 mL and 50mL sterile tubes	3188 / 3189	Manual DNA IQ™; Off-deck Lysis
1000 µL ART or clip tips	3188 / 3189	Manual DNA IQ™; Off-deck Lysis
1.5mL and/or 2mL tubes	3188 / 3189	Manual DNA IQ™; Off-deck Lysis
Spin baskets and 2 mL collection tubes	3189	Manual DNA IQ™; Off-deck Lysis
Inoculation sticks	3189	Manual DNA IQ™; Off-deck Lysis
300 µL ART, 200 µL One-touch tips, 200µL Clip tips	3188 / 3189	Manual DNA IQ™
Nunc Bank-it™ tubes	3189	Manual DNA IQ™; Off-deck Lysis
Nunc Bank-it™ caps	3189	Off-deck lysis
Sharps bin	3188 / 3189	Manual DNA IQ™; Off-deck Lysis

Further consumables can be found in the Store Room (3184).

4.2.2 Equipment and consumables required for Automated DNA IQ™

Table 4 outlines the equipment with asset number and location required for Automated DNA IQ™.

Table 4 Equipment with asset number and location for Automated DNA IQ™

Equipment	Location
STORstar (A)	3190
STORstar (B)	3194
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (EXT MPII-A)	3191
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (EXT MPII-B)	3191
DPC shaker x 2	3191
Automated temperature controller, heat block tiles and heat block adapters x 2	3191
Milli-Q Integral 3 (A10) water purification system	3194
Eppendorf 5804 centrifuge Labogene Scanspeed 1248	3194 / 3191
Fridge	3191
Freezer	3189
MixMate	319 1/ 3194
Capit-All automated decapper	3191
4titude 4seal sealer	3191

Table 5 outlines the consumables and location required for Automated DNA IQ™.

Table 5 Consumables and location for Automated DNA IQ™

Consumables	Location
175µL Clear Non-Conductive Filter RoboRack tips – Pre-Sterilised	3191
MβP Pure 1000µL Tips – Pre-Sterilised	3191
Abgene 96-deep well plate	3191
Axygen 2mL deep well storage plate	3191
96 well Half Skirt PCR Microplate	3191
4titude Piercing Film	3191
12 Channel plate	3191
Nunc Bank-it™ tubes	3191
Nunc Bank-it™ caps	3191
Sterile 50mL Falcon tubes	3188
Sterile 10mL or 5mL tubes	3188
Autoclaved 100mL glass bottles	3188
Aluminium sealing film	3191
300uL ART tips, one-touch or clip tips	3189
1000µL ART tips or Clip tips	3189

Further consumables can be found in the Store Room (3184).

5 SAFETY

As per the standard laboratory procedure QIS 22857 Anti-Contamination Procedure, PPE is to be worn by all staff when performing this procedure. This includes the use of safety glasses.

The MPII instruments, labware, biohazard cabinets and centrifuges should be wiped with 5% v/v Trigene Advance **only**, followed by 70 % v/v ethanol.
Work benches and non-metallic equipment should be wiped with 0.5 % v/v CleanTech bleach, followed by 70 % v/v ethanol.

Warning: Do not place any part of the body inside the cabinet while the MPII is running a procedure.

If it is necessary to access the deck, pause the program using the computer software or the emergency STOP button located on the front of the instrument.

Warning: Tris base, EDTA, Sarcosyl (contained in the Extraction buffer which is prepared) and Lysis Buffer are irritants and hazardous. DTT is a reducing agent that destroys disulfide bonds. Wash Buffer (which contains 2 x Wash Buffer, 2-Propanol and Ethanol) is flammable and hazardous. Handle carefully and wear appropriate PPE. It is recommended that Wash Buffer is not prepared / handled if pregnant, and therefore the procedures using these reagents should not be performed by pregnant women.

Lysis Buffer contains guanidinium thiocyanate (GuSCN) which is toxic and can be harmful if inhaled, swallowed or comes in contact with skin. Left over Lysis Buffer-DTT is to be disposed of in a brown Winchester bottle in the ventilated cupboard in Room 3191. Never

dispose down the sink. If spillage occurs, absorb with liquid-binding material (such as sand or kitty litter) and dispose in a biohazard bin.

Do not combine bleach with Lysis Buffer and do not wipe areas of Lysis Buffer spillage with bleach, as the two reagents produce cyanide gas when combined. Handle carefully and wear appropriate PPE, including safety glasses, when handling Lysis Buffer. If Lysis Buffer is spilt onto PPE (eg. Gloves, gowns), remove and discard the contaminated item/s in a biohazard bin.

6 SAMPLING AND SAMPLE PREPARATION

6.1 SAMPLE LOCATIONS

Samples waiting to be extracted are stored in freezers as detailed in Table 6.

Table 6 Sample storage locations

Sample type	Storage Device	Storage Location
Urgent/High/Medium Priority Samples	Freezer	3190 or Block 6 walk-in freezer*
Lysates in 1.0mL Nunc Bank-it™ tubes	Freezer	3190
Extracts in 1.0mL Nunc Bank-It™ tubes	Freezer	3194 A

* Some storage boxes containing samples are located in Block 6, within the Exhibit Room (6106).

6.2 QC SAMPLES

One negative reagent control and one positive blood control (from a known donor) must be registered for all extraction batches. Additionally, off-deck lysis batches require five blank controls as detailed in Table 7.

Table 7 Extraction Quality Controls

Batch Type	Control
Off-deck lysis	Positive control (x1)
	Negative control (x1)
	Blank control (x5)
Manual DNA IQ™	Positive control (x1)
	Negative control (x1)
Retain supernatant DNA IQ™	Positive control (x1)
	Negative control (x1)

6.3 REGISTRATION OF QC SAMPLES

Register control samples according to the standard laboratory procedure QIS 24919 Forensic DNA Analysis Workflow Procedure. CQ samples for off-deck lysis are to be registered with a 9PLEX test code. QC samples for a DNA IQ Maxwell or a Retain Supernantant DNA IQ extraction batch are to be registered using the XPLEX test code.

6.4 CREATION OF EXTRACTION BATCHES

Create extraction batches according to the standard laboratory procedure QIS 24919 Forensic DNA Analysis Workflow Procedure. All samples that are registered using a 9PLEX test code will be extracted using the automated DNA IQ method with the exception of tapelifts that will be processed using the DNA IQ Maxwell procedure.

6.5 LOCATING SAMPLES

Locate samples according to the standard laboratory procedure QIS 23959 Storage Guidelines for Forensic DNA Analysis.

6.6 SEQUENCE CHECKING AUTOMATED EXTRACTION BATCHES

Sequence check lysates in Nunc Bank-It tubes for automated extraction batches according to the standard laboratory procedure QIS 24256 Procedure for the use of the STORstar unit for automated sequence checking.

6.7 ELECTRONIC WORKFLOW DIARY

Within the electronic workflow diary I:\AAA Electronic Workflow Diary record details of the batch sample creation and processing.

7 MANUAL METHODS FOR EXTRACTION USING DNA IQ™

7.1 MANUAL DNA IQ™ (NO RETAIN SUPERNTANT)

Note: All manual extractions are to be performed within a biohazard cabinet in room 3189.

1. Prepare fresh reagents within the biohazard hood of the clean room (3188) prior to commencing the procedure. Refer to Section 4.1 for preparation of reagents. Use Table 8 in Appendix 18.1 for reagent volumes.

Warning: The 2 x Wash Buffer, 2-Propanol and Ethanol is flammable and hazardous. It may cause serious eye irritation, drowsiness or dizziness if vapours are inhaled. Please use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling. It is recommended that this reagent is not to be prepared / handled if pregnant, and therefore this procedure should not be performed by pregnant women.

2. Turn on the Eppendorf Thermomixer and set to 37 ˚C and 1400 rpm.

3. Label for each sample:
   - Original sample tube;
   - Spin basket (if required);
   - 2mL tube; and
   - Nunc™ Bank-It™ tube.

Note: A spin basket is required for swabs, fabric, paper and cigarette butts. Samples that do not require a spin basket are tape-lifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

4. Have a second operator perform a sequence check of all tubes. Record the sequence check in AUSLAB.

5. Add 300 µL of Extraction Buffer to each sample. Ensure that large substrates including tape-lifts are fully submerged.

6. Vortex and incubate at 37 °C on the Thermomixer at 1400 rpm for 45 minutes.

7. Remove from the Thermomixer and increase the temperature on the Thermomixer to 65 °C for use in the elution steps.

8. Transfer the substrate to spin basket (if required) or transfer the lysate to an appropriately labelled 2.0 mL tube. Retain the original tube containing the substrate if no spin basket is used.

9. Centrifuge the spin basket at 15800 xg for 2 minutes.

10. Transfer the lysate from the original sample tube and the flow-through from the collection tube of the spin basket to an appropriately labelled 2 mL tube. Transfer the substrate from the spin basket back into the original sample tube.

    Note: If original sample tube is an appropriate 2 mL tube, transfer the substrate into the new 2 mL tube and add the flow-through from the spin basket to the lysate still in the original sample tube.

11. Add 550 µL of Lysis Buffer-DTT solution to the lysate.

12. Add 50 µL of DNA IQ™ Resin-Lysis Buffer-DTT solution to the lysate. Pipette mix or vortex the solution frequently to keep a homogenous suspension of resin within the solution.

13. Vortex and place on the Multitube shaker set at 1200 rpm for 5 minutes.

14. Remove from the Multitube shaker, vortex and immediately place into the magnetic stand. Separation will occur instantly.

    Note: If resin does not form a distinct pellet on the side of the tube, or if the magnetic beads have re-suspended while in the stand, vortex the tube and quickly place back in the stand.

15. Carefully remove and discard the liquid, ensuring that the resin is not disturbed. Remove from the magnetic stand.

    Note: If some resin is drawn up in the pipette tip, gently expel resin back into tube to allow re-separation.

16. Add 125 µL of Lysis Buffer-DTT solution. Vortex and place into the magnetic stand.

17. Once separation has occurred, remove and discard the liquid. Remove the tube from the magnetic stand.

18. Add 100 µL of 1X Wash Buffer, vortex and place into the magnetic stand. Once separation has occurred remove and discard the liquid. Remove the tube from the magnetic stand.

19. Repeat step 18 twice for a total of three Wash Buffer washes. Ensure that all of the liquid has been removed after the last wash. Remove the tube from the magnetic stand.

20. Uncap the tubes and place the lids down onto a clean rediwipe within the biohazard cabinet. Allow the resin to air-dry in the hood for 15 minutes and then recap the tubes.

    Note: Do not allow the resin to dry for more than 20 minutes as this may inhibit DNA elution.

21. Add 50 µl of Elution Buffer by carefully pipetting the solution to the side of the tube, above the pellet. **Do not mix**.

22. Incubate in the Thermomixer at 65°C for 3 minutes and then continue to incubate for a further 3 minutes shaking at 1100 rpm. If no thermomixer is available, incubate samples within a hot block and vortex mix at the start and end of the second 3 minute incubation. Remove samples.

23. Vortex and immediately place into the magnetic stand while hot to ensure maximum DNA yield during elution.

24. Carefully transfer the DNA extract to the corresponding labelled Nunc™ Bank-It™ tube.

25. Remove from the magnetic stand and repeat steps 21-23 once. The final volume after the second elution is approximately 95 µL of DNA extract.

26. Store the DNA extracts in temporary storage located within the Pre-PCR sorting room 3194-A in the upright freezer with equipment number 30512883.

27. Store tubes containing substrates in Spin Basket boxes located within the Pre-PCR sorting room 3194-A in the upright freezer with equipment number 30512883.

7.2 PROCEDURE FOR MANUAL DNA IQ™ (RETAIN SUPERNATANT)

Note: All manual extractions are to be performed within a biohazard cabinet in room 3189.

1. Prepare fresh reagents within the biohazard hood of the clean room (3188) prior to commencing the procedure. Refer to Section 4.1 for preparation of reagents. Use Table 9 in the Appendix 18.2 for reagent volumes.

Warning: The 2 x Wash Buffer, 2-Propanol and Ethanol is flammable and hazardous. It may cause serious eye irritation, drowsiness or dizziness if vapours are inhaled. Please use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling. It is recommended that this reagent is not to be prepared / handled if pregnant, and therefore this procedure should not be performed by pregnant women.

2. Turn on the Eppendorf Thermomixer and set to 37 ˚C and 1400 rpm.

3. Label for each sample:
   - Original sample tube;
   - 2 mL tube for sample transfer and processing;
   - 1.5mL tube labelled ‘SUP’ for supernatant;
   - Spin basket (if required); and
   - Nunc™ Bank-It™ tube.

   Note: A spin basket is required for swabs, fabric, paper and cigarette butts. Samples that do not require a spin basket are tape-lifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

4. Have a second operator perform a sequence check of all tubes. This person must enter the sequence check in AUSLAB.

5. Add 450µL of TNE buffer to the sample and vortex.

6. Incubate at room temperature for 30 minutes.

7. Vortex, then centrifuge at 15800 xg for 3 minutes.

8. Remove 150 µL of supernatant and place into the respective 1.5 mL tube labelled with 'SUP' (for further testing).

9. Add 14µL of 20mg/mL Proteinase K and 7µL 40% (w/v) Sarcosyl to the original sample tube containing substrate and TNE buffer, then vortex.

10. Vortex, then incubate at 37°C on the Thermomixer at 1400 rpm for 45 minutes. If no thermomixer available, incubate samples within a hot block, vortex mix at the start and end of the incubation and at least one during the incubation.

11. Remove from the Thermomixer and increase the temperature on the Thermomixer to 65°C (for use in the Elution steps).

12. Transfer substrate to spin basket if required or transfer the lysate to an appropriately labelled 2.0mL tube. Retain original tube containing the substrate in if no spin basket used.

13. Centrifuge spin basket at 15800 g for 2 minutes.

14. Transfer the substrate in the spin basket into a new and labelled 2mL tube to be retained. Transfer the flow through (lysate) back into the original sample tube.

15. Add 550µL of Lysis-DTT Buffer solution.

16. Add 50µL of DNA IQ™ Resin-Lysis solution. Pipette mix the Resin-Lysis solution between each addition to keep the resin suspended in solution.

17. Vortex and place on the Multitube shaker set at 1200 rpm for 5 minutes.

18. Remove from the Multitube shaker, vortex and immediately place into the magnetic stand. Separation will occur instantly.

    Note: If resin does not form a distinct pellet on the side of the tube, or if the magnetic beads have re-suspended while in the stand, vortex the tube and quickly place back in the stand.

19. Carefully remove and discard the solution, ensuring that the resin is not disturbed. Remove from the magnetic stand.

    Note: If some resin is drawn up in the pipette tip, gently expel resin back into tube to allow re-separation.

20. Add 125µL of Lysis-DTT Buffer solution. Vortex and place into the magnetic stand.

21. Once separation has occurred, remove and discard the Lysis-DTT Buffer. Remove from the magnetic stand.

22. Add 100µL of 1X Wash Buffer, vortex and place into the magnetic stand. Once separation has occurred remove and discard the Wash Buffer. Remove from the magnetic stand.

23. Repeat the Wash Buffer step (step 23) two times for a total of three washes. Ensure that all of the solution has been removed after the last wash. Remove from the magnetic stand.

24. Uncap the tubes and place the lids down onto a clean rediwipe. Air-dry the resin in the hood for 15 minutes and then recap tubes

    Note: Do not dry for more than 20 minutes, as this may inhibit the elution of DNA.

25. Add 50µl of Elution Buffer by carefully pipetting the solution to the side of the tube, above the pellet. **Do not mix**.

26. Incubate in the Thermomixer at 65°C for 3 minutes and then continue to incubate for a further 3 minutes shaking at 1100 rpm. If no thermomixer available, incubate samples within a hot block, vortex mix at the start and end of 2nd 3 minute incubation. Remove samples.

27. Vortex and immediately place into the magnetic stand while hot to ensure maximum DNA yield during elution.

28. Carefully transfer the DNA extract to the corresponding labelled Nunc™ Bank-It™ tube.

29. Remove from the magnetic stand and repeat the Elution Buffer steps (step 26-29). The final volume after the double elution is approximately 95µL of DNA extract.

30. Store the DNA extracts in temporary storage located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

31. Store tubes containing substrates in Spin Basket boxes located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

32. Store supernatants in the S/N Retention boxes located within the Extraction sorting room in the Orford Freezer 30512883 (Room 3190).

33. Ensure that the negative extraction control from the batch is also inserted onto the **'Saliva List':**

    - Log into Auslab
    - 3 for Patient Enquiry
    - Type/Scan the lab number for the negative extraction control
    - From the 9Plex page or the XPLEX Page press Shift F12
    - A prompt will appear 'Enter List Name'
    - Type Saliva or use the F1 lookup list.
    - Press Enter

8 OFF-DECK LYSIS PROCEDURE

1. All reagents are to be made fresh within the clean room (3188) prior to commencing each process. Use Table 10 in Appendix 18.3 for reagent volumes.

2. All manual extractions are to be performed within a biohazard cabinet in Room 3189

3. For each sample label:
   - Original sample tube
   - Spin basket as required
   - 1.0mL Nunc Bank-It™ tube
   - 1.5mL or 2.0mL tube

   Note: Samples that require a spin basket are: swabs, fabric, paper and cigarette butts. Samples that do not require a spin basket are: tapelifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

4. Have a second operator sequence check the tubes and enter the sequence check in AUSLAB.

5. Add 300µL of Extraction Buffer and vortex briefly. Ensure that substrates are immersed in the Extraction Buffer.

6. Incubate for 45 minutes at 37 degrees on a hotblock. It is best practice to vortex the samples intermittently throughout the duration of the incubation period.

7. Remove from the hotblock. Vortex and pulse spin samples to remove condensation from the lids.

8. Transfer substrate to spin basket if required or transfer the lysate to an appropriately labelled 1.5mL or 2mL tube. Retain original tube containing the substrate if no spin basket is used.

9. Centrifuge spin baskets at 15800 g for 2 minutes.

10. Transfer the substrate in the spin basket to a new and appropriately labelled 1.5mL or 2mL tube and retain.Transfer the flow through back to original lysis tube.

11. Vortex lysate, then incubate on hotblock at 65°C for 10 minutes. Record temperature on worksheet.

12. Centrifuge at 15800 g for 1 minute.

13. Transfer 300µL of lysate to the corresponding Nunc Bank-It™ tube.

Note: If more than 300µL of lysate is present, retain the remaining lysate in the 1.5mL tube. In AUSLAB, write a specimen note for that sample (eg. Extra lysate retained from sample XXXXXXXXX.). Store the retained 1.5mL lysate tube in appropriate box in freezer.

14. In AUSLAB, enter reagent and hotblock temperature details and complete the batch.

15. Store lysates in temporary storage boxes located within the extraction sorting room in the orford Freezer with equipment number 102384430 (Room 3190).

16. Store tubes containing substrates or extra lysate in Spin Basket boxes located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

9 AUTOMATED EXTRACTION OF LYSED SAMPLES

9.1 MPII EXTRACTION PROCEDURE

Automated setup of DNA IQ extractions in 96-deep well format is performed using the MultiPROBE® II PLUS HT EX EP-A and EP-B platforms located in Room 3191.

Refer to QIS 23939 Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform for instructions on the use and maintenance of the MultiPROBE® II PLUS HT EX platforms.

9.2 PREPARATION OF REAGENTS FOR THE AUTOMATED EXTRACTION PROCESS

All reagents are to be made fresh within the clean room (3188) prior to commencing each process. Use Table 11 in the Appendix 18.4 for reagent volumes.

Warning: The 2 x Wash Buffer, 2-Propanol and Ethanol is flammable and hazardous. It may cause serious eye irritation, drowsiness or dizziness if vapours are inhaled. Please use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling. It is recommended that this reagent is not to be prepared / handled if pregnant, and therefore this procedure should not be performed by pregnant women.

9.3 SUMMARY OF DNA IQ™ EXTRACTION VERSION 6.7_ODL

9.3.1 Transfer of lysates from Nunc Bank-It™ tubes into the Abgene 96-deep well plate

Lysates from the off-deck lysis protocol, contained in individual Nunc Bank-It™ tubes, are transferred automatically into an Abgene 96-deep well plate prior to commencing automated sample processing. Instead of a 96-deep well plate, the use of individual Nunc Bank-It™ tubes for storage of lysates removes the need for an adhesive film and therefore reduces the risk of well-to-well contamination from the action of peeling back an adhesive plate cover.

9.3.2 Automated addition of DNA IQ™ Resin and Lysis Buffer

DNA IQ™ Resin is added automatically into the Abgene 96-deep well plate on the platform. The program uses a waste mode dispensing step to add 53µL DNA IQ™ Resin-Lysis Buffer solution to each well in either half the plate or the whole plate. Because of this, batch sizes are restricted to either 48 or 96 samples in order to maintain efficiency and economy. Two volumes of Lysis Buffer are then added to promote binding of DNA to the paramagnetic resin.

9.3.3 Mixing using a MixMate to bind DNA to resin

Manual intervention is required to seal the Abgene 96-deep well plate with a 4titude Pierce Seal film using the 4titude sealer pre-heated at 175 °C. The plate is then transferred onto a MixMate instrument for mixing at 1100rpm for 5 minutes, followed by centrifugation on an Eppendorf 5804 centrifuge at 3000rpm for 2 minutes. The seal is then carefully pierced with a 96 well PCR microplate and the Abgene plate is returned to the Applied Biosystems magnet on the MPII platform.

9.3.4 Removing lysis reagents for storage

At this point, most of the DNA is bound to the paramagnetic resin. With the positioning of the Abgene plate on the ABI magnet, DNA IQ™ Resin becomes immobile at the bottom of the plate. The lysis reagents from each well are transferred automatically to a storage plate on the MPII platform without disturbing the DNA IQ™ Resin.

9.3.5 Washing of the resin-DNA complex

Washing steps are performed to remove any inhibitors in solution. The first wash uses 125µL Lysis Buffer with shaking at room temperature for 1 minute on the DPC shaker to ensure that the DNA is bound to the paramagnetic resin. The plate is moved to the ABI magnet and the supernatant is transferred into the storage plate. The next three washes incorporate 100µL of 1x Wash Buffer with shaking at room temperature for 1 minute on the DPC shaker. During each wash cycle, the Abgene 96-deep well plate is moved to the ABI magnet and the supernatant is discarded into the tip chute.

9.3.6 Removing any excess of 1x Wash Buffer

The samples are allowed to air dry at room temperature for 5 minutes in order to remove all traces of Ethanol from the Wash Buffer. The presence of Ethanol may potentially inhibit both the elution process and also downstream PCR.

9.3.7 Elution of DNA from the resin-DNA complex

A double elution method is employed in this procedure. At each elution step, 60µL of Elution Buffer is added to each sample, followed by incubation at 65°C for 6 minutes (3 minutes without shaking followed by 3 minutes shaking on the DPC shaker). The Abgene plate is moved to the ABI magnet and the eluted solution (supernatant containing eluted DNA) is transferred to fresh Nunc™ Bank-It™ tubes.

9.3.8 Flushing of liquid pathway

As a decontamination measure, the liquid pathway is washed with Amphyl and Nanopure water at the conclusion of the automated extraction process.

9.4 SETTING UP THE MPII PLATFORMS FOR AUTOMATED DNA IQ™ PROCESSING

The following steps are carried out in the automated extraction room (Room 3191).

1. Remove the Nunc Bank-It™ tubes containing lysates from the fridge to allow to come to room temperature before commencing the extraction procedure.

   Note: If the lysates are frozen, remove them from the freezer and thaw in Room 3191.

2. Restart or turn on the instrument PC.
3. Log onto the network using the **Robotics** login.
4. Open WinPrep® by double clicking icon on the computer desktop (Figure 1).
5. Log onto the WinPrep® software by entering your username and password, then press **"Enter"**.

Figure 1.
The WinPrep® icon.

6. Ensure that the daily/weekly start-up and maintenance has been performed before running any program. If WinPrep® has been closed or been idle for a long period of time initialise the MP II platform as described in QIS 23939.
7. Ensure the **System Liquid reservoir is FULL** and tubing is fully submerged in the system liquid before every run and perform a Flush/Wash. If visible air bubbles have appeared in tubing or in syringes between setting up the deck and executing the test, another flush wash will need to be performed before starting the MPII extraction run.

8. Open the Extraction setup MP II test file in WinPrep® by selecting:

   - **File**
   - **Open**, navigate to **C:\PACKARD\MULTIPROBE\BIN\QHSS PROTOCOLS**
   - Select "**DNA IQ Extraction_Ver 6.7_ODL.mpt**"
   - Click the "**Open**" button

9. Check the tree panel of the whole program for any bold fonts. See the Analytical Senior Scientist if bold fonts are present.

10. Copy the required plate map from the network folder **I:\EXTRACTION** into the local folder **C:\PACKARD\EXT PLATE MAPS.** Open the plate map using Excel and check that the plate map is complete, and make changes if necessary. If there are blanks (samples that have been removed), make all volumes to 0. If it is not a full batch, delete the rows of blank samples at the end of the platemap. Save all changes made to the platemap (as a .txt file).

11. Decontaminate the required labware with 5% TriGene Advance followed by 70% Ethanol and place onto the instrument deck in the designated grid positions (as shown in the WinPrep® software). Additionally, ensure the DPC shaker is positioned properly.

12. Ensure that the DPC shaker and Heater Controller Box are switched on.
    For EP-A: Tile 1 at F22 (850C).
    For EP-B: Tile 2 at F22 (850C).

    Note: Press the start/stop button twice at the front of the DPC shaker to ensure that it displays zero on the screen.

13. Ensure the heat transfer tile is clicked into the plate adapter tile properly.

    Note: This is critical to ensure correct incubation temperatures.

14. To the Amphyl wash station in position **A10**, add 1% Amphyl to the far left hand side, 0.2% diluted Amphyl to the middle left and place an empty (waste) reagent trough in the middle right position of the station.

15. Add Wash Buffer to the far right hand side trough of the Amphyl wash station in position **A10**.

16. Add Lysis Buffer to the labelled 150mL reagent trough on the left hand side of the 2 trough holder in position **A13**.

17. Place the 12 channel plate into position **A16**. Add Elution Buffer to the plate by dividing the Elution Buffer between channels 11 and 12.

18. Add Resin to channel 1 of the 12 channel plate in position **A16**. It is important to add the resin in a uniform fashion to ensure equal distribution of resin along the channel.

19. **Nunc Bank-It™ lysate tubes:** The lysates should now be at room temperature. Ensure that the rack is labelled with the correct **AUSLAB batch ID** and that the label matches the batch ID on the worksheet and platemap. Additionally, randomly check some visible barcodes on the side of lysate tubes against the positions on the worksheet to ensure the correct batch of samples is to be processed.

20. Place the rack onto the MixMate to shake for 1 minute at 1000rpm.

21. Centrifuge the rack at 3000rpm for 2 minutes in the Eppendorf 5804 centrifuge and then place into position **C13**.

22. **Abgene 96-deep well plate:** With the plate in its correct orientation label the **left hand side** of the plate with both the correct **AUSLAB batch ID** and **batch ID barcode**. With a marker, print the word "**FRONT-lysate"** on the front of the plate. Place the plate in its correct orientation in position **E13**.

23. **2mL 96-deep well storage plate:** With the plate in its correct orientation label the **left hand side** of the plate with both the correct **AUSLAB batch ID** and **batch ID barcode**. With a marker, print the word **"FRONT-store"** on the front of the plate. Place the plate in its correct orientation in position **E16**.

24. **Nunc Bank-It™ extract tubes:** Ensure that the rack is labelled with the correct **AUSLAB batch ID** on the **front** of the Nunc Bank-It™ tube rack. Additionally, randomly check some visible barcodes on the side of lysate tubes against the positions on the worksheet to ensure the correct batch of samples is to be processed. Place the rack in its correct orientation in position **G16**.

25. Add Nanopure water to the 160mL trough in the Flush/Wash station in position **G13**.

26. Ensure that all necessary labware have been positioned correctly as displayed within WinPrep®, then click "**EXECUTE TEST"**. Record run information in the Run Log book.

27. The following message will appear (Figure 2 below):

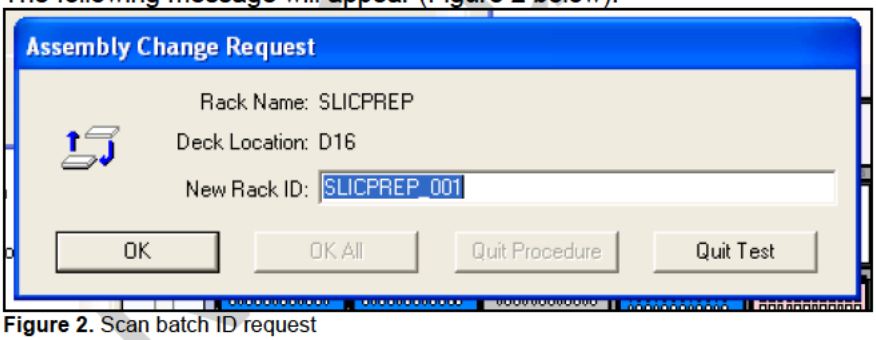

Figure 2. Scan batch ID request

Into **"New Rack ID:"** scan barcode off the worksheet. It is important that this barcode is scanned as it corresponds to the labelling of labware on the deck and the plate maps used.

28. Click "**Reset Tip Boxes**" and ensure that the number of tips displayed in this window match those as available on the deck platform. Fill new tips if necessary. Click "**Close**" to accept the tip count, and then click "**Next**".

29. Select the correct platemap by browsing to **C:\PACKARD\EXT PLATE MAPS**. Ensure that the platemap selected corresponds to the labelling of labware on the deck, the paperwork used and the **"New Rack ID"** entered.

30. For a full batch of 76 samples, ensure that all nodes are checked. Click "**Next**" to check all other nodes.

31. For a batch of 48 samples or less, uncheck the node: **"Add resin to second half of plate"**. Click "**Next**" to check all other nodes.

32. Click "**Start**" to continue.
    - The MPII instrument will begin and user prompts will appear as a reminder to confirm the deck setup. **Always decap tubes from positions H1 to A1, H2 to A2 etc.**
    - Ensure all steps on the first prompt have been complete, Click **OK** to continue.
    - Ensure all steps on the second prompt have been complete, Click **OK** to continue.

33. The program will progress to transfer the lysates followed by automated addition of the Lysis-DTT buffer and the DNA IQ™ Resin solution. The next user prompt will appear. Follow the steps as outlined in the user prompt. Then click **OK** when ready.

    Note: Ensure that the plate is heated and sealed properly with the Pierce Seal on the 4titude Heat Sealer. Mix the plate on the mixmate for 5min at 1100rpm and centrifuge. Pierce the plate with a PCR Microplate and then discard. (A new plate is to be used each time). Ensure the pierced plate is returned to the magnet.

34. Once lysis steps have been completed, a user prompt will appear. Follow the directions as outlined in the user prompt. Then click **OK** when ready.

    Note 1: The Lysate Nunc tubes (including the rack) should be placed into a clipseal bag, sealed and discarded into a biohazard waste bin. The Store plate can also be removed, heat sealed and discarded into a biohazard waste bin.

    Note 2: When the supernatant storage plate is sealed, check the plate for the transfer of beads. To do this, place the plate onto the ABI magnet (if beads are present they will settle to the bottom of the plate) and then inspect the bottom of the plate. If no beads are present, discard the store plate. If, however, beads are present, refer to the Section 15, Troubleshooting.

35. Once the wash steps have been completed, a user prompt will appear. Follow the directions as outlined in the user prompt. Then click **OK** when ready.

    Note: The Nunc extract tubes can be decapped on the bench in the MPII cabinet while the samples are drying and the heating tile is stabilising. At this point, the operator has approximately **12 minutes** to decap the tubes. If the operator has been interrupted and requires more time to decap the tubes, the program may be paused at this step.

36. A message will appear waiting for the heating tile to reach 85°C (for incubation at 65°C). **DO NOT PRESS CONTINUE** as the program will continue automatically when the temperature has been reached with sufficient stability.

37. A user prompt will appear. Follow the directions as outlined in the user prompt. Then click **OK** when ready.

38. Once the elution is completed, a user prompt appears. Follow the directions as outlined in the user prompt. Then click **OK** when ready.

39. The Lysate Plate is heat sealed and kept in temporary storage for one month.

40. Once all plates are removed from the deck and sealed. Click "**OK**" to proceed to the Amphyl wash step.

41. A final message will advise that the run has completed. Click "**OK**".

9.5 FINALISING THE MPII RUN

1. Transfer left over Resin solution from the 12 channel plate and the Lysis Buffer-DTT (wearing safety glasses) into the glass Lysis-DTT bottle previously used. Discard the 12 channel plate in the biohazard waste bin. Transfer the contents of glass bottle into the brown Winchester bottle located in the safety cupboard in room 3191.

2. Discard the contents of the Amphyl wash station into the sink and rinse the troughs with Nanopure water.

3. Remove all labware from the deck and clean with 5% TriGene™ followed by 70% Ethanol, and setup for the next run if necessary. In addition, clean the work area around the MPII instrument with 0.5% (v/v) Bleach and 70% Ethanol.

4. Import the platemap into Auslab and then delete it.

9.6 IMPORTING MPII LOG FILE INTO AUSLAB

1. Click on the Microsoft Access icon in the WinPrep® main menu to open the MultiPROBE log database.

2. Click **"Open"** when prompted.

3. Click on the relevant run Test ID in the Test Selection box. In the Report/Query/Action Selection dropdown menu, select **"Report: Test Summary (Sorted by Destination Rack ID)"**

4. In the Output Selection dropdown menu, select **"File"**. Save the output file as *.csv format to **C:\PACKARD\ LOGS** with the same name as the AUSLAB batch ID and click "**Apply**".

5. Open the log file and check for any errors that may have arisen during the extraction process. Compare the listed errors to any that were encountered during the run. Report any critical errors to the line manager.

6. Import the log file, entering the path, filename and extension (e.g. C:\Packard \logs….) and press **[Enter]**. Delete the log file after importing.

7. For more details on importing files into AUSLAB refer to Batch functionality in AUSLAB SOP (QIS 24469).

9.7 IMPORTING EXTRACTION "RESULTS" INTO AUSLAB

1. Import the results file, entering the filename and .txt extension (e.g. CWIQEXT20100917_06.txt). For more details on importing files into AUSLAB refer to Batch functionality in AUSLAB SOP (QIS 24469).

2. The file will be imported into AUSLAB and appear in the Results Import Table.

3. Highlight entry and press **[Enter]**, for access to the Results Table.

4. Page down through the table and check that all sample results have been imported.

5. Press **[SF8]** ***Table Sort Order***, this sorts the table, sorting samples that have failed Autovalidation to the top. Samples that have failed are those that have processing comments present.

6. For samples that have failed, check the **Processing Comments,** by entering into the sample.

7. If processing comments state sample is to be sent to another batch type **other** than quant. Proceed with the following steps:
   a. Request the appropriate rework test code via the **[SF7]** results history table and the **[SF8]** request rework functions (e.g. samples requiring Microcon, NucleoSpin and pooling).
   b. Press **[Esc]** to exit back to the DNA results table. Do not toggle accept.
   c. Add the extraction batch ID ONLY into the 9PLEX or 9FTAR completed date fields in AUSLAB.

8. If processing comments do not state next step the sample will be processed as normal:
   a. Press **[Esc]** to exit back to the DNA results table.
   b. Highlight any entries to be changed and press **[SF7]** ***Toggle Accept.***

9. Press **[F7]** ***Complete Batch***, all of the samples with a ***Yes*** in the Accept column will be transferred to the outstanding Quant Casework or Quant Reference lists.

10. File the extraction worksheet into the relevant intray for scanning in room 3194A (Pre PCR-Sorting).

10 SAMPLE STORAGE

Refer to QIS 23959 Storage Guidelines for Forensic DNA Analysis for how to store the DNA extract Nunc™ Bank-It™ tubes and Abgene 96-deep well. Refer to QIS 24919 Forensic DNA Analysis Workflow Procedure for storage of extracts.

11 TROUBLESHOOTING WITH THE MPII

1. If the resin is not pipette mixing correctly (eg. Resin is not being drawn up into the pipette tip), pause the Winprep program and check that the support tile is clicked onto the MPII deck correctly. Also, pipette mix resin manually one more times in the corresponding columns of the 12 channel plate.

2. If the Gripper is not placing the rack/plate properly on the deck, pause the program and manually adjust the rack/plate properly on the adapter support tile.

3. If the Gripper appears to be not gripping a plate correctly, or the pipetting alignment of a labware appears to be incorrect, the deck and / or labware can be re-calibrated. This is covered in QIS 23939 Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform

12 QUALITY ASSURANCE/ACCEPTANCE CRITERIA

- Positive and negative (reagent blank) controls are included in each extraction batch. However, for off deck-lysis batches, an additional five negative (reagent blank) controls are included. These controls are processed as normal samples through to completion.
- If any results are obtained from the reagent blank, either at quantification or during the CEQ check, then the possible source of the contamination is investigated. The samples extracted with this control are thoroughly checked and repeated if required. This is covered in QIS 17130 CE Quality Check and QIS 24012 Miscellaneous Analytical Section Tasks

13 VALIDATION

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- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 11: Report on the Validation of the Automated Extraction Chemistry Kit using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Casework Platform." 2007.
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- Muharam I., Hlinka V., Gallagher, B., Lundie, G., Iannuzzi, C., Nurthen T., McNevin A., Ientile V. "Project 22: A Modified DNA IQ™ Method for Off-Deck Lysis Prior to Performing Automated DNA Extraction" 2008

14 STORAGE OF DOCUMENTS

The off-deck lysis worksheets are to be stored within the extraction sorting room (3190) in the nominated intray. It is essential that ALL off-deck lysis worksheets go within this intray so the operational officers are aware that batches are ready and waiting to be combined for extraction on the automated robots.

All worksheets, after auto extraction, manual IQ and Retain supernatant batches have been completed, are stored within the Pre-PCR sorting room (3194-A) in an allocated in-tray for scanning by the operational officers.

15 REFERENCES

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- Promega, DNA IQ™ System –Small Casework Protocol. Promega Technical Bulletin #TB296 2006. Rev 4/06: p. 1-14.
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- Promega, Tissue and Hair Extraction Kit (for use with DNA IQ™) Protocol. Promega Technical Bulletin #TB307, 2006. Rev 5/06: p. 1-11.
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- Vandenberg, N., van Oorchot., R. A. H., & Mitchell, R. J., An evaluation of selected DNA extraction strategies for short tandem repeat typing. Electrophoresis, 1997. 18: p. 1624-

16 ASSOCIATED DOCUMENTS

QIS 17165 Receipt, Storage and Preparation of Chemicals, Reagents and Test Kits
QIS 22857 Anti-Contamination procedure
QIS 23939 Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform
QIS 23959 Storage Guidelines for Forensic DNA Analysis
QIS 24256 Sequence Checking with the STORstar Instrument
QIS 24469 Batch functionality in AUSLAB
QIS 24919 Forensic DNA Analysis Workflow Procedure
QIS 17130 CE Quality Check
QIS 24012 Miscellaneous Analytical Section Tasks

17 AMENDMENT HISTORY

Version	Date	Author/s	Amendments
R0	23 Oct 2007	B. Gallagher, T. Nurthen, C. Iannuzzi, V. Hlinka, G. Lundie, I Muharam.	First Issue

Version	Date	Author/s	Amendments
R1	12 Dec 2007	M Harvey, C Iannuzzi, A McNevin	Reviewed and updated after initial training
R2	19 March 2008	M Harvey, B Andersen, C Iannuzzi, A McNevin	Addition of Off-deck Lysis procedure, Retention of fully automated method as Appendix, addition of reagent record tables into Appendix
R3	April 2008	QIS2 Migration Project	Headers and Footers changed to new CaSS format. Amended Business references from QHSS to FSS, QHPSS to CaSS and QHPS to Pathology Queensland
4	13 March 2009	QIS2 migration	Version incremented by one on migration to QIS2
5	03 June 2009	M Aguilera, B Micic, C Iannuzzi, A. Cheng, V. Hlinka, I. Muharam, G. Lundie, C. Weber	Major changes to reflect new procedure. Updated to reflect changes in procedure as an outcome of internal and external audits. Created ver.6.4 ODL in MPII Platforms. Minor changes in procedures using 4titude 4seal heat sealer to seal plates.
6	29 June 2009	A McNevin, K Lancaster	Removed references to retaining lysate and beads, fixed minor formatting errors. Created ver6.5 ODL in MPII Platforms. Substrates now to be retained in 2mL tube
7	30 September 2010	M.Cipollone, M.Mathieson	Major changes made RE: room numbers to reflect the move to Block 3. Re-formatted entire SOP. New Appendices added which outline all reagent volumes. All equipment and associated asset numbers have been included. Use of the electronic diary now included as an additional section. Preparation of reagents within the clean room (3188) now to be done prior to starting each process. Storage of worksheets updated. New software version 6.6 on automated extraction robots. S/N Retention Boxes now stored in Manual Ext Room. Associated Documents and hyperlinks updated. Consumables and Equipment table added for Manual DNA IQ.
8	24 May 2012	A. Speirs M. Cipollone	Major revision of document. Revision of sections 1-3 to improve clarity. Updated Tables 1 to 7. Removed redundant sections on locating samples and creating batches. Removed procedure for retain supernatant Off-Deck Lysis and thus associated Appendix. Table of Contents amended to reflect all

Version	Date	Author/s	Amendments
			changes. Moved the Manual DNA IQ procedures before the Off-Deck Lysis procedure. Re-formatted spacing and alignments of headings. Re-numbered Tables in Appendices. Updated sections to include which relevant Appendix to use in regards to reagents. Minor grammatical and spelling errors fixed.
9	4 Dec 2013	B. Andersen, M. Cipollone	Updated Section 7 of the Manual DNA IQ Retain Supernatant procedure to reflect differences in list inserting between P+ and PP21 samples. Updated Section 6 to clarify 9PLEX and XPLEX test codes. New template applied and minor formatting and wording changes.
10	April 2015	M. Aguilera M.Cipollone	Adjusted Table 9 for Retain Supernatant batches to indicated TNE, Prok and Sarcosyl are not to be combined at reagent prep stage and to be added to samples separately. Changed reference to Isopropyl Alcohol to 2-Propanol. Included warning for the preparation and use of the Wash Buffer for pregnancy, and amended Risk phrases for reagents used as per risk assessment completed by JSM. Updated equipment tables and removed asset numbers. Changed template to HSSA Fixed Document headers; Fixed formatting, removed steps no longer used; Added two more associated documents QIS 17130 CE Quality Check QIS 24012 Miscellaneous Analytical Section Tasks

18 APPENDICES

18.1 REAGENT VOLUMES FOR MANUAL DNA IQ™ (NO RETAIN SUPERNATANT)

Table 8 - Reagent volumes for manual method

Reagent	Volume for 12 samples (mL)	Volume for 24 Samples (mL)
TNE buffer	4.0	8.0
Proteinase K (20mg/mL)	0.216	0.432
Sarcosyl (40%)	0.108	0.216
Lysis-DTT Buffer		
DNA IQ™ Lysis Buffer	10	20
DTT (1M)	0.1	0.2
DNA IQ™ Resin solution		
Lysis-DTT Buffer	0.645	1.29
DNA IQ™ Resin	0.105	0.210
DNA IQ™ 1x Wash Buffer	4.0	8.0
DNA IQ™ Elution Buffer	1.4	2.8

Note: The volume of Lysis-DTT Buffer calculated includes the volume used in the Resin-Lysis solution preparation.

18.2 REAGENT VOLUMES FOR MANUAL DNA IQ™ (RETAIN SUPERNATANT)

Table 9 - Reagent volumes for manual method (retain supernatant)

Reagent	Volume per sample (uL)	Volume for 12 samples (mL)
TNE buffer	450	5.4
Proteinase K (20mg/mL)	14	0.168
Sarcosyl (40%)	7	0.084
Lysis-DTT Buffer		
DNA IQ™ Lysis Buffer	660	10
DTT (1M)	6.6	0.1
DNA IQ™ Resin solution		
Lysis-DTT Buffer	43	0.645
DNA IQ™ Resin	7	0.105
DNA IQ™ 1x Wash Buffer	300	4.0
DNA IQ™ Elution Buffer	100	1.4

Note: The volume of Lysis-DTT Buffer calculated includes the volume used in the Resin-Lysis solution preparation. For retain supernatant batches, please ensure that TNE, Pro K, and Sarcosyl are not combined at reagent preparation stage and are to be added to samples separately.

18.3 REAGENT VOLUMES FOR THE OFF DECK LYSIS PROCEDURE

Table 10 - Reagent volumes for off-deck procedure

Reagent	Volume for 39 Samples (mL)
Extraction Buffer	
TNE buffer	12
Proteinase K (20mg/mL)	0.648
Sarcosyl (40%)	0.324

Note: Do not directly pipette from reagent stock bottles. Stock reagents should be decanted into working containers where appropriate.

18.4 REAGENT VOLUMES FOR AUTOMATED DNA IQ™

Table 11 - Reagent volumes for automated procedure

Reagent	Volume for 78 samples (mL)
Lysis-DTT Buffer	
DNA IQ™ Lysis Buffer	70
DTT (1M)	0.700
DNA IQ™ Resin solution	
Lysis-DTT Buffer	6.0
DNA IQ™ Resin	1.0
DNA IQ™ 1x Wash Buffer	30.0
DNA IQ™ Elution Buffer	12.0

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DNA IQ™ Method of Extracting DNA from Casework and Reference Samples

1 PURPOSE AND SCOPE

This document outlines the manual procedure for extracting DNA from reference and casework samples using the DNA IQ™ kit (Promega Corp., Madison, WI, USA) within Forensic DNA Analysis Unit.

Reference samples and casework samples must be extracted on separate batches.

This procedure applies to all staff members who are required to extract DNA from samples using DNA IQ™ methods

2 DEFINITIONS

DTT	1,4 Dithiothreitol
EDTA	Ethylenediaminetetraacetatic acid
Extracts	Samples that have undergone DNA extraction
Lysates	Samples that have undergone off-deck lysis but not DNA extraction
Paramagnetic	Becomes magnetic with the application of a magnetic force
Pro K	Proteinase K
Samples	Sample substrates in tubes awaiting a DNA extraction process
Sarcosyl	N-Lauroylsarcosine
TNE	Tris, NaCl and EDTA buffer

3 PRINCIPLE

3.1 DNA IQ™ KIT

The DNA IQ™ system (Promega Corp., Madison, WI, USA) is designed to extract and purify DNA from a number of different substrates and has been optimised for trace DNA samples.

Cell lysis is performed using DNA IQ™ Lysis Buffer containing Dithiothreitol (DTT). 1,4 Dithiothreitol is a reducing agent used in extractions to break disulfide bonds of proteins. The Lysis Buffer used is a proprietary buffer containing chaotropic salts required for binding of DNA to the magnetic beads. According to the MSDS (Promega Corporation 2006), the Lysis Buffer contains 50-75% guanidinium thiocyanate, < 2.5% EDTA, < 2% 3-[(3-Choalamidopropryl)dimethylammonio] propanesulfonic and < 2% polyethylene glycol tert-octylphenyl ether.

The foundation of the DNA IQ™ system is the magnetic bead resin which contains novel paramagnetic particles covered with silica. The resin has a negative charge at basic and near neutral pH. However, the addition of Lysis Buffer changes the pH and salt concentration of the solution, resulting in the silica on the magnetic beads becoming positively charged and able to bind free DNA.

The magnetic bead resin has a DNA binding capacity of 100ng and binding is more efficient with samples containing small amounts of DNA rather than samples with large amounts of DNA. As a result, small sample size is necessary to ensure efficient recovery of DNA.

Inhibitors are removed from the DNA through several washing steps. The first washing step is performed using Lysis Buffer to ensure the DNA is bound to the magnetic bead resin and to wash out inhibitors. The next three washing steps incorporate the use of DNA IQ™ Wash Buffer. This buffer contains an alcohol/aqueous mixture which dehydrates the DNA to ensure it is not eluted during washing, while the aqueous phase washes out inhibitors.

The DNA IQ™ Elution Buffer changes the salt content which, in conjunction with heating to 65 °C, allows the DNA to be released from the magnetic beads. The Elution Buffer is a low ionic strength buffer that reduces the affinity of the DNA for the silica that covers the magnetic beads by re-hydration of the phosphate backbone. To prevent PCR inhibition caused by the presence of very small DNA fragments within extracts the DNA IQ™ System selectively isolates DNA fragments greater than 80bp.

3.2 OFF-DECK LYSIS PROCEDURE

Samples must undergo a pre-extraction Off-Deck Lysis step before processing with the DNA IQ™ reagents on the MPII instrument.

The Extraction Buffer used for the Off-Deck Lysis is designed to digest cellular material and release DNA. It contains TNE buffer (Tris, NaCl, EDTA, pH 8.0), Sarcosyl and Pro K.

TNE acts as a basic buffer with EDTA chelating ions in solution.

Sarcosyl is a detergent that releases cellular material from the substrate.

Pro K is an enzyme from the fungus *Engyodontium album* (formerly *Tritirachium album*). As a highly active endopeptidase, it cleaves peptide bonds adjacent to the carboxyl group of N-substituted (blocked alpha amino) hydrophobic, aliphatic and aromatic amino acids. Pro K is added to the extraction buffer to digest cellular material, helping the DNA to be released. It also digests proteins that interfere with the DNA binding capacity of the resin and rapidly inactivates enzymatic activity that could potentially degrade DNA (e.g. nucleases).

4 REAGENTS AND EQUIPEMENT

4.1 REAGENTS

Table 1 outlines all the reagents and storage locations required for Manual DNA IQ™ extraction.

Table 1 Reagents with storage room and location

Reagent	Room	Location
Amphyl (0.2 and 1%)	3191	Sink
DNA IQ™ Elution Buffer	3188	In-use tray or shelf
DNA IQ™ Lysis Buffer	3188	In-use tray or shelf
DNA IQ™ Resin	3188	In-use tray or shelf
DNA IQ™ 2x Wash Buffer	3188	In-use tray or shelf
DTT (1M)	3188	Freezer
70 % v/v ethanol	3188, 3189, 3191	Sink or bench
AnalR 100 % v/v Ethanol	3188	Shelf
0.5 % v/v Bleach	3188, 3189, 3191	Sink or bench
2- Propanol	3188	Shelf
Nanopure water	3188, 3194	Sink
40 % w/v Sarcosyl	3188	In-use tray or shelf
Pro K (20 mg/mL)	3188	Freezer
TNE	3188	In-use tray or shelf
5 % v/v Trigene Advance	3191, 3188	Sink

4.1.1 Extraction Buffer

Prepare Extraction Buffer just prior to commencing the Manual DNA IQ™ or Off-Deck Lysis procedures.

Warning: The TNE, Pro K and Sarcosyl contained in the Extraction buffer may cause irritation or damage to eyes if contact occurs, may cause irritation to skin and respiratory system, or sensitisation if inhaled. Please use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling.

1. Use the correct table in the appendices (Section 18) for the required procedure to determine the reagent volumes.
2. Remove the appropriate aliquot/s of 20 mg/mL Pro K from the freezer and thaw. Vortex mix and briefly centrifuge before use.
3. Ensure that the 40 % w/v Sarcosyl is completely dissolved and appears clear in the stock solution. If not dissolved, invert the container a few times and leave at room temperature.
4. In the biohazard cabinet, aliquot the required amount of TNE buffer into a 50 mL tube. Do not use directly from the stock bottle, use a smaller working aliquot instead.
5. Add the appropriate volume of 20 mg/mL Pro K and 40% w/v Sarcosyl to the TNE buffer and invert gently to mix.
6. Label the tube with **"Extraction Buffer"**, your initials and the date.
7. Note down all reagent lot numbers.

4.1.2 Lysis Buffer with DTT Solution

Lysis Buffer is supplied with the DNA IQ™ kit. The Lysis Buffer with DTT solution is prepared just prior to commencing the Manual DNA IQ™ procedures.

Warning: Lysis Buffer is harmful if swallowed or inhaled, and causes severe skin burns and eye damage. DTT is also harmful if swallowed and irritating to eyes, respiratory system and skin. DTT is a reducing agent that destroys disulfide bonds. Please use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling.

1. Use the correct table in the appendices (Section 18) for the required procedure to determine the reagent volumes.
2. Remove the appropriate aliquot/s of DTT from the freezer and thaw. Vortex mix and centrifuge briefly before use.
3. In the biohazard cabinet, add the required volume of Lysis Buffer to a sterilised glass Schott bottle or 50mL tube.
4. Add the required volume of DTT to the Lysis Buffer and gently swirl to mix.

5. Label the container with **"Lysis Buffer + DTT"**, your initials and the date.
6. Note down all reagent lot numbers.

4.1.3 DNA IQ™ Resin

DNA IQ™ Resin is supplied with the DNA IQ™ kit. The resin solution is prepared using the Lysis Buffer with DTT solution (from section 4.1.2) just prior to commencing the Manual DNA IQ™ procedures.

Warning: Resin may cause an allergic skin reaction. Lysis Buffer is harmful if swallowed or inhaled, and causes severe skin burns and eye damage. DTT is also harmful if swallowed and irritating to eyes, respiratory system and skin. DTT is a reducing agent that destroys disulfide bonds. Please use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling.

Note: Ensure the resin is properly mixed by vortex mixing prior to use.

1. Use the correct table in the appendices (Section 18) for the required procedure to determine the reagent volumes.
2. In the biohazard cabinet, aliquot the required amount of Lysis Buffer with DTT solution (from 4.1.2) into a 10, 5 or 2 mL tube.
3. Thoroughly vortex mix the DNA IQ™ resin and immediately add the required volume to the Lysis Buffer with DTT solution.
4. Mix by gentle inversion.
5. Label the tube with **"Resin"**, your initials and the date.
6. Note down all reagent lot numbers.

4.1.4 1x Wash Buffer

Note: 2x Wash Buffer is supplied with the DNA IQ™ kit.

Warning: The 2 x Wash Buffer, 2-Propanol and Ethanol is flammable and hazardous. It may cause serious eye irritation, drowsiness or dizziness if vapours are inhaled. Please use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling. It is recommended that this reagent is not to be prepared / handled by pregnant staff.

To prepare 1x Wash Buffer:

1. In a biohazard cabinet, add 35 mL of *AnalR* 100 % Ethanol to the 2x Wash Buffer bottle.
2. Add 35 mL of 2- Propanol to the 2x Wash Buffer bottle.
3. Update the information on the bottle to indicate that it now contains 1x Wash Buffer and when and by whom it was prepared by. Update the 1x Wash Buffer reagent audit trail in AUSLAB by:

    - From the main page press <2> "Sample Processing"
    - Press <8> "Materials Processing"
    - Press <2> "Consumable Inventory"
    - Highlight "DNA IQ Wash Buffer" in the list and press <enter>
    - Highlight the correct lot number and press <SF8> "Consumable Lot Audit"
    - Add audit entry that specifies the 1x Wash Buffer aliquot (e.g. A1) and the lot numbers of the ethanol and isopropyl alcohol used.

Note: 1x Wash Buffer can be prepared and stored at room temperature prior to use in a manual DNA IQ™ procedure.

1. Use the correct table in the appendices (Section 18) for the required procedure to determine the reagent volume.
2. In the biohazard cabinet, aliquot the required amount of 1x Wash Buffer into a 50 or 10 mL tube.
3. Label the tube with **"Wash Buffer"**, your initials and the date.
4. Note down the reagent lot number.

4.1.5 Elution Buffer

Elution Buffer is supplied with the DNA IQ™ kit.

1. Use the correct table in the appendices (Section 18) for the required procedure to determine the reagent volume.
2. In the biohazard cabinet, aliquot the required amount of 1x Wash Buffer into a 50 or 10 mL tube.
3. Label the tube with **"Wash Buffer"**, your initials and the date.
4. Note down the reagent lot number.

4.2 EQUIPMENT

4.2.1 Equipment and consumables required for Manual DNA IQ™

Table 2 outlines the equipment and location required for Manual DNA IQ™ procedures.

Table 2 Equipment with asset number and location for each procedure

Equipment	Location	Procedure
Fridge	3189	Manual DNA IQ™; Off-deck Lysis
Freezers	3189 / 3190	Manual DNA IQ™; Off-deck Lysis
Biological safety cabinet class II x **4**	3188 x 1 3189 x 3	Manual DNA IQ™; Off-deck Lysis
PCR cabinet x 1	3189	Manual DNA IQ™; Off-deck Lysis
96 well tube racks	3189	Manual DNA IQ™; Off-deck Lysis
Vortex x 4	3189	Manual DNA IQ™; Off-deck Lysis
Hot block x 4	3189 / 3191 / 3194	Manual DNA IQ™; Off-deck Lysis
Centrifuge x 4	3189/3194	Manual DNA IQ™; Off-deck Lysis
Mini/Micro centrifuges x 4	3189	Manual DNA IQ™; Off-deck Lysis
Finnpipettes 100 – 1000 µL	3189	Manual DNA IQ™; Off-deck Lysis
Finnpipette 20 – 200 µL	3189	Manual DNA IQ™
Thermo mixer x 2	3189	Manual DNA IQ™
Magnetic rack	3189	Manual DNA IQ™
Multitube Shaker	3191	Manual DNA IQ™

Table 3 outlines the consumables and location required for Manual DNA IQ™ procedures.

Table 3 Consumables and location for each procedure

Consumables	Location	Procedure
5 mL, 10 mL and 50mL sterile tubes	3188 / 3189	Manual DNA IQ™; Off-deck Lysis
1000 µL ART or clip tips	3188 / 3189	Manual DNA IQ™; Off-deck Lysis
1.5mL and/or 2mL tubes	3188 / 3189	Manual DNA IQ™; Off-deck Lysis
Spin baskets and 2 mL collection tubes	3189	Manual DNA IQ™; Off-deck Lysis
Inoculation sticks	3189	Manual DNA IQ™; Off-deck Lysis
300 µL ART, 200 µL One-touch tips, 200µL Clip tips	3188 / 3189	Manual DNA IQ™
Nunc Bank-it™ tubes	3189	Manual DNA IQ™; Off-deck Lysis
Nunc Bank-it™ caps	3189	Off-deck lysis
Sharps bin	3188 / 3189	Manual DNA IQ™; Off-deck Lysis

Further consumables can be found in the Store Room (3184).

5 SAFETY

As per the standard laboratory procedure QIS 22857 Anti-Contamination Procedure, PPE is to be worn by all staff when performing this procedure. This includes the use of safety glasses.

The MPII instruments, labware, biohazard cabinets and centrifuges should be wiped with 5% v/v Trigene Advance **only**, followed by 70 % v/v ethanol.
Work benches and non-metallic equipment should be wiped with 0.5 % v/v bleach, followed by 70 % v/v ethanol.

Warning: Do not place any part of the body inside the cabinet while the MPII is running a procedure.

If it is necessary to access the deck, pause the program using the computer software or the emergency STOP button located on the front of the instrument.

Warning: Tris base, EDTA, Sarcosyl (contained in the Extraction buffer which is prepared) and Lysis Buffer are irritants and hazardous. DTT is a reducing agent that destroys disulfide bonds. Wash Buffer (which contains 2 x Wash Buffer, 2-Propanol and Ethanol) is flammable and hazardous. Handle carefully and wear appropriate PPE. It is recommended that Wash Buffer is not prepared / handled if pregnant, and therefore the procedures using these reagents should not be performed by pregnant women.

Lysis Buffer contains guanidinium thiocyanate (GuSCN) which is toxic and can be harmful if inhaled, swallowed or comes in contact with skin. Left over Lysis Buffer-DTT is to be disposed of in a brown Winchester bottle in the ventilated cupboard in Room 3191. Never dispose down the sink. If spillage occurs, absorb with liquid-binding material (such as sand or kitty litter) and dispose in a biohazard bin.

Do not combine bleach with Lysis Buffer and do not wipe areas of Lysis Buffer spillage with bleach, as the two reagents produce cyanide gas when combined. Handle carefully and wear appropriate PPE, including safety glasses, when handling Lysis Buffer. If Lysis Buffer

is spilt onto PPE (eg. Gloves, gowns), remove and discard the contaminated item/s in a biohazard bin.

6 SAMPLING AND SAMPLE PREPARATION

6.1 SAMPLE LOCATIONS

Samples waiting to be extracted are stored in freezers as detailed in Table 6.

Table 6 Sample storage locations

Sample type	Storage Device	Storage Location
Urgent/High/Medium Priority Samples	Freezer	3190 or Block 6 walk-in freezer*
Lysates in 1.0mL Nunc Bank-it™ tubes	Freezer	3190
Extracts in 1.0mL Nunc Bank-It™ tubes	Freezer	3194 A

* Some storage boxes containing samples are located in Block 6, within the Exhibit Room (6106).

6.2 QC SAMPLES

One negative reagent control and one positive blood control (from a known donor) must be registered for all extraction batches. Additionally, off-deck lysis batches require five blank controls as detailed in Table 7.

Table 7 Extraction Quality Controls

Batch Type	Control
Off-deck lysis	Positive control (x1)
	Negative control (x1)
	Blank control (x5)
Manual DNA IQ™	Positive control (x1)
	Negative control (x1)
Retain supernatant DNA IQ™	Positive control (x1)
	Negative control (x1)

6.3 REGISTRATION OF QC SAMPLES

Register control samples according to the standard laboratory procedure QIS 24919 Forensic DNA Analysis Workflow Procedure. CQ samples for off-deck lysis are to be registered with a 9PLEX test code. QC samples for a DNA IQ Maxwell or a Retain Supernantant DNA IQ extraction batch are to be registered using the XPLEX test code.

6.4 CREATION OF EXTRACTION BATCHES

Create extraction batches according to the standard laboratory procedure QIS 24919 Forensic DNA Analysis Workflow Procedure.

6.5 LOCATING SAMPLES

Locate samples according to the standard laboratory procedure QIS 23959 Storage Guidelines for Forensic DNA Analysis.

6.6 EXCESS SUBSTRATE

If excess substrate is noticed before extraction buffer is added, remove the sample from the extraction batch and return to Evidence Recovery for sampling. If excess substrate is

identified after the extraction buffer has been added then proceed with extraction and notify Analytical HP5 for reporting to QPS via EXH.

6.7 ELECTRONIC WORKFLOW DIARY

Within the electronic workflow diary I:\AAA Electronic Workflow Diary record details of the batch sample creation and processing.

7 MANUAL METHODS FOR EXTRACTION USING DNA IQ™

7.1 MANUAL DNA IQ™ (NO RETAIN SUPERNTANT)

Note: All manual extractions are to be performed within a biohazard cabinet in room 3189.

1. Prepare fresh reagents within the biohazard hood of the clean room (3188) prior to commencing the procedure. Refer to Section 4.1 for preparation of reagents. Use Table 8 in Appendix 18.1 for reagent volumes.

Warning: The 2 x Wash Buffer, 2-Propanol and Ethanol is flammable and hazardous. It may cause serious eye irritation, drowsiness or dizziness if vapours are inhaled. Please use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling. It is recommended that this reagent is not to be prepared / handled if pregnant, and therefore this procedure should not be performed by pregnant women.

2. Turn on the Eppendorf Thermomixer and set to 37 ˚C and 1400 rpm.

3. Label for each sample:
   - Original sample tube;
   - Spin basket (if required);
   - 2mL tube; and
   - Nunc™ Bank-It™ tube.

Note: A spin basket is required for swabs, fabric, paper and cigarette butts. Samples that do not require a spin basket are tape-lifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

4. Have a second operator perform a sequence check of all tubes. Record the sequence check in AUSLAB.

5. Add 300 µL of Extraction Buffer to each sample. Ensure that large substrates including tape-lifts are fully submerged.

6. Vortex and incubate at 37 °C on the Thermomixer at 1400 rpm for 45 minutes.

7. Remove from the Thermomixer and increase the temperature on the Thermomixer to 65 °C for use in the elution steps.

8. Transfer the substrate to spin basket (if required) or transfer the lysate to an appropriately labelled 2.0 mL tube. Retain the original tube containing the substrate if no spin basket is used.

9. Centrifuge the spin basket at 15800 xg for 2 minutes.

10. Transfer the lysate from the original sample tube and the flow-through from the collection tube of the spin basket to an appropriately labelled 2 mL tube. Transfer the substrate from the spin basket back into the original sample tube.

    Note: If original sample tube is an appropriate 2 mL tube, transfer the substrate into the new 2 mL tube and add the flow-through from the spin basket to the lysate still in the original sample tube.

11. Add 550 µL of Lysis Buffer-DTT solution to the lysate.

12. Add 50 µL of DNA IQ™ Resin-Lysis Buffer-DTT solution to the lysate. Pipette mix or vortex the solution frequently to keep a homogenous suspension of resin within the solution.

13. Vortex and place on the Multitube shaker set at 1200 rpm for 5 minutes.

14. Remove from the Multitube shaker, vortex and immediately place into the magnetic stand. Separation will occur instantly.

    Note: If resin does not form a distinct pellet on the side of the tube, or if the magnetic beads have re-suspended while in the stand, vortex the tube and quickly place back in the stand.

15. Carefully remove and discard the liquid, ensuring that the resin is not disturbed. Remove from the magnetic stand.

    Note: If some resin is drawn up in the pipette tip, gently expel resin back into tube to allow re-separation.

16. Add 125 µL of Lysis Buffer-DTT solution. Vortex and place into the magnetic stand.

17. Once separation has occurred, remove and discard the liquid. Remove the tube from the magnetic stand.

18. Add 100 µL of 1X Wash Buffer, vortex and place into the magnetic stand. Once separation has occurred remove and discard the liquid. Remove the tube from the magnetic stand.

19. Repeat step 18 twice for a total of three Wash Buffer washes. Ensure that all of the liquid has been removed after the last wash. Remove the tube from the magnetic stand.

20. Uncap the tubes and place the lids down onto a clean rediwipe within the biohazard cabinet. Allow the resin to air-dry in the hood for 15 minutes and then recap the tubes.

    Note: Do not allow the resin to dry for more than 20 minutes as this may inhibit DNA elution.

21. Add 50 µl of Elution Buffer by carefully pipetting the solution to the side of the tube, above the pellet. **Do not mix**.

22. Incubate in the Thermomixer at 65°C for 3 minutes and then continue to incubate for a further 3 minutes shaking at 1100 rpm. If no thermomixer is available, incubate samples within a hot block and vortex mix at the start and end of the second 3 minute incubation. Remove samples.

23. Vortex and immediately place into the magnetic stand while hot to ensure maximum DNA yield during elution.

24. Carefully transfer the DNA extract to the corresponding labelled Nunc™ Bank-It™ tube.

25. Remove from the magnetic stand and repeat steps 21-23 once. The final volume after the second elution is approximately 95 µL of DNA extract.

26. Store the DNA extracts in temporary storage located within the Pre-PCR sorting room 3194-A in the upright freezer with equipment number 30512883.

27. Store tubes containing substrates in Spin Basket boxes located within the Pre-PCR sorting room 3194-A in the upright freezer with equipment number 30512883.

7.2 PROCEDURE FOR MANUAL DNA IQ™ (RETAIN SUPERNATANT)

Note: All manual extractions are to be performed within a biohazard cabinet in room 3189.

1. Prepare fresh reagents within the biohazard hood of the clean room (3188) prior to commencing the procedure. Refer to Section 4.1 for preparation of reagents. Use Table 9 in the Appendix 18.2 for reagent volumes.

Warning: The 2 x Wash Buffer, 2-Propanol and Ethanol is flammable and hazardous. It may cause serious eye irritation, drowsiness or dizziness if vapours are inhaled. Please use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling. It is recommended that this reagent is not to be prepared / handled if pregnant, and therefore this procedure should not be performed by pregnant women.

2. Turn on the Eppendorf Thermomixer and set to 37 ˚C and 1400 rpm.

3. Label for each sample:
   - Original sample tube;
   - 2 mL tube for sample transfer and processing;
   - 1.5mL tube labelled 'SUP' for supernatant;
   - Spin basket (if required); and
   - Nunc™ Bank-It™ tube.

   Note: A spin basket is required for swabs, fabric, paper and cigarette butts. Samples that do not require a spin basket are tape-lifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

4. Have a second operator perform a sequence check of all tubes. This person must enter the sequence check in AUSLAB.

5. Add 450µL of TNE buffer to the sample and vortex.

6. Incubate at room temperature for 30 minutes.

7. Vortex, then centrifuge at 15800 xg for 3 minutes.

8. Remove 150 µL of supernatant and place into the respective 1.5 mL tube labelled with 'SUP' (for further testing).

9. Add 14µL of 20mg/mL Proteinase K and 7µL 40% (w/v) Sarcosyl to the original sample tube containing substrate and TNE buffer, then vortex.

10. Vortex, then incubate at 37°C on the Thermomixer at 1400 rpm for 45 minutes. If no thermomixer available, incubate samples within a hot block, vortex mix at the start and end of the incubation and at least one during the incubation.

11. Remove from the Thermomixer and increase the temperature on the Thermomixer to 65°C (for use in the Elution steps).

12. Transfer substrate to spin basket if required or transfer the lysate to an appropriately labelled 2.0mL tube. Retain original tube containing the substrate in if no spin basket used.

13. Centrifuge spin basket at 15800 g for 2 minutes.

14. Transfer the substrate in the spin basket into a new and labelled 2mL tube to be retained. Transfer the flow through (lysate) back into the original sample tube.

15. Add 550µL of Lysis-DTT Buffer solution.

16. Add 50µL of DNA IQ™ Resin-Lysis solution. Pipette mix the Resin-Lysis solution between each addition to keep the resin suspended in solution.

17. Vortex and place on the Multitube shaker set at 1200 rpm for 5 minutes.

18. Remove from the Multitube shaker, vortex and immediately place into the magnetic stand. Separation will occur instantly.

    Note: If resin does not form a distinct pellet on the side of the tube, or if the magnetic beads have re-suspended while in the stand, vortex the tube and quickly place back in the stand.

19. Carefully remove and discard the solution, ensuring that the resin is not disturbed. Remove from the magnetic stand.

    Note: If some resin is drawn up in the pipette tip, gently expel resin back into tube to allow re-separation.

20. Add 125µL of Lysis-DTT Buffer solution. Vortex and place into the magnetic stand.

21. Once separation has occurred, remove and discard the Lysis-DTT Buffer. Remove from the magnetic stand.

22. Add 100µL of 1X Wash Buffer, vortex and place into the magnetic stand. Once separation has occurred remove and discard the Wash Buffer. Remove from the magnetic stand.

23. Repeat the Wash Buffer step (step 23) two times for a total of three washes. Ensure that all of the solution has been removed after the last wash. Remove from the magnetic stand.

24. Uncap the tubes and place the lids down onto a clean rediwipe. Air-dry the resin in the hood for 15 minutes and then recap tubes

    Note: Do not dry for more than 20 minutes, as this may inhibit the elution of DNA.

25. Add 50µl of Elution Buffer by carefully pipetting the solution to the side of the tube, above the pellet. **Do not mix**.

26. Incubate in the Thermomixer at 65°C for 3 minutes and then continue to incubate for a further 3 minutes shaking at 1100 rpm. If no thermomixer available, incubate samples within a hot block, vortex mix at the start and end of 2nd 3 minute incubation. Remove samples.

27. Vortex and immediately place into the magnetic stand while hot to ensure maximum DNA yield during elution.

28. Carefully transfer the DNA extract to the corresponding labelled Nunc™ Bank-It™ tube.

29. Remove from the magnetic stand and repeat the Elution Buffer steps (step 26-29). The final volume after the double elution is approximately 95µL of DNA extract.

30. Store the DNA extracts in temporary storage located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

31. Store tubes containing substrates in Spin Basket boxes located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

32. Store supernatants in the S/N Retention boxes located within the Extraction sorting room in the Orford Freezer 30512883 (Room 3190).

33. Ensure that the negative extraction control from the batch is also inserted onto the **'Saliva List':**

    - Log into Auslab
    - 3 for Patient Enquiry
    - Type/Scan the lab number for the negative extraction control
    - From the 9Plex page or the XPLEX Page press Shift F12
    - A prompt will appear 'Enter List Name'
    - Type Saliva or use the F1 lookup list.
    - Press Enter

8 QUALITY ASSURANCE/ACCEPTANCE CRITERIA

- Positive and negative (reagent blank) controls are included in each extraction batch. However, for off deck-lysis batches, an additional five negative (reagent blank) controls are included. These controls are processed as normal samples through to completion.
- If any results are obtained from the reagent blank, either at quantification or during the CEQ check, then the possible source of the contamination is investigated. The samples extracted with this control are thoroughly checked and repeated if required. This is covered in QIS 17130 CE Quality Check and QIS 24012 Miscellaneous Analytical Section Tasks

9 VALIDATION

- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 9. Automation: Report on the Evaluation of DNA Extraction Chemistries." June 2007.
- Muharam I., Hlinka V., Gallagher, B., Lundie, G., Iannuzzi, C., Nurthen T., McNevin A., Ientile V. "Project 21: A Modified DNA IQ™ Method Consisting of Off-Deck Lysis to Allow Supernatant Retention for Presumptive Identification of α-Amylase" 2008

10 STORAGE OF DOCUMENTS

All worksheets, after auto extraction, manual IQ and Retain supernatant batches have been completed, are stored within the Pre-PCR sorting room (3194-A) in an allocated in-tray for scanning by the operational officers.

11 REFERENCES

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- Marko, M.A., Chipperfield, R., & Birnboim, H.C., A Procedure for the Large Scale Isolation of Highly purified Plasmid DNA using alkaline extraction and binding to glass powder. Anal. Biochem., 1982. 121: p. 382-387.
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- Promega, FAQs –DNA IQ™ System.
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- Promega, DNA IQ™ System –Small Casework Protocol. Promega Technical Bulletin #TB296 2006. Rev 4/06: p. 1-14.
- Promega, DNA IQ™ System-Database Protocol. Promega Technical Bulletin #TB297, 2006. Rev 4/06: p. 1-14.
- Promega, Tissue and Hair Extraction Kit (for use with DNA IQ™) Protocol. Promega Technical Bulletin #TB307, 2006. Rev 5/06: p. 1-11.
- Promega Corporation 2006 Material Safety Data Sheet. Lysis Buffer. Article number: A826.
- Schiffner, L.A., Bajda, E. J., Prinz, M., Sebestyen, J., Shaler, R. & Caragine, T.A., Optimisation of a Simple, Automatable Extraction Method to Recover Sufficient DNA from

Low Copy Number DNA Samples for Generation of Short Tandem Repeat Profiles. Croat Med J, 2005. 46(4): p. 578 -586.

- Vandenberg, N., van Oorchot., R. A. H., & Mitchell, R. J., An evaluation of selected DNA extraction strategies for short tandem repeat typing. Electrophoresis, 1997. 18: p. 1624-

12 ASSOCIATED DOCUMENTS

QIS 17165 Receipt, Storage and Preparation of Chemicals, Reagents and Test Kits
QIS 22857 Anti-Contamination procedure
QIS 23959 Storage Guidelines for Forensic DNA Analysis
QIS 24256 Sequence Checking with the STORstar Instrument
QIS 24469 Batch functionality in AUSLAB
QIS 24919 Forensic DNA Analysis Workflow Procedure
QIS 17130 CE Quality Check
QIS 24012 Miscellaneous Analytical Section Tasks

13 AMENDMENT HISTORY

Version	Date	Author/s	Amendments
R0	23 Oct 2007	B. Gallagher, T. Nurthen, C. Iannuzzi, V. Hlinka, G. Lundie, I Muharam.	First Issue
R1	12 Dec 2007	M Harvey, C Iannuzzi, A McNevin	Reviewed and updated after initial training
R2	19 March 2008	M Harvey, B Andersen, C Iannuzzi, A McNevin	Addition of Off-deck Lysis procedure, Retention of fully automated method as Appendix, addition of reagent record tables into Appendix
R3	April 2008	QIS2 Migration Project	Headers and Footers changed to new CaSS format. Amended Business references from QHSS to FSS, QHPSS to CaSS and QHPS to Pathology Queensland
4	13 March 2009	QIS2 migration	Version incremented by one on migration to QIS2
5	03 June 2009	M Aguilera, B Micic, C Iannuzzi, A. Cheng, V. Hlinka, I. Muharam, G. Lundie, C. Weber	Major changes to reflect new procedure. Updated to reflect changes in procedure as an outcome of internal and external audits. Created ver.6.4 ODL in MPII Platforms. Minor changes in procedures using 4titude 4seal heat sealer to seal plates.
6	29 June 2009	A McNevin, K Lancaster	Removed references to retaining lysate and beads, fixed minor formatting errors. Created ver6.5 ODL in MPII Platforms. Substrates now to be retained in 2mL tube
7	30 September 2010	M.Cipollone, M.Mathieson	Major changes made RE: room numbers to reflect the move to Block 3. Re-formatted entire SOP. New Appendices added which outline all reagent volumes. All equipment and

Version	Date	Author/s	Amendments
			associated asset numbers have been included. Use of the electronic diary now included as an additional section. Preparation of reagents within the clean room (3188) now to be done prior to starting each process. Storage of worksheets updated. New software version 6.6 on automated extraction robots. S/N Retention Boxes now stored in Manual Ext Room. Associated Documents and hyperlinks updated. Consumables and Equipment table added for Manual DNA IQ.
8	24 May 2012	A. Speirs M. Cipollone	Major revision of document. Revision of sections 1-3 to improve clarity. Updated Tables 1 to 7. Removed redundant sections on locating samples and creating batches. Removed procedure for retain supernatant Off-Deck Lysis and thus associated Appendix. Table of Contents amended to reflect all changes. Moved the Manual DNA IQ procedures before the Off-Deck Lysis procedure. Re-formatted spacing and alignments of headings. Re-numbered Tables in Appendices. Updated sections to include which relevant Appendix to use in regards to reagents. Minor grammatical and spelling errors fixed.
9	4 Dec 2013	B. Andersen, M. Cipollone	Updated Section 7 of the Manual DNA IQ Retain Supernatant procedure to reflect differences in list inserting between P+ and PP21 samples. Updated Section 6 to clarify 9PLEX and XPLEX test codes. New template applied and minor formatting and wording changes.
10	April 2015	M. Aguilera M.Cipollone	Adjusted Table 9 for Retain Supernatant batches to indicated TNE, Prok and Sarcosyl are not to be combined at reagent prep stage and to be added to samples separately. Changed reference to Isopropyl Alcohol to 2-Propanol. Included warning for the preparation and use of the Wash Buffer for pregnancy, and amended Risk phrases for reagents used as per risk assessment completed by JSM. Updated equipment tables and removed asset

Version	Date	Author/s	Amendments
			numbers. Changed template to HSSA Fixed Document headers; Fixed formatting, removed steps no longer used; Added two more associated documents QIS 17130 CE Quality Check QIS 24012 Miscellaneous Analytical Section Tasks
11	January 2017	L. Ryan	Removed Automated Extraction

14 APPENDICES

14.1 REAGENT VOLUMES FOR MANUAL DNA IQ™ (NO RETAIN SUPERNATANT)

Table 8 - Reagent volumes for manual method

Reagent	Volume for 12 samples (mL)	Volume for 24 Samples (mL)
TNE buffer	4.0	8.0
Proteinase K (20mg/mL)	0.216	0.432
Sarcosyl (40%)	0.108	0.216
Lysis-DTT Buffer		
DNA IQ™ Lysis Buffer	10	20
DTT (1M)	0.1	0.2
DNA IQ™ Resin solution		
Lysis-DTT Buffer	0.645	1.29
DNA IQ™ Resin	0.105	0.210
DNA IQ™ 1x Wash Buffer	4.0	8.0
DNA IQ™ Elution Buffer	1.4	2.8

Note: The volume of Lysis-DTT Buffer calculated includes the volume used in the Resin-Lysis solution preparation.

14.2 REAGENT VOLUMES FOR MANUAL DNA IQ™ (RETAIN SUPERNATANT)

Table 9 - Reagent volumes for manual method (retain supernatant)

Reagent	Volume per sample (uL)	Volume for 12 samples (mL)
TNE buffer	450	6.5
Proteinase K (20mg/mL)	14	0.168
Sarcosyl (40%)	7	0.1
Lysis-DTT Buffer		
DNA IQ™ Lysis Buffer	660	10
DTT (1M)	6.6	0.1
DNA IQ™ Resin solution		
Lysis-DTT Buffer	43	0.645
DNA IQ™ Resin	7	0.105
DNA IQ™ 1x Wash Buffer	300	4.0
DNA IQ™ Elution Buffer	100	1.4

Note: The volume of Lysis-DTT Buffer calculated includes the volume used in the Resin-Lysis solution preparation. For retain supernatant batches, please ensure that TNE, Pro K, and Sarcosyl are not combined at reagent preparation stage and are to be added to samples separately.

Promega talk – Dr David Grasso (Tuesday, 28 June 2005)

Please note David Grasso is attempting to give us an electronic copy of his talk.

SlicPrep

- 96-well spin basket (basically 96 spin baskets glued together) to go into a 96-well deep plate – requires centrifugation.
- Digestion (165uL) and spin positions (165uL + 500uL).
- Lyse blood cards or buccal samples in the 96-well spin basket + deep-well plate, centrifuge to bring down DNA solution, put deep-well plate on robot (minus the spin basket).
- Makes Differex amenable to automation.
    - Manual Differex: 90 mins including 60 mins Prot K digestion (37, 56ºC), depends on number of samples (more samples will increase processing time).
    - Automated Differex: 40 samples (80 fractions) in 120 mins (10 mins hands-on). Use liquid sensing and barrier tips, ie program tips to go in at certain depth.
    - High-throughput with SlicPrep.
    - Compatible for use with phenol/chloroform.
    - Yellow dye in one of the buffers doesn't inhibit STR PCR.

Wizard SV96 Genomic (fixed solid phase)

- SV = spin/vac; membrane columns.
- 1 or 96 samples.
- Manual or auto (some membrane clogging).
- Easy, rapid, high yield at fixed cost per column.
- Mainly used in research fields (eg tissue/cell).

DNA IQ

- Amenable to automation.
- Use what you need – scalable; more sample/DNA: increase volume of resin.
- Cost effective and most flexible.
- Validated methods, used in forensics. Proven with Biomek 2000, PE and Tecan instruments.
- Overloading can happen due to too much sample/biomass.
- 7uL resin binds 100ng of DNA.
- High efficiency, small samples.
- Hair/tissue Prot K = removes material that competes with binding to resin.
- To be efficient, should process ~>16 samples
- Elution volume 25-100uL in strip tubes or polypropylene 96-well.
- For blood samples: ~95uL (?), for >100uL you need to change the program.
- For forensic usage, see Palm Beach County Sheriff's Office (Julie Conover); 7th International Symposium, Wellington, NZ; Greenspoon paper.
- For DNA normalisation, see Profiles in DNA on Biomek 2000 (but Biomek 3000 has different s/ware).

Slicprep™ 96 Device

High-Throughput Processing of Samples on Solid Supports Using the Slicprep™ 96 Device

By Allan Tereba, Julia Krueger, Ryan Olson, Paraj Mandrekar and Bob McLaren
Promega Corporation

INTRODUCTION

Crime statistics now support the effectiveness of aggressively analyzing breaking-and-entering cases and developing genotype databases of these felons. Unfortunately, this approach has created an ever-expanding workload and increasing backlogs. Over the last few years, automation has played an important and expanding role in handling the increased work, but the upfront processing of these samples still creates a bottleneck. Most casework, reference and database samples are on solid supports that do not lend themselves to robotic manipulation. FTA® cards have provided one solution for reference and database samples. However, because of the high DNA-binding capacity, they typically give poor genotype profiles unless the DNA is removed. Although automated methods exist for buccal swabs, they can give variable results and are prone to clogged pipette tips. Until now, casework samples have had to be processed individually to separate the solid support from the eluted DNA solution.

Promega has solved this bottleneck by developing the Slicprep™ 96 Device. This device allows the simultaneous centrifugation of 96 samples and is designed so that both the digestion or lysis and centrifugation can be performed in the same device.

The Slicprep™ 96 Device allows the simultaneous centrifugation of 96 samples and is designed so that both the digestion or lysis and centrifugation can be performed in the same device.

THE SLICPREP™ 96 DEVICE

The Slicprep™ 96 Device consists of 3 components: a 2.2ml 96 Deep Well Plate, a 96 Spin Basket and a U-Shaped Collar (Figure 1). The 7 holes in the rounded bottom of the baskets ensure good removal of DNA and cells from the solid support. Samples such as cotton swabs, blood punches or pieces of clothing are inserted into the baskets, which are big enough to accept an entire dried cotton swab. In the digestion position, the 96 Spin Basket is fully inserted into the 96 Deep Well plate, allowing space for approximately 165µl of solution below the basket in each well (Figure 2, Panel A). After the incubation, the baskets are raised approximately 1cm (the spin position, Figure 2, Panel B) to create space for an additional 500µl of solution.

MATERIALS AND METHODS

With the device in the digestion position, samples were placed in the baskets. For reference samples, 400µl of DNA IQ™ Lysis Buffer was added, and the device was sealed with a foil seal and heated in a 70°C water bath for 1 hour. For touch samples, 400µl of a 1.8µg/µl proteinase K solution was added, and samples were incubated in a 56°C oven for 1 hour. After incubation, the Slicprep™ 96 Device was removed from the water bath or oven, and the U-Shaped Collar was inserted. The device was then centrifuged at 1,450 × *g* for 5 minutes in a swinging plate rotor. The collar and baskets were then discarded, leaving the DNA-containing solution in the 96 Deep Well plate. The 96 Deep Well Plate was placed on a Beckman Coulter Biomek® 2000 workstation, and a BioWorks™ method directed DNA purification using the DNA IQ™ System[a]. DNA was eluted in 100µl (reference samples) or 40µl (touch samples) of TE^{-4} buffer (10mM Tris [pH 8.0], 0.1mM EDTA). Finally, a

Slicprep™ 96 Device

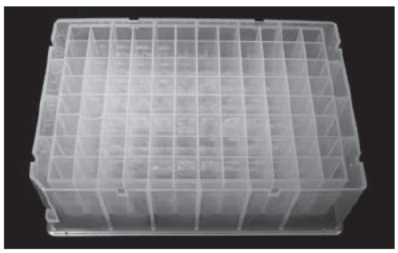
total of 1µl of the eluted DNA or 10µl of eluted control (blank) DNA was amplified with the PowerPlex® 16 System[b–d] and analyzed on an ABI PRISM® 3100 Genetic Analyzer.

TESTING FOR CONTAMINATION

The Slicprep™ 96 Device is made and packaged in a HEPA-filtered clean room to reduce the risk of DNA contamination. To detect any contamination introduced during the manufacturing process or the robotic method, the Slicprep™ 96 Device was incubated with DNA IQ™ Lysis Buffer, and the solution was then processed using the DNA IQ™ System and amplified with the PowerPlex® 16 System. To check for cross-contamination between samples, FTA® blood punches and cotton buccal swabs were placed in the baskets in a checkerboard pattern. Blank FTA® punches and cotton swabs were placed in the remaining baskets. Samples were processed as described in the Materials and Methods section. None of the blanks contained discernible peaks (data not shown).

96 Spin Basket

U-Shaped Collar



96 Deep Well Plate

S2447A

Figure 1. Components of the Slicprep™ 96 Device. The Slicprep™ 96 Device contains an ABgene 2.2ml, 96-well, deep-well plate (the 96 Deep Well Plate), a 96 Spin Basket and a U-Shaped Collar.

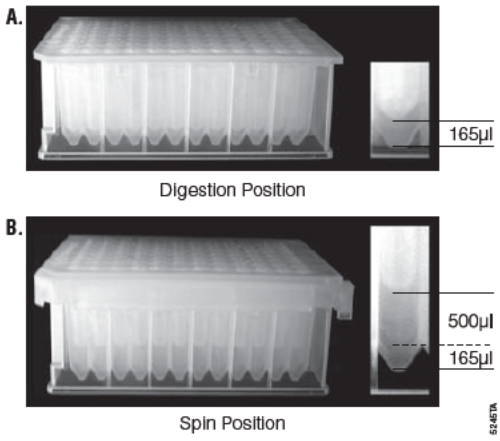
PROCESSING FTA® CARD PUNCHES AND BUCCAL SWABS

The Slicprep™ 96 Device is ideally suited for high-throughput extraction of DNA from database samples such as blood cards and buccal swabs. One 4mm-diameter or three 2mm-diameter FTA® punches containing blood, and whole cotton and paper (CEP) buccal swabs were processed as described in the Materials and Methods section using 400µl of DNA IQ™ Lysis Buffer (500µl for paper swabs).

The results are shown in Table 1, and representative genotypes from cotton swabs are shown in Figure 3. All three sample sets gave the expected yields with tight standard deviations. The FTA® blood cards yielded, on average, 70ng of DNA. Because DNA is most efficiently removed from FTA® paper by denaturation, heating this sample type to 90°C may give slightly higher yields. The buccal swabs gave average DNA yields of 151ng and 396ng for whole cotton and paper swabs, respectively. The higher DNA:protein ratios in these sample types (compared to blood) result in a higher DNA-binding capacity for the DNA IQ™ Resin. These yields are similar to those obtained using single spin baskets. Paper swabs can collect a large number of cells, forcing higher recovered yields. Using a fraction of the paper swabs would give yields closer to those obtained with cotton swabs but is less convenient when processing large numbers of this sample type.

Table 1. Average DNA Yields Using the Slicprep™ 96 Device.

Support Type	Average Yield (ng)	Standard Deviation (ng)
FTA® Blood Cards	70	10
Cotton Swabs	151	56
Paper Swabs	396	60



S2457A

Figure 2. Operational modes of the Slicprep™ 96 Device. Panel A. The digestion position. The 96 Spin Basket is fully inserted into the 96 Deep Well Plate to allow incubation of solid supports with digestion or lysis buffer. **Panel B.** The spin position. The U-Shaped Collar is inserted between the 96 Deep Well Plate and the 96 Spin Basket. This raises the baskets and allows an additional 500µl below the baskets.

Slicprep™ 96 Device

To test whether touch samples can be processed in a high-throughput manner, samples were collected on cotton swabs from soda cans, phones and computer keyboards. The swabs were placed in the 96 Spin Basket, and the shafts were broken off and used to push the swab head to the bottom of the basket. Samples were processed as described in Materials and Methods. The resulting DNA solutions were purified manually using the DNA IQ™ chemistry. As expected, some samples provided more DNA than others, but many of the samples gave full profiles (Figure 4).

AUTOMATION WITH THE SLICPREP™ 96 DEVICE

The Slicprep™ 96 Device is designed to provide a higher throughput than individual spin baskets for upfront processing of samples on solid supports. For database and reference samples where the sample is soaked in DNA IQ™ Lysis Buffer, the extracted fractions in the 96 Deep Well Plate can be processed using existing methods. Touch samples are best processed with up to 500µl of proteinase K digestion solution, which must be diluted with two volumes of DNA IQ™ Lysis Buffer before DNA purification. The resulting large volumes are not efficiently processed by most workstations. However, Dr. Susan Greenspoon from the Virginia Division of Forensic Sciences has collaborated with Promega to develop a Biomek® 2000 method that can efficiently handle aqueous sample volumes up to 0.5ml as efficiently as manual DNA IQ™ System or phenol:chloroform purification. A method to incorporate the Slicprep™ 96 Device for processing large volumes is being developed.

ORDERING INFORMATION

Slicprep™ 96 Device	Cat.# V1391

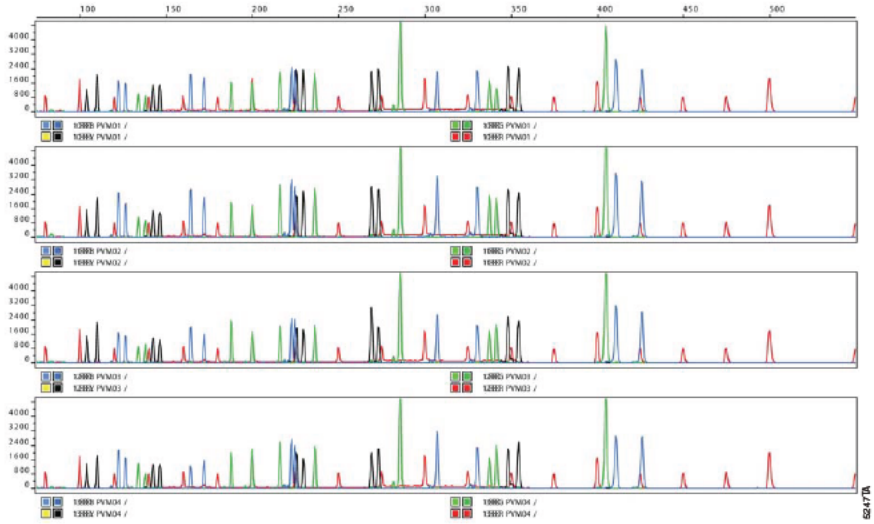


Figure 3. Analysis of buccal swabs. Cotton swabs were placed in a checkerboard pattern between buccal swabs in the Slicprep™ 96 Device. DNA IQ™ Lysis Buffer was added, and the device was sealed with a Beckman Coulter Biomek® seal & sample aluminum foil lid (Cat.# 538619) and incubated at 70°C for 1 hour. The device was centrifuged for 5 minutes at 1,450 × *g* (3,000rpm) in a Beckman Coulter Allegra 6R centrifuge with a GH-3.8 rotor containing Microplus plate carriers. The samples were processed on a Beckman Coulter Biomek® 2000 workstation using DNA IQ™ chemistry, and the DNA was eluted in 100µl of TE^{-4} buffer. One microliter was amplified with the PowerPlex® 16 System and analyzed on an ABI PRISM® 3100 Genetic Analyzer. Quantitation of the DNA was not necessary.

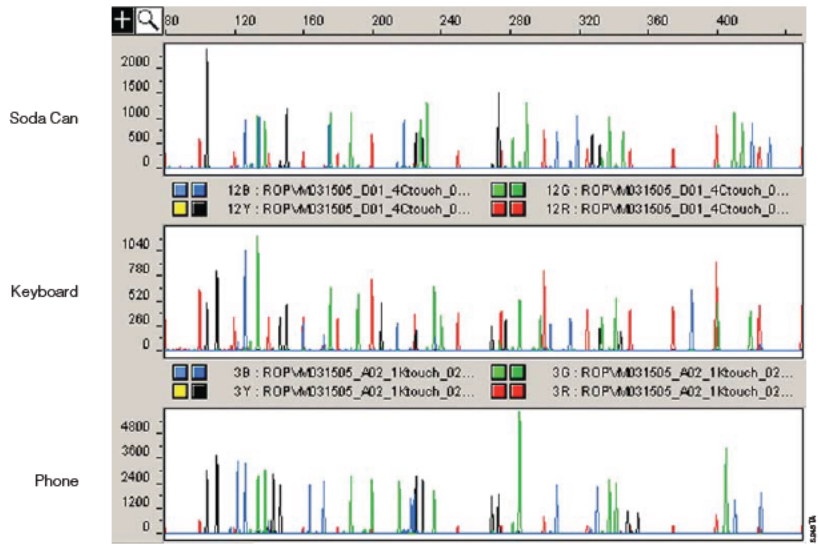


Figure 4. Analysis of touch samples. Soda cans **(Panel A)**, keyboards **(Panel B)** and phones **(Panel C)** were swabbed with cotton swabs, and the swabs were placed in a Slicprep™ 96 Device after drying. A total of 400µl of proteinase K digestion buffer was added, and the samples were incubated for 1 hour at 56°C in an oven with a foil seal. The device was centrifuged to recover the DNA. Samples were processed manually using the DNA IQ™ System, and DNA was eluted in 40µl of TE^{-4} buffer. The samples were amplified with PowerPlex® 16 and analyzed on an ABI PRISM® 3100 Genetic Analyzer.

The DNA IQ™ System on the Tecan Freedom EVO® 100

By Cristopher Cowan
Promega Corporation

These new automated scripts incorporate the ability to process aqueous and swab-type samples, start with samples in microcentrifuge tubes or deep-well plates, and process any number of samples between 1 and 96 to minimize reagent waste.

INTRODUCTION

Since its introduction, the automated DNA IQ™ System[a] for isolating genomic DNA has become a workhorse for the forensic community. Promega now introduces scripts for the use of this valuable chemistry on the Tecan Freedom EVO®100 automated liquid handler (Figure 1). The Tecan instrument boasts a number of advantages, including independent liquid-handling and gripping arms, adjustable tip spacing, independent volume control on each tip, and disposable-tip volumes of up to one milliliter. In addition, the Tecan Freedom EVO®100 for the DNA IQ™ System is configurable as either a lower cost 4-tip instrument or a higher throughput 8-tip instrument. The user can select the instrument configuration based on the need for lower cost or higher throughput.

Promega has developed and optimized scripts for DNA isolation on both the 4-tip and 8-tip Tecan Freedom EVO®100 instruments using the DNA IQ™ System for casework and databasing samples. These scripts are able to process liquid samples preprocessed with a proteinase K incubation (designated "aqueous" samples) and samples on solid supports preprocessed by incubation with DNA IQ™ Lysis Buffer (designated "swab" samples). For "swab" samples, the scripts are compatible with the Slicprep™ 96 Device (Cat.# V1391), which allows removal of the incubation liquid and solubilized material from the solid support without having to transfer material to another tube or plate. In addition, both scripts are able to process 1–96 samples, and samples can be placed directly in a deep-well plate to start or can be transferred from microcentrifuge tubes.

TECAN FREEDOM EVO® 100

The DNA IQ™ System scripts take advantage of the many unique features of the Tecan Freedom EVO®100.

- **Number of Tips:** One of the most basic options on the instrument is the number of tips on the liquid-handling arm. We have created DNA IQ™ System scripts for both the lower cost 4-tip option and the higher throughput 8-tip option. While the process remains the same for both numbers of tips, the 4-tip script requires 2 hours and 20 minutes, while the 8-tip script is complete in 1 hour and 30 minutes. (Times are based on processing 96 swab samples starting in a deep-well plate. Starting with samples in microcentrifuge tubes will increase the time.)
- **Tip Sizes:** Disposable tips are used for all experiments and are adequate in all liquid-handling steps. We used two different types of tips depending on what type of pipetting is being done. For all reagent transfers, we used 1ml filter, disposable tips, which allow for time and cost savings through multipipetting. These tips are also conductive, so the instrument can sense liquid levels in the reagent troughs, ensuring enough reagent for each step in the process. Two sets of 200µl filter, disposable tips are used, one for all sample transfer and waste removal steps and a second set for all elution steps. Here we use the less expensive, nonconductive tips to reduce the overall costs of the script.

FREEDOM EVO® 100

- **Adjustable Tip Spacing:** The liquid-handling arm of the Tecan Freedom EVO®100 has the ability to adjust individual tip spacing. We have taken advantage of this feature by allowing users to present samples either in a 2.2ml deep-well plate or individual microcentrifuge tubes.
- **Independent Tip Control:** Since each tip on the Freedom EVO® is controlled independently in terms of height and volume, we can process 1–96 samples without wasting reagents on empty wells in a column. Using the Tecan Freedom EVO®100 allowed us to process individual samples rather than an entire column.
- **Powerful Software:** EVOware® is the powerful software that controls the Tecan Freedom EVO®100. This software allows a single script to have many customizable features and allows rapid customization for the chosen hardware. The single scripts for the 4- and 8-tip instruments query the user for information about sample type (aqueous or swab), starting sample vessel (plate or tubes) and sample number (1–96). With this information, the script is customized to meet the user's needs. In addition to the core EVOware® functionality, users can take advantage of new options that enable effective tracking of all sample-handling steps and simple programming of normalization tasks.

RESULTS

Aside from the special features of the Tecan Freedom EVO®100 scripts, sample processing and results from the DNA IQ™ System remain unchanged. Here we report results from the DNA IQ™ System scripts run on the Tecan Freedom EVO®100.

CONSISTENT CONCENTRATION

To examine consistency of DNA yield, we purified DNA from 48 □ 20µl liquid whole blood samples using the 4-tip Tecan Freedom EVO®100 DNA IQ™ script. To minimize variability in the starting material, all blood used in these experiments was from the same individual. Prior to extraction the samples were preprocessed by incubating at 56°C for 1 hour with 80µl of proteinase K solution [as described in the *Tissue and Hair Extraction Kit (for use with DNA IQ™) Technical Bulletin* #TB307]. Samples were processed using the "aqueous" DNA IQ™ System script on the Tecan Freedom EVO® 100. Samples were eluted with 100µl of DNA IQ™ Elution Buffer and quantitated using the Quant-iT™ PicoGreen® dsDNA reagent. The average DNA concentration was found to be 1.76ng/µl with a standard deviation of 0.38ng/µl.

SAMPLE SIZE VERSUS DNA YIELD

The DNA IQ™ System allows DNA purification in two formats. When samples have limited amounts of DNA, the DNA IQ™ System provides a consistent amount of DNA; when samples have DNA in excess of the binding capacity of the DNA IQ™ Resin, a fixed yield is provided. To illustrate this using the DNA IQ™ script on the Tecan Freedom EVO®100, purification was performed from a dilution series of liquid whole blood samples. Triplicate samples of 0.05–20µl of whole blood were preprocessed by incubating at 56°C with 80µl of proteinase K solution. As shown in Figure 2, DNA yield increased with sample amount up to approximately 10µl of blood, where the resin becomes saturated.

Figure 1. The Tecan Freedom EVO® 100 instrument.

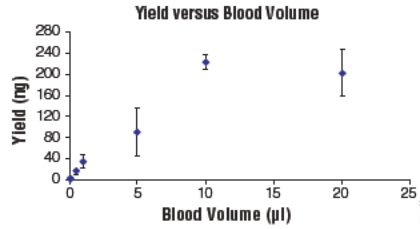


Figure 2. A plot of DNA yield versus input blood volume. DNA was purified from blood samples (0.05–20µl) in triplicate using the DNA IQ™ System method on the Tecan Freedom EVO®100. Quantitation of the eluates was performed using the Quant-iT™ PicoGreen® dsDNA reagent.

CROSS-CONTAMINATION

To assess cross-contamination in the DNA IQ™ script, we used the Tecan Freedom EVO®100 to purify DNA from a checkerboard pattern of buccal and blank swabs. Samples were preprocessed using the Slicprep™ 96 Device. Four hundred microliters of DNA IQ™ Lysis Buffer was added to each well of the Slicprep™ 96 Device containing either a buccal or blank swab. The plate was sealed, incubated at 70°C for 1 hour and centrifuged at 1,500 □ *g* for 5 minutes. Samples were processed using the "swab" DNA IQ™ script on the Tecan Freedom EVO® 100. One hundred microliters of DNA IQ™ Elution Buffer was used to elute the samples, and DNA from buccal swabs was diluted to a

concentration of approximately 1ng/µl. One microliter of each buccal swab sample and 10µl of each undiluted blank was amplified in a 25µl PowerPlex®16 System[b–d] reaction. Electropherograms from representative buccal swab and blank samples are shown in Figure 3. DNA from buccal swabs gave robust amplification, whereas there were no detectable amplification products in reactions with blank swabs.

CONCLUSIONS

The Tecan Freedom EVO®100 automated liquid handler is the newest and most flexible instrument available to automate the DNA IQ™ System. These new automated Tecan scripts incorporate the ability to process "aqueous" and "swab" samples, start with samples in microcentrifuge tubes or deep-well plates, and process any number of samples between 1 and 96 to minimize reagent waste.

Using these scripts, we observed the expected, linear DNA yields with limiting samples and consistent, fixed DNA concentrations when samples contained DNA in excess of the binding capacity of the resin. We have not observed any cross-contamination attributable to this script. Based on the data presented here, the 4-tip and 8-tip DNA IQ™ automated scripts provide the quality performance you would expect from the DNA IQ™ System while taking advantage of the strengths of the Tecan Freedom EVO®100.

For more information about the instrumentation necessary to run these scripts, contact Tecan. Additional hardware and plates are available from Promega.

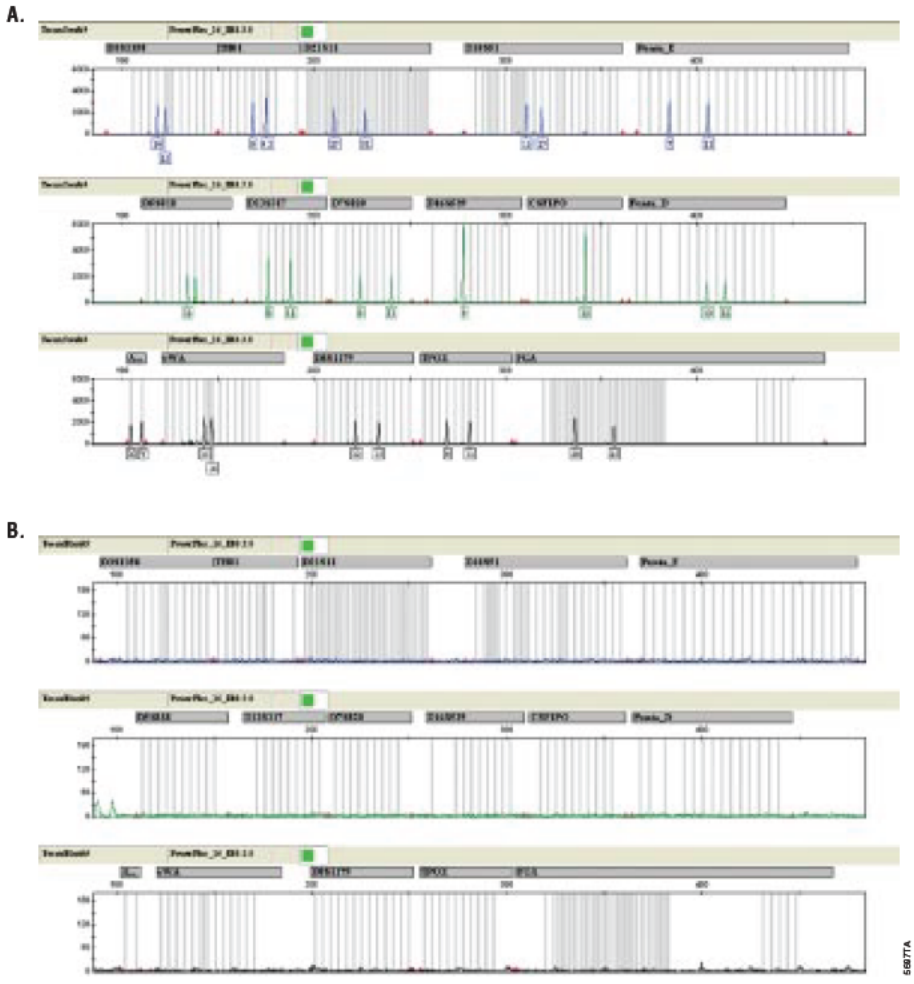


Figure 3. Cross-contamination results. Electropherograms showing representative traces from buccal swab (**Panel A**) and blank samples (**Panel B**). Samples were placed in a 96-well, deep-well plate in a checkerboard pattern and preprocessed by incubating with DNA IQ™ Lysis Buffer in the Slicprep™ 96 Device. DNA from buccal swab samples was adjusted to a final concentration of 1ng/µl. One microliter of swab samples or 10µl of blanks were amplified using the PowerPlex®16 System with 10/22 cycling on the GeneAmp®9700 PCR System. Amplification products were detected with the ABI PRISM®3100 Genetic Analyzer.

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Project 13. Report on the Verification of an Automated DNA IQ™ Protocol using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform

Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C., Ientile, V.
Automation/LIMS Implementation Project, DNA Analysis FSS (August 2008)

1. Abstract

A manual method for extracting DNA from forensic samples using the DNA IQ™ system (Promega Corp., Madison, WI, USA) was validated for routine use in DNA Analysis (FSS). We have verified an automated DNA IQ™ protocol in 96-well format for use on the MultiPROBE® II PLUS HT EX Forensic Workstation platforms (PerkinElmer, Downers Grove, IL, USA). Data indicate that results from the automated procedure are comparable to those from the manual procedure. Contamination checks were performed using samples prepared in checkerboard and zebra-stripe format, and results were as expected. We recommend the use of the MultiPROBE® II PLUS HT EX platforms to perform automated DNA extraction using the DNA IQ™ system.

2. Introduction

The MultiPROBE® II PLUS HT EX FORENSIC WORKSTATION platforms (PerkinElmer, Downers Grove, IL, USA) are equipped to perform automated DNA extractions, as they include a DPC shaker and individual heat controllers to enable on-board lysis and incubation steps. Currently in DNA Analysis, the MultiPROBE® platforms allow walk-away operation of PCR setup protocols for DNA quantitation and amplification.

The DNA IQ™ protocol has been verified or validated by various laboratories for use on the MultiPROBE® II PLUS platform. The laboratories that perform an automated DNA IQ™ protocol include PathWest (Western Australia), Forensic Science South Australia (South Australia) and Centre of Forensic Sciences in Toronto (Ontario). The MultiPROBE® II PLUS instrument comes pre-loaded with an automated DNA IQ™ protocol. Unlike the other laboratories, however, we did not validate the included protocol, but instead validated a manual DNA IQ™ protocol which was based on the CFS automated protocol (PerkinElmer, 2004), followed by verification of an automated protocol based on the validated manual method.

The verified automated DNA IQ™ protocol is identical to the validated manual protocol used in-house: there are no differences in reagents or volumes. The adopted DNA IQ™ protocol differs slightly, however, from the manufacturer's protocol, as it includes a lysis step using Extraction Buffer (10mM Tris, 1mM EDTA, 100mM NaCl, 20% w/v SDS) in the presence of Proteinase K, before incubating in the DNA IQ™ Lysis Buffer. Furthermore, the lysis incubation conditions were lowered from 70°C to 37°C in order to accommodate extraction of DNA from heat labile materials such as nylon and polyester. In addition, the automated protocol utilises the SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA) for simultaneous processing of samples in a 96-well format.

3. Aim

To verify an automated DNA IQ™ protocol for use on the MultiPROBE® II PLUS HT EX platforms to allow extraction of DNA from various sample types.

4. Equipment and Materials

- MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (PerkinElmer, Downers Grove, IL, USA)
- Gravimetric Performance Evaluation Option with Mettler SAG285/L balance (Mettler-Toledo, Greifensee, Switzerland)
- DNA IQ™ System (Promega Corp., Madison, WI, USA)
- Extraction Buffer (10mM Tris, 1mM EDTA, 100mM NaCl, 20% SDS)
- SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA)
- Nunc™ Bank-It tubes (Nunc A/S, Roskilde, Denmark)
- 175µL non-conductive sterile filter RoboRack tips (PerkinElmer, Downers Grove, IL, USA)
- 1000µL Conductive sterile filter Robotix tips (Molecular BioProducts, San Diego, CA, USA)
- ABI Prism® 7000 SDS (Applied Biosystems, Foster City, CA, USA)
- Quantifiler™ Human DNA Quantification kits (Applied Biosystems, Foster City, CA, USA)
- AmpFℓSTR® Profiler Plus Amplification kits (Applied Biosystems, Foster City, CA, USA)
- GeneAmp® 9700 thermalcycers (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 96-well optical reaction plates (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA)
- GeneScan™ 500 ROX™ Size Standard (Applied Biosystems, Foster City, CA, USA)
- Hi-Di™ Formamide (Applied Biosystems, Foster City, CA, USA)
- 3100 POP-4™ Polymer (Applied Biosystems, Foster City, CA, USA)
- Cytobrush® Plus Cell Collector (Cooper Surgical, Inc., Trumbull, CT, USA)
- 0.9% saline solution (Baxter Healthcare, Old Toongabbie, NSW, Australia)
- Stem digital tilting head thermometer
- For mock samples:
  - FTA™® Classic Card (Whatman Inc., Florham Park, NJ, USA)
  - Sterile cotton swabs (Medical Wire & Equipment, Corsham, Wiltshire, England)
  - Sterile rayon swabs (Copan Italia SPA, Brescia, Italy)

5. Methods

5.1 Gravimetric Evaluation of Pipetting Accuracy and Precision

Gravimetric analysis was performed by placing the SAG285/L balance on the platform deck and instructing the MP II to repeatedly pipette certain volumes of system liquid onto the balance pan. Readings were taken automatically by the software and compiled into a results table, which was then used to automatically generate an Excel-based results chart containing mean, %CV and %inaccuracy values. The mean values obtained were used to calculate R^2, slope and Y-intercept (offset) values to calibrate the system's pipetting.

Pipetting performance was assessed for various volumes using three different tips in order to calculate appropriate R^2, slope and Y-intercept (offset) values which were then added to the performance file. Values were calculated for both Blowout (single-liquid transfer) and Waste (multidispense) modes for the 1000µL conductive tips, and Blowout mode only for the 175µL non-conductive tips and fixed tips.

For the addition of resin, a specialised performance file was created based on the performance file for 175µL tips in blowout mode, except the "Blowout Volume" column values were set to 0 to allow pipetting performance that is similar to waste mode. Retesting was performed to confirm accurate and precise pipetting with these settings.

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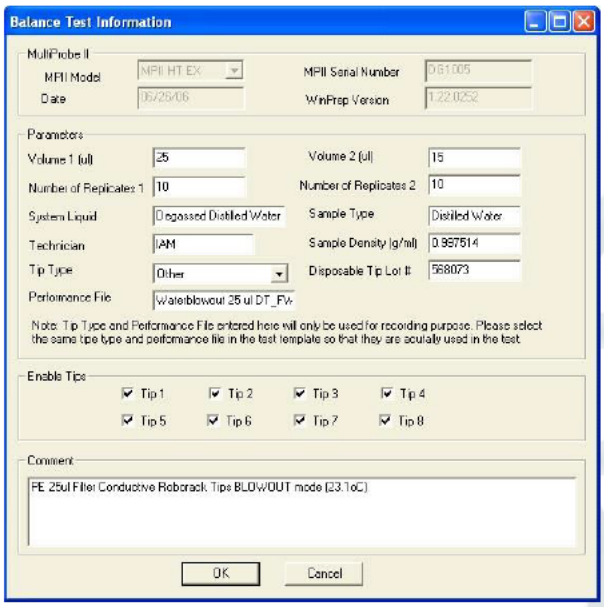

Figure 1. The Balance Test Information Window as present within the Balance Test DT program. All pipetting parameters are entered here and are subsequently transferred to the result output file.

All gravimetric testing was performed using the Balance Test DT test program within WinPrep®. Parameter values that needed to be entered into the Balance Test Information Window (Figure 1) included those as outlined in Table 1.

Table 1. Input values that are required for the various Balance Test Information parameters.

Parameter(s)	Value
Volume 1 and Volume 2	For 175µL tips: 175, 100, 50, 15µL For 1000µL tips: 1000, 700, 400, 100µL For fixed tips: 1000, 700, 400, 100µL
Number of Replicates	10
System Liquid	Degassed Nanopure Water
Sample Type	Nanopure Water
Technician	Initials of the operator performing the test
Sample Density (g/ml)	The density of water at environmental temperature*
Tip Type	Other
Disposable Tip Lot #	The lot number of the particular tips in use
Performance File	The appropriate Performance File for the tip (175µL, 1000µL or fixed tips) and pipetting mode (Blowout or Waste) in use
Enable Tips (checkboxes)	Select the actual tips (1 to 8) to be tested
Comments	Free text box to add additional information (eg. Tip type, mode, current environmental room temperature, etc).

* Water density values were obtained from http://www.simetric.co.uk/si_water.htm

Pipetting accuracy and precision were examined at four different volumes for each tip size: 175, 100, 50, 15µL for 175µL tips and 1000, 700, 400, 100µL for the 1000µL and fixed tips. In order to calculate unbiased values for each set of volumes, the slope and offset values in the relevant Performance File were changed to the default 1 and 0 respectively prior to

testing. The mean volumes that were pipetted by each tip (10 replicates per tip) at the four designated volumes were used to generate a standard curve. The slope and offset calculated from this curve was used to calibrate the relevant Performance File. The final Performance File settings were then tested at the highest and lowest volumes (as per Table 1) to confirm accurate and precise pipetting.

5.2 Blood Collection

Blood samples were collected from 2 staff donors (DJC/VKI) by a phlebotomist as per normal in three 4mL EDTA vials. Blood samples were stored at 4ºC.

5.3 Cell Collection

Buccal cells were collected using a modified Cytobrush® protocol (Mulot *et al*., 2005; Satia-Abouta *et al*., 2002). The donor was instructed to brush the inside of one cheek for one minute using a Cytobrush®. Then, with another Cytobrush®, the other cheek was also sampled. Once each cheek was swabbed, the cells on the brush were suspended in 2mL of 0.9% saline solution. Buccal cell samples were stored at 4ºC.

5.4 FTA cell Collection

Cells were collected from two staff donors (VKI/CJA) by using a "lolly-pop" swab to sample the inside of the donor's cheek for 15 seconds before pressing the swab onto the FTA™ paper to transfer the DNA. FTA™ cards were stored at room temperature.

5.5 Heater tile temperature verification

Heat tiles supplied with the MultiPROBE® II PLUS HT EX platforms were modified to accept the SlicPrep™ 96 Device. For testing, 1mL of nanopure water (at room temperature) was added to each well. The plate was then placed on a heater tile (controlled by the MP II heater controller) and allowed to reach temperature. The temperatures tested were 37ºC and 65ºC. Temperature readings for specific outer and inner wells (i.e. A1, A6, A12, D1, D6, D12, H1, H6, H12) were taken at regular intervals up to and including 45 minutes, using calibrated stem digital tilted head thermometer probes. The data were collated and means calculated to determine the distribution of heat over the tile.

5.6 Verification of automated DNA IQ™ Protocol

The automated DNA IQ™ protocol, based on the validated manual method (refer to Project 11), was programmed in WinPrep™ software. The final, optimised protocol was named "DNA IQ Extraction_Ver1.1.mpt". A screenshot of the Test Outline window for this protocol is depicted below in Figure 2. The deck layout is illustrated in Figure 3.

The automated DNA IQ™ protocol was designed to mimic the validated manual method, with minor modifications. Briefly, the changes include:

- Increasing the volume of Extraction Buffer to 500µL;
- A SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA) was used for sample lysis;
- Incubation steps and any shaking steps were performed on the integrated DPC shaker;
- CRS toroid magnet (P/N 5083175) was used for isolating the DNA IQ™ resin.
- Instead of a single elution of 100µL, a double elution method (2 x 50µL) is used.

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Reagents used in the automated protocol were as per the manual method.

Figure 2. The Test Outline window displaying individual nodes within the DNA IQ Extraction_Ver1.1.mpt program test file.

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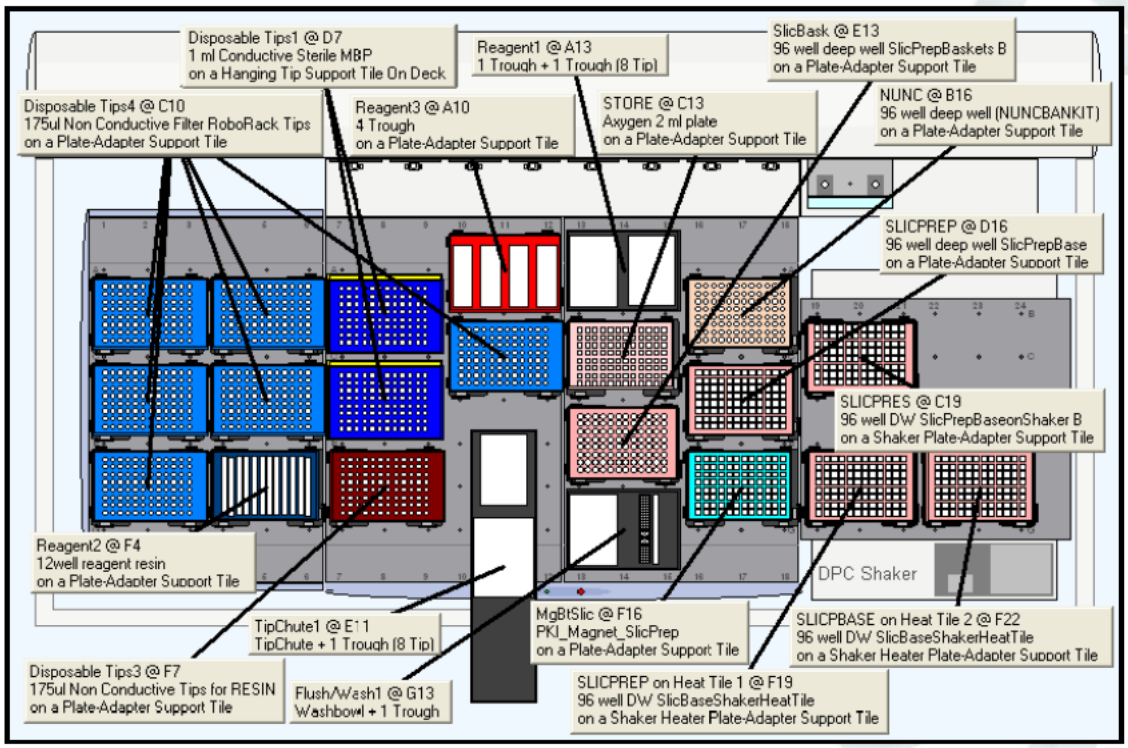

Figure 3. The deck layout for DNA IQ Extraction_Ver1.1.mpt, displaying the required labware on the platform deck.

The automated DNA IQ™ protocol was used to perform the following tests.

5.6.1. Contamination Check via Checkerboard and Zebra-stripe Patterns

Samples consisting of two 3.2mm FTA® discs (containing blood, buccal cells, or blank cards) were arranged in a checkerboard and zebra-stripe pattern (Figure 4) in SlicPrep™ plates using the BSD Duet 600 instrument (BSD Robotics, Brisbane, QLD, Australia) and extracted on the MultiPROBE® II PLUS HT EX platforms using the automated DNA IQ™ protocol. One checkerboard and one zebra-stripe plate was processed on each platform.

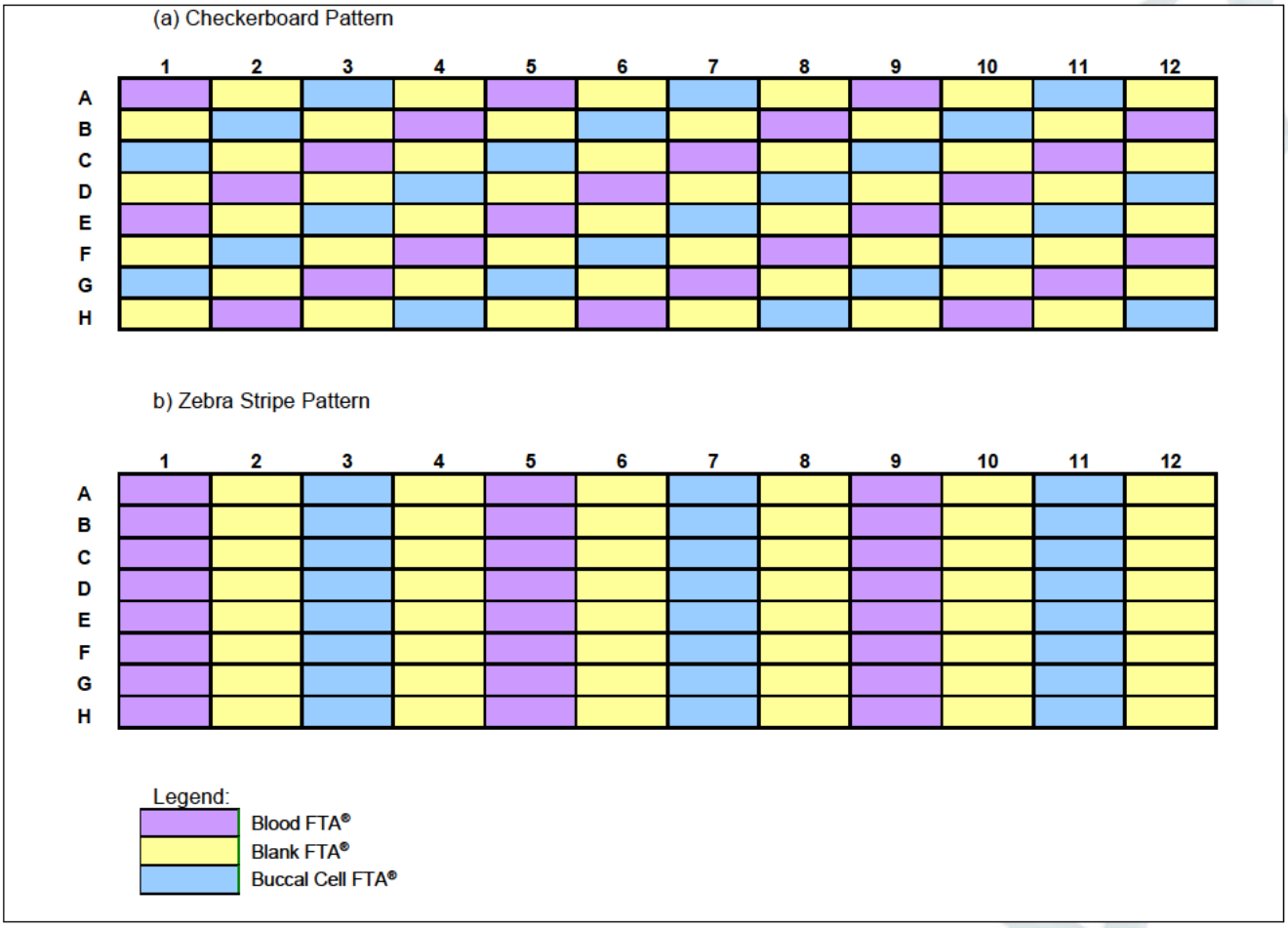

Figure 4. Checkerboard and zebra-stripe patterns utilised in the contamination check.

5.6.2. Comparisons with the manual DNA IQ™ method

Comparisons were made between results generated by the automated and manual methods to verify the performance of the automated DNA IQ™ protocol.

Verification samples consisted of different dilutions of blood and cells spotted in 30µL aliquots onto quartered cotton and rayon swabs. Four blood dilutions of neat, 1/10, 1/100 and 1/1000 and four cell dilutions of neat, 1/5.2, 1/52.2 and 1/522 were used to test the sensitivity of both the manual and automated methods. Dilutions were created using 0.9% saline solution for both sample types. Four replicates of each dilution were made up for each substrate and sample type.

The blood was collected using the same method as in 5.2. Four separate extractions were performed for the manual set based on the combination of sample type and swab type: Blood Rayon, Blood Cotton, Cell Rayon and Cell Cotton. For the automated verification, all

sample types were extracted together after being transferred to a SlicPrep™ 96 Device to allow automated processing.

5.6.3. Resin volume

The performance of the automated DNA IQ™ protocol was assessed when either 7µL or 14µL of DNA IQ™ resin was used in the protocol to extract blood samples.

5.6.4. Modifying extraction volumes

The performance of the automated DNA IQ™ protocol was assessed for varying volumes of extraction buffer at 300, 350, 400, 450 and 500µL. In each case, the volume of DNA IQ™ Lysis Buffer was kept at 2x the volume of extraction buffer. Samples extracted were blood swabs, prepared as per ???.

5.6.5. Sensitivity of the automated DNA IQ™ protocol

The sensitivity of the automated DNA IQ™ protocol was assessed using dilutions of whole blood at neat, 1:10, 1:50, 1:100 and 1:1000.

6. Results and Discussion

6.1 Gravimetric Evaluation of Pipetting Accuracy and Precision

Pipetting on both automated platforms was assessed gravimetrically as per laboratory practice. Gravimetric results indicate that pipetting performance for five different pipetting behaviours using 500µL syringes on the instruments is accurate and precise to within the established threshold of ±5% (Table 2). The maximum CV at the maximum volume was 0.78%, whereas the maximum CV at the minimum volume was 1.1%. The CV for pipetting at lower volumes is expected to be slightly higher than the CV at higher volumes using 500µL syringes, because accuracy at small volumes is harder to achieve with larger syringe sizes. Nevertheless, pipetting on the extraction platforms is limited to a minimum of 50µL, which exhibited a CV of 0.36%.

Table 2. Gravimetric evaluation results for various performance files used on either MP II EXTN A or MP II EXTN B.

Performance File	Max. Vol. µL	Min. Vol. µL	Max. Vol. µL Mean	Max. Vol. %CV	Max. Vol. %Inac.	Min. Vol. µL Mean	Min. Vol. %CV	Min. Vol. %Inac.
EXTN A								
Water Blowout 175µL DT_FW _13112007RESIN prf	50µL	N/A	49.98	0.36	0.0	N/A	N/A	N/A
Water Blowout 175µL DT_FW QHSS _13112007.prf	175µL	15µL	172.26	0.21	1.6	12.47	3.38	16.19
WaterWaste 1mL_FW_QHSS 12112007.prf	1000µL	100µL	999.11	0.24	0.1	99.22.	0.71	0.8
Water Blowout 1mL DT_QHSS _09112007.prf	1000µL	100µL	1001.02	0.27	0.1	100.65	0.63	0.7
Water Blowout Fixed Tips_08112007.prf	1000µL	100µL	995.97	0.31	0.4	99.6	0.71	0.4
EXTN B								
Water Blowout 175µL DT_FW_ 25102007RESIN prf	50µL	N/A	50.12	0.36	0.2	N/A	N/A	N/A
Water Blowout 175µL DT_FW_ 25102007.prf	175µL	15µL	175.58	0.14	0.3	15.23	1.1	1.5
WaterWaste 1mLDT_FW_QHSS 24102007 prf	1000µL	100µL	1002.39	0.78	0.2	99.56	0.89	0.4
Water Blowout 1mL DT_QHSS 23102007.prf	1000µL	100µL	998.2	0.44	0.2	99.44	0.68	0.6
Water Blowout Fixed Tips_FW 26102007.prf	1000µL	100µL	998.87	0.68	0.1	100.37	0.74	0.4

6.2 Heater tile temperature verification

Two heater tiles on each MP II platform was verified to reach either 37ºC or 65ºC, the optimum incubation temperatures for sample lysis and DNA elution respectively (using the DNA IQ™ kit). Each tile, upon completion of the verification process, could only be used for a specific temperature, and as such was labelled appropriately to ensure use of the correct tile for specific incubation steps (Table 3).

Table 3. Verified heater tiles for use in the automated DNA IQ™ protocol.

Extraction platform	Tile number	Heater Controller Setting	Average ºC reached	Verified temperature	Incubation Step
EXTN A	3 (45W)	50ºC	37ºC	37ºC	Sample Lysis
EXTN A	1 (45W)	85ºC	[redacted]	65ºC	DNA Elution
EXTN B	1 (45W)	50ºC		37ºC	Sample Lysis
EXTN B	2 (45W)	85ºC	65ºC	65ºC	DNA Elution

A slight variation in the incubation temperature to achieve sample lysis is acceptable, because Proteinase K exhibits stable activity and broad specificity over a wide range of temperatures between 20-60ºC, at which the serine protease still retains greater than 80% of its activity (Sweeney & Walker, 1993).

The efficiency of the elution step is dependent on heating the sample to 65ºC in the presence of DNA IQ™ Elution Buffer (Huston, 2002). If the sample is not sufficiently heated, the extraction yield may be lower than expected. Two heater tiles were able to be verified for this crucial incubation step, with both tiles exhibiting minimal variation.

6.3 Contamination Check via Checkerboard and Zebra-stripe Patterns

Table 4 below lists the Extraction Batch ID's of the contamination checks.

Table 4. Extraction Batch ID's for the various contamination check plates that were processed on the MP II platforms using the automated DNA IQ™ protocol.

Type of plate	Extraction batch Id	Extraction Platform	Check passed
Checkerboard 1	VALB20070817_02	Extraction A	Invalidated
Checkerboard 2	VALB20070803_02	Extraction B	Yes
Zebra-Stripe 1	VALB20070803_03	Extraction A	Yes
Zebra-Stripe 2	VALB20070817_03	Extraction B	Yes
Checkerboard/Zebra	VALB20071022_01	Extraction A	Yes

Checkerboard 1

Position E3 (Sample Cells 6) was known to have been contaminated prior to the start of the extraction, during the STORstar process (???). The result showed a mixed DNA profile, with contributing alleles originating from the expected wells (Table 5). In addition to this contamination event, eight of the designated blank samples (positions D3, A10, F1, H5, C4, E4, B7 and E6), two of the cell samples (A1 and B10) and two of the blood samples (F4 and G7) all exhibited a partial DNA profile that was previously unknown (Table 5). This profile did not match any of the positive control samples present on the batch. The DNA profile was searched against the Staff Database and no matches were found. The source of this contaminating DNA profile could not be identified.

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None of the other blank samples yielded any DNA profile. The rest of the cell and blood samples yielded the correct DNA profile. Although there is no evidence of well-to-well contamination, the unknown DNA profile obtained has invalidated this plate. A further checkerboard/Zebra-Stripe combination plate was performed to ensure…

Table 5. The DNA profile of the unknown contaminant that was observed in Checkboard-1.

Sample description	D3	vWA	FGA	Amel	D8	D21	D18	D5	D13	D7
Blk23-E6	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	11,13
Blk25-B7	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	
Blk15-E4	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	11,13
Blk14-C4		14		X	11	32.2		9		
Blk20-H5	14,14	17,17	20,21	X,X	13,16	29,30	14,16	11,13	11,12	11,11
Blk3-F1	14	17		X	13	29,30	14		12	11
Blk10-D3	14,17	14		X,Y	11	29,32.2	14	9,11		11,13
Blk37-A10	14,17	14	22,24	X,Y	11	29	14	9,11		
Cells19-B10	14,17	14,17	20,21,22,24	X,Y	11,13,16	29,30,	14,15,16	11,15	11,12	11,11
Cells13-A1	14,17	14,17	20,21,22,24	X,Y	11,13,16	29,30,32,32.2,33	14,15,16	9,11,13	11,12	11,13
Blood14-G7	NR,17,18	NR,16	20,21	X,Y	NR,13,14	29,30,31,NR	NR,14	NR,12	10,10	10,NR,12
Blood8-F4	NR,17,18	NR,16,17	20,21,NR,24	X,Y	11,13,14	29,30,NR,NR	14,14	9,11,12	10,NR	10,NR,12
Cells 6-E3	14,17,18	16,17	20,21	X,Y	13,14,16	29,30,31	NR,14,16	11,12,13	10,11,12	10,11,12

Checkerboard 2

None of the blank samples yielded DNA profiles; all of the positive cell and positive blood samples yielded the correct DNA profile. Figure 5 illustrates the DNA quantitation results from this plate. DNA was not detected in any of the blank samples.

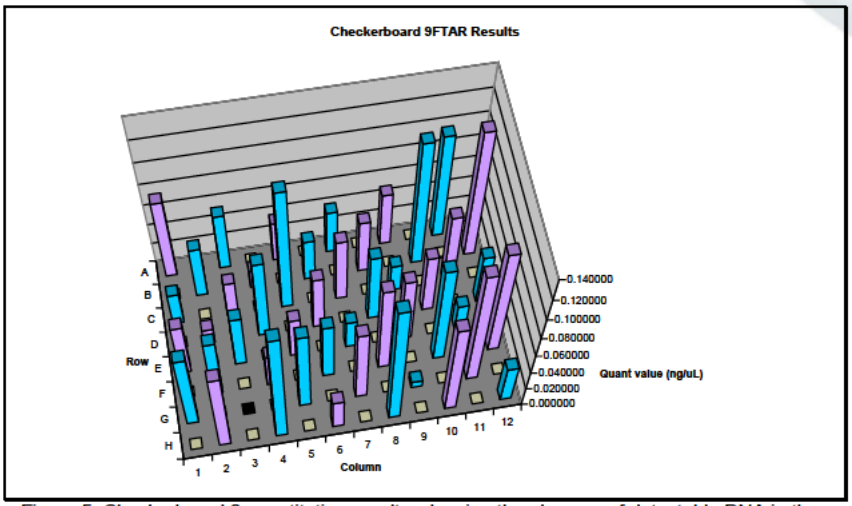

Figure 5. Checkerboard 2 quantitation results, showing the absence of detectable DNA in the blank samples (grey).

Zebra-Stripe 1
None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile. Figure 6 illustrates the absence of detectable DNA in the blank samples.

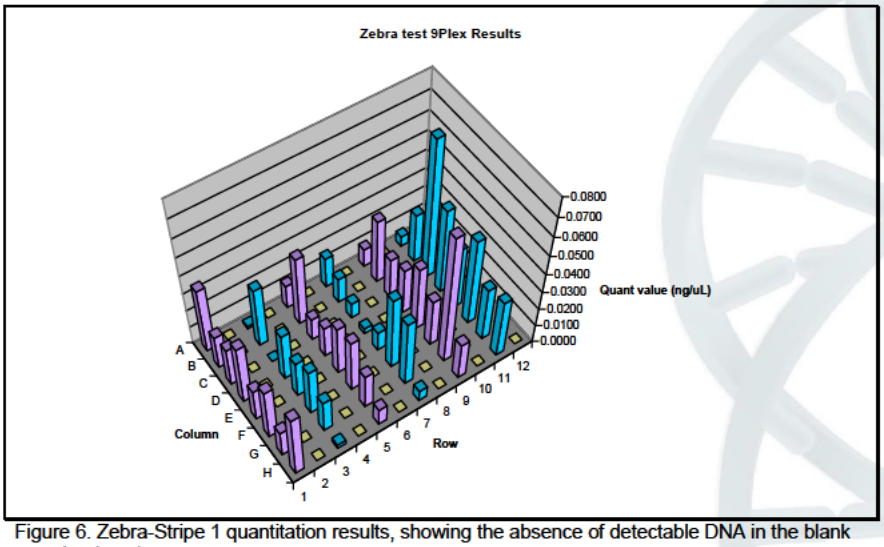

Figure 6. Zebra-Stripe 1 quantitation results, showing the absence of detectable DNA in the blank samples (grey).

Zebra-Stripe 2
None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile. Figure 7 shows the absence of detectable DNA in the blank samples.

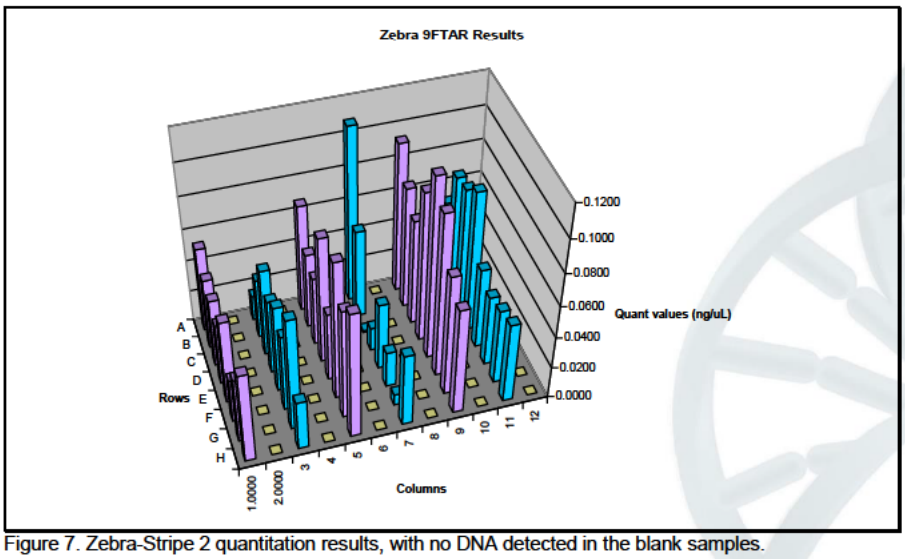

Figure 7. Zebra-Stripe 2 quantitation results, with no DNA detected in the blank samples.

Checkerboard/Zebra

None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile. DNA was undetected in the blank samples (Figure 8).

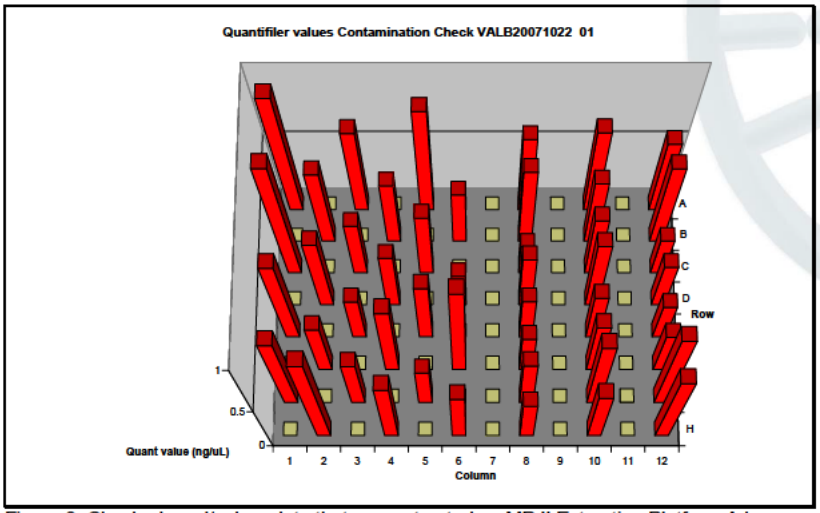

Figure 8. Checkerboard/zebra plate that was extracted on MP II Extraction Platform A because the previous plate was invalidated. DNA was not detected in the blank samples (grey).

6.4 Comparisons with the manual DNA IQ™ method

When dilutions of either blood or cells were applied on to either rayon or cotton swabs, followed by extraction using the DNA IQ™ method, the results of the automated method were always lower in yield compared to the manual method. For blood samples on rayon swabs, the automated method generated yields that were on average around 8% (SD

8.45%) of the automated method. For blood on cotton swabs, the yield from the automated method was also around 8% (SD 3.62%). The yields for cell samples were higher at around 33% (SD 16.29%) and 25% (10.32%) for cells on rayon and cotton swabs respectively.

The manual method was found to be more sensitive than the automated method. Out of five replicates at the 1/100 and 1/1000 dilutions for blood on rayon swabs that were processed using the manual method, five and three replicates respectively were detected (and none from the automated method) (see Figure 9). The trend is repeated for blood on cotton swabs (Figure 10). For cell samples on either rayon or cotton swabs, the automated method was found to be more sensitive as evidenced by detection of DNA at the 1/522 dilutions (Figure 11 and 12).

Cell clumping may have occurred with the cell dilutions, therefore causing inaccurate dilutions as can be observed in the ratios between each dilution.

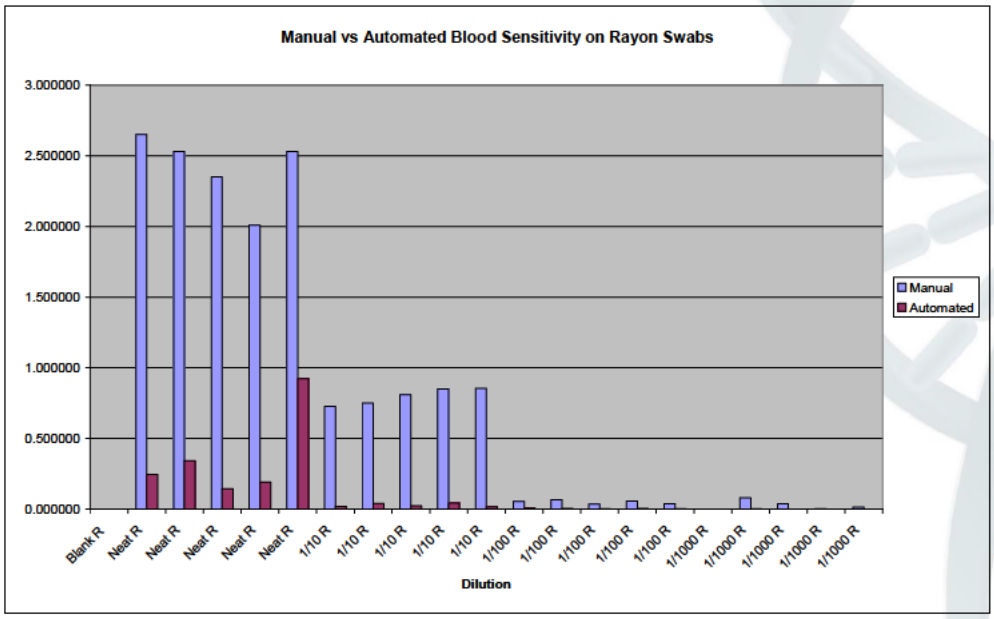

Figure 9. Comparison of sensitivity between the manual and automated DNA IQ™ methods for blood samples on rayon swabs.

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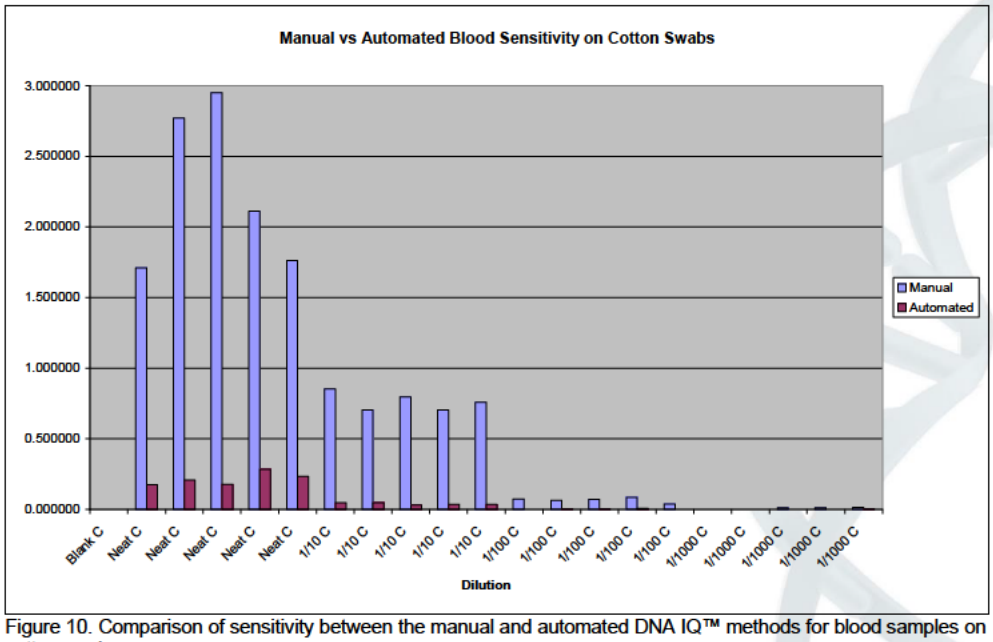


Figure 10. Comparison of sensitivity between the manual and automated DNA IQ™ methods for blood samples on cotton swabs.

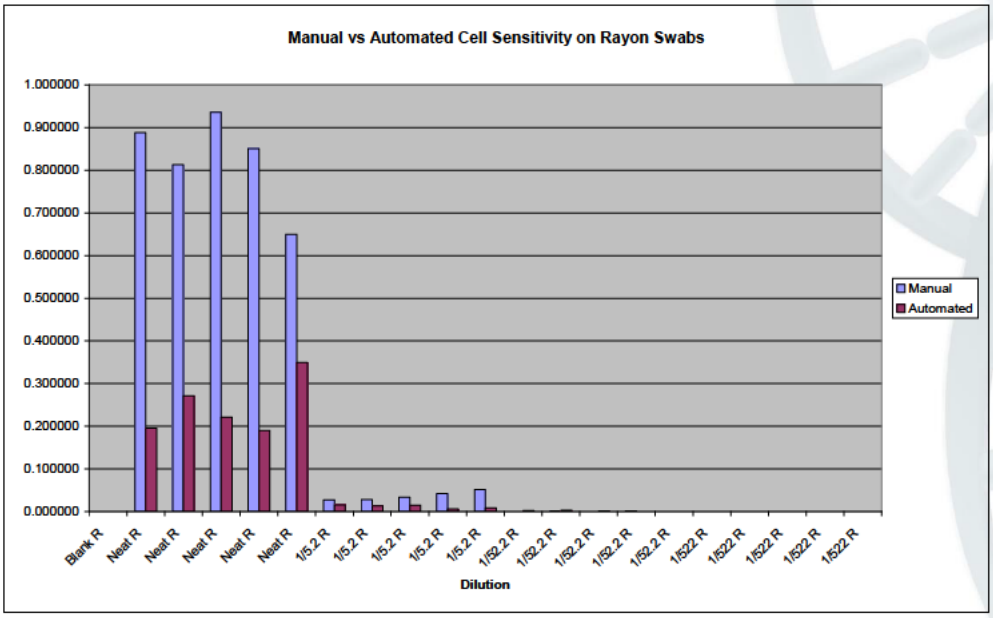


Figure 11. Comparison of sensitivity between the manual and automated DNA IQ™ methods for cells samples on rayon swabs.

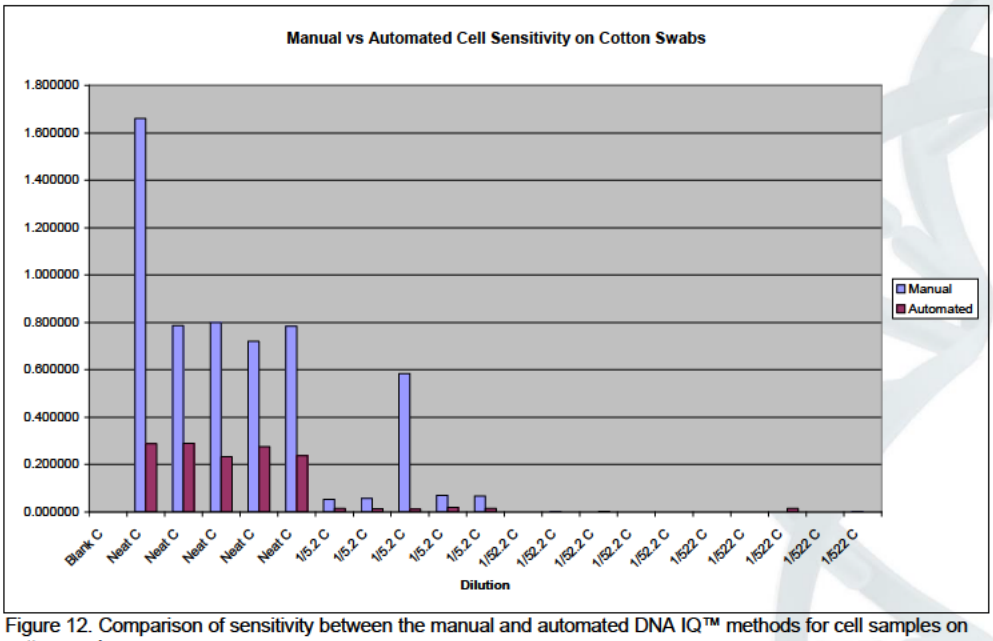


Figure 12. Comparison of sensitivity between the manual and automated DNA IQ™ methods for cell samples on cotton swabs.

6.5 Investigating resin volume

Promega recommends the use of 7µL of DNA IQ™ resin with their protocol. We investigated the performance of the protocol with double the amount of resin (14µL) in order to assess any benefits that may be gained in terms of the resulting yield and quality of the STR profile.

It was observed that doubling the resin resulted in a proportional doubling of the yield. On average, doubling the resin increased the yield by an additional 77.28% (*n*=4). The average yield from an extraction using 7µL of resin was 64.725ng (SD 32.21ng, *n*=4), whereas 14µL resin generated 114.75ng (SD 10.72ng, *n*=4) (Table 6). At the higher resin concentration, the amount of DNA isolated appears to be capped at around 100ng, indicating no change in the ability of the reaction to isolate more DNA due to saturation of resin.

Table 6. Comparison of the effects of doubling the amount of recommended DNA IQ™ resin.

Sample ID	Resin volume	[DNA] ng/µL	Reportable alleles
33383-4216	7µL	0.701	18/18
33383-4225		1.070	18/18
33383-4239		0.319	18/18
33383-4248		0.499	18/18
33383-4252	14µL	1.140	18/18
33383-4261		1.270	18/18
33383-4270		1.010	18/18
33383-4284		1.170	18/18

Samples extracted using either amount of resin generated concordant full DNA profiles (18/18 alleles). Samples processed using the 14µL method produced peaks that were slightly higher. The difference in peak heights between alleles within the same loci ranged from 59-86%, with a mean of 71%, indicating minimal difference between the two methods.

Doubling the amount of resin did not appear to provide any additional benefits compared to the original recommended protocol. More importantly, full DNA profiles were resolved using either method. Therefore, the costs associated with increasing the amount of resin cannot be justified at this stage.

6.6 **Modifying extraction volumes**

An investigation into optimising extraction volumes ranging from 300µL to 500µL was performed in order to ensure that buffer coverage over the samples was sufficient to enable optimal lysis and release of DNA. In addition, the use of an optimum volume of extraction reagents increases efficiency and economy, therefore potentially lowering laboratory costs.

Although the higher extraction volume generated higher yields when processed using the automated DNA IQ™ protocol (Table 7), DNA profile results were comparable across the various extraction volumes tested for eight replicates each (Table 8). Three instances of allelic imbalance were encountered in two samples from the 300µL and 450µL tests. In all instances, allelic imbalance was greater than 69%.

Table 7. DNA profile results for samples extracted using various volumes of Extraction Buffer, for 8 replicates.

Extraction Buffer Volume (µL)	Mean [DNA] (ng/µL)	SD
300	2.04	0.07
350	2.16	0.09
400	1.69	0.10
450	3.14	0.13
500	3.64	0.17

Table 8. DNA profile results for samples extracted using various volumes of Extraction Buffer, for 8 replicates.

Sample	Extraction Buffer Volume (µL)	DNA Profile Result
300-1 swab	300	OK
300-2 swab		OK
300-3 swab		OK
300-4 swab		OK
300-5 swab		OK
300-6 swab		OK
300-7 swab		AI D13
300-8 swab		OK

350-1 swab	350	OK
350-2 swab		OK
350-3 swab		OK
350-4 swab		OK
350-5 swab		OK
350-6 swab		OK
350-7 swab		OK
350-8 swab		OK
400-1 swab	400	OK
400-2 swab		OK
400-3 swab		OK
400-4 swab		OK
400-5 swab		OK
400-6 swab		OK
400-7 swab		OK
400-8 swab		OK
450-1 swab	450	OK
450-2 swab		OK
450-3 swab		OK
450-4 swab		OK
450-5 swab		OK
450-6 swab		OK
450-7 swab		AI vWA, D18
450-8 swab		OK
500-1 swab	500	OK
500-2 swab		OK
500-3 swab		OK
500-4 swab		OK
500-5 swab		OK
500-6 swab		OK
500-7 swab		OK
500-8 swab		OK

6.7 **Sensitivity of the automated DNA IQ™ protocol**

DNA was detected from samples that were diluted down to 1:1000 (Figure 13).

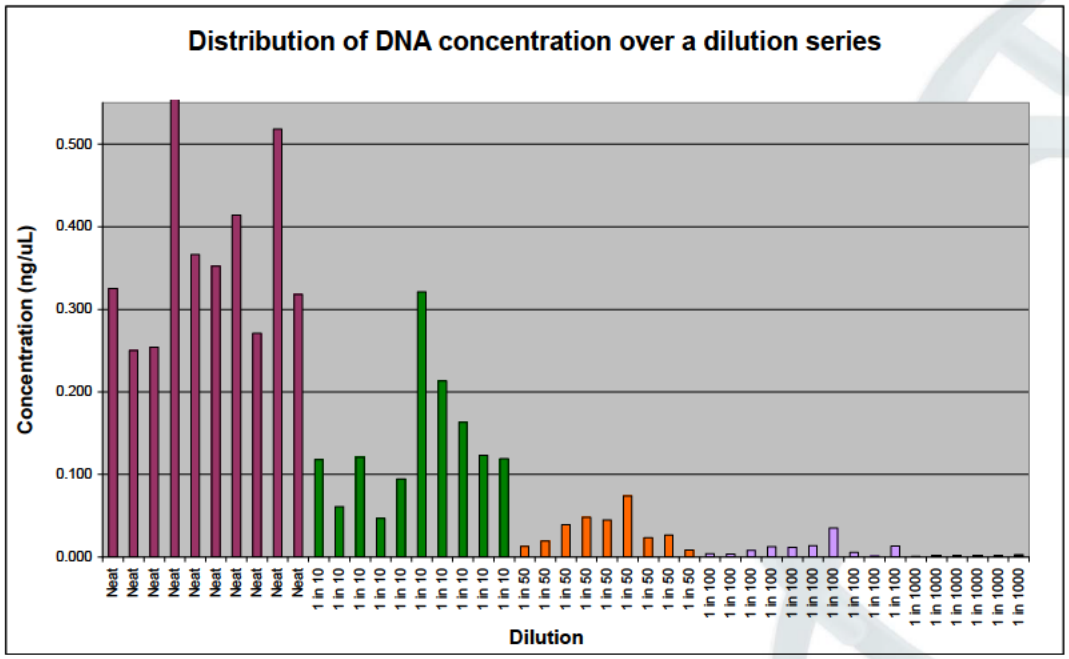


Figure 13. DNA IQ™ sensitivity across various dilutions

as

7. Summary and Recommendations

We recommend the following:

- Use of MPII for automated extraction of reference samples
- Use of MPII for automated extraction of casework samples
- Ongoing development of the automated extraction program to increase the efficiency of the extraction

Sweeney, P.J. and Walker, J.M., Burrell, M.M., Enzymes of molecular biology. *Methods Mol. Biol.* Towanam NJ , (1993) **16**, 306

Project 13: Report on the Verification of automated Extraction Chemistry Kit/s using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Case Work Platform

Iman Muharam, Allan McNevin, Thomas Nurthen, Cathie Allen, Vanessa Ientile
Automation Project, Forensic Biology QHSS (August 2006)

1. Abstract

The Extraction of forensic DNA samples within Forensic Biology QHSS is performed using the BioRad Chelex-100 process (company details). The extraction procedure is currently performed manually ??in 96-well format. We have verified the use of the MultiPROBE® II PLUS HT EX with Gripper™ Integration Case Work Platform to automatically to preform extractions in a 96-well plate format. Data indicate that results from the automated procedure are comparable to those from the manual procedure. Contamination checks were performed using samples prepared in checkerboard and zebra-stripe format, and results were as expected.

2. Introduction

The Queensland Government has allocated 11 million dollars to be spent over 3 years as part of the February 2004 “Law and Order” election commitment to clear the DNA backlog at QHSS. Forensic Biology QHSS has formulated a number of initiatives to address the backlog, including the recruitment of new scientific staff members, the refurbishment of existing laboratory space to enhance workflow efficiency, and the purchasing of additional instrumentation and equipment to improve efficiency and turn around times for DNA profiling.

In October 2005, Forensic Biology decided to purchase four MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms from PerkinElmer after an extensive tender evaluation process. The four platforms and their intended applications are tabulated below (Table 1). The PCR platforms were installed in Forensic Biology on 7 December 2005, while the extraction platforms were installed soon after on 3 January 2006. Installation was performed by Fraser Smith (PerkinElmer engineer) and training was delivered by Desley Pitcher (PerkinElmer Automated Liquid Handling Product Specialist). Forensic Biology’s purchase of the four platforms represents the largest automation project undertaken in a forensic biology facility within Australia. Whereas other Australian forensic laboratories have only purchased automated platforms with the view of automating specific steps within their DNA processing workflows, Forensic Biology QHSS aims to automate all DNA analysis steps from DNA extraction up to post-PCR setup and the analysis of data by expert systems.

Table 1. The proposed functions of the four MultiPROBE® II platforms in Forensic Biology.

Platform	Function
Extraction Platform A	To extract and purify DNA from mainly reference or non-casework samples in the form of FTA cards, blood stains or cellular materials.
Extraction Platform B	To extract and purify DNA from mainly casework samples, originating from a variety of matrices and sample types.
Pre-PCR	i. To perform set-up of DNA quantitation using Quantifiler real-time quantitative PCR (Applied Biosystems, Foster City, CA, USA), involving the addition of reaction mastermix, DNA standards and controls, and extracted DNA samples, into a 96-well reaction plate. ii. To perform set-up of STR genotyping reactions using Profiler Plus® and COfiler® AmpFℓSTR® PCR (Applied Biosystems, Foster City, CA, USA), involving the addition of reaction mastermix, DNA controls, and extracted DNA samples, into a 96-well reaction plate.
Post-PCR	To perform the set-up of 96-well plates containing a sub-sample of amplified DNA (PCR products) for capillary electrophoresis using the ABI Prism 3100 Genetic Analyzer (ABI Foster City, CA, USA).

The MultiPROBE® II PLUS HT EX platform is a computer-controlled Cartesian X-Y-Z liquid handling system designed for the efficient automation of sample preparation. For the Forensic Biology platforms, liquid transfer is performed by an 8-tip system with VersaTip® and Varispan™ options, allowing the use of fixed and/or disposable tips, with variable probe spacing to access a wide variety of labware including microplates, tubes, vials and reagent containers. Each sample probe is capable of independent motion in the Z direction due to independent Z-drives. Accusense™ technology enables multichannel liquid level sensing using a capacitive mode of detection that also allows detection of low or non-ionic polar solutions and solvents. To perform pipetting, the platforms transfer liquid using a positive displacement of system liquid (nanopure H_2O) driven by 8 individually-controlled syringes. The Gripper™ Integration platform (installed on all systems except the post-PCR instrument) allows relocation of labware across the deck and automated identification of labware via the scanning of barcodes. All platforms include the Expanded Left Extension deck to provide increased microplate tile capacity.

Control of the platforms is performed via WinPrep® application software using pull-down menus and simple user prompts. The application interface includes a virtual representation of the platform deck and functions via drag-and-drop operations. Standard tests (programs) are created by performing three basic steps: placing labware on deck, creation of procedures, and linking labware to procedure operation steps. A library of standard labware definitions is available which allows labware to be placed anywhere on the deck without the need to define additional labware parameters. Additional labware can be added to the labware library to meet user-specific application requirements. New labware only needs to be defined once for unlimited future use. Data logs or audit reports are generated by the software for every test run. An Enhanced Security Option upgrade to the existing WinPrep® application software was purchased to enable individual log-in options and account restrictions.

3. Aim

To verify the performance of the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform ("Casework MP II") to prepare extractions of forensic DNA samples. This includes a verification of the Casework MP II's pipetting accuracy and precision, as determined colorimetrically and gravimetrically, and an assessment or comparison of the output data to the manual setup method.

4. Equipment and Materials

- MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (Casework) (PerkinElmer Life Sciences, Downers Grove, IL, USA)
- Gravimetric Performance Evaluation Option with Mettler SAG285/L balance (Mettler-Toledo, Greifensee, Switzerland), PN 7601823
- Tecan Spectra Reader (Tecan GmbH, Salzburg, Austria)
- DNA IQ™ Kit – 400 samples (Promega)
- 175µL Non – Conductive MP II sterile filter RoboRack tips (PerkinElmer)
- 1000µL Conductive sterile filter Robotix tips (MBP)
- Slicprep 96 device (Promega)
- Nunc Bank-It tubes (Nunc)
- ABI Prism® 7000 SDS (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 7100 SDS (Applied Biosystems, Foster City, CA, USA)
- Quantifiler™ Human DNA Quantification kits (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 96-well optical reaction plates (Applied Biosystems, Foster City, CA, USA)
- For mock samples:
  - FTA® Classic Card (Whatman Inc., Florham Park, NJ, USA)
  - Sterile cotton swabs (Medical Wire & Equipment, Corsham, Wiltshire, England)
  - Sterile rayon swabs
- 3130 machine
- BSD-duet ???
- Other general consumables were supplied by Analytical Section, Forensic Biology QHSS

5. Methods

5.1. Gravimetric Evaluation of Pipetting Accuracy and Precision

The Gravimetric Performance Evaluation Option with Mettler SAG285/L balance (Mettler-Toledo, Greifensee, Switzerland), hereby referred to as the “gravimetric kit”, was purchased from PerkinElmer (PN 7601823) as an optional accessory upon recommendations made by Forensic Science South Australia staff members. The gravimetric kit consists of a SAG285/L balance and various accessories including a humidified chamber and software for integration with the WinPrep® software. All components were installed by a qualified PerkinElmer service engineer, and subsequently calibrated by a Mettler-Toledo engineer for NATA certification. A NATA certificate for the kit (Report Number Q0737-001-1) was produced on 13 March 2006.

Gravimetric analysis is performed by placing the balance on the platform deck and instructing the MP II to repeatedly pipette certain volumes of system liquid onto the balance pan. Readings are taken automatically by the software and compiled into a results table, which is then used to automatically generate an Excel-based results chart containing mean, %CV and %inaccuracy values. The mean values obtained are used to calculate R^2, slope and Y-intercept (offset) values to calibrate the system’s pipetting.

The pipette tips required for use on the Extraction Platforms include 175µL non-conductive MPII sterile filter RoboRack tips, 1000µL conductive sterile Robotix tips and the Fixed tips of the MPII itself. 175µL tips are used for the purpose of removing reagents and the transfer of DNA sample. The 1000µL tips were chosen for the purpose of removing supernatant and the fixed tips are used only for the addition of reagents. Pipetting performance was assessed for various volumes using the three different tips in order to calculate appropriate R^2, slope and Y-intercept (offset) values which were then added to the performance file. Values were calculated for both Blowout (single-liquid transfer) and Waste (multidispense) modes for the 1000µL tips, and Blowout mode only for the 175µL and fixed tips. A specialised performance file was created for the addition of resin. The performance file for the 175µL tips uses the already calibrated information of the Blowout mode, however the Blowout volume column values are set to 0, making it preform in a waste like mode. Retesting was performed to confirm accurate and precise pipetting with these settings.

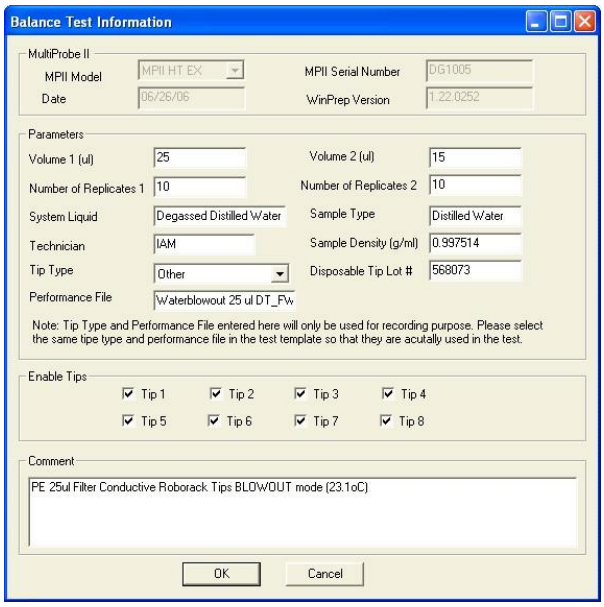


Figure 1. The Balance Test Information Window as present within the Balance Test DT program. All pipetting parameters are entered here and are subsequently transferred to the result output file.

All gravimetric testing was performed using the Balance Test DT test program within WinPrep®. Parameter values that needed to be entered into the Balance Test Information Window (Figure 1) included those as outlined in Table 2.

Table 2. Input values that are required for the various Balance Test Information parameters.

Parameter(s)	Value
Volume 1 and Volume 2	For 175µL tips: 175, 100, 50, 15µL For 1000µL tips: 1000, 700, 400, 100µL For Fixed tips: 1000, 700, 400, 100µL
Number of Replicates	10
System Liquid	Degassed Distilled Water
Sample Type	Distilled Water
Technician	Initials of the operator performing the test
Sample Density (g/ml)	The density of water at environmental temperature*
Tip Type	Other
Disposable Tip Lot #	The lot number of the particular tips in use
Performance File	The appropriate Performance File for the tip (175µL, 1000µL or fixed tips) and pipetting mode (Blowout or Waste) in use
Enable Tips (checkboxes)	Select the actual tips (1 to 8) to be tested
Comments	Free text box to add additional information (eg. Tip type, mode, current environmental room temperature, etc).

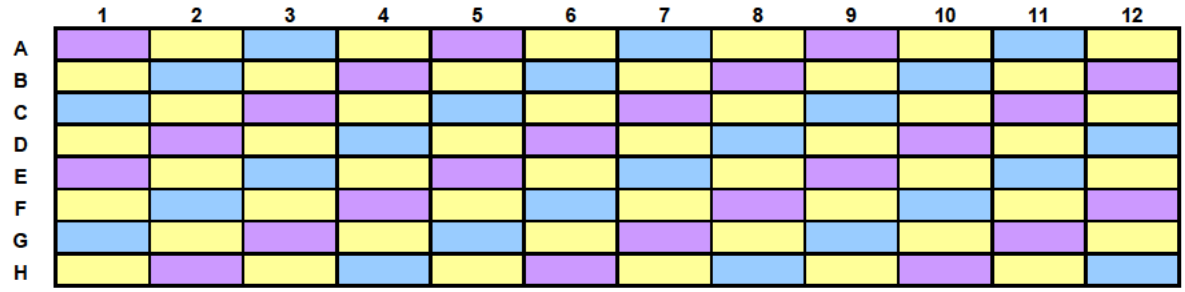
* Water density values were obtained from http://www.simetric.co.uk/si_water.htm

Pipetting accuracy and precision were examined at four different volumes for each tip size: 175, 100, 50, 15µL for 175µL tips and 1000, 700, 400, 100µL for the 1000µL and Fixed tips. In order to calculate unbiased values for each set of volumes, the slope and offset values in the relevant Performance File were changed to the default 1 and 0 respectively prior to testing. The mean volumes that were pipetted by each tip (10 replicates per tip) at the four designated volumes were used to generate a standard curve. The slope and offset calculated from this curve was used to calibrate the relevant Performance File. The final Performance File settings were then tested at the highest and lowest volumes to confirm accurate and precise pipetting. In the case of the specialised 175µL Blowout performance file, only high and low volumes are tested as it uses the settings from the original 175µL Blowout performance file.

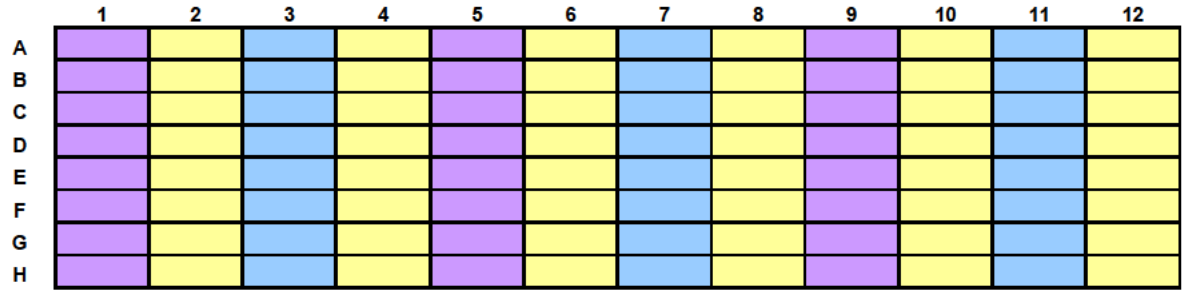
5.2. Contamination Check via Checkboard and Zebra-stripe Patterns using Quantifiler Assays

Three sample types were chosen to create the contamination check plates: Blood FTA, Cell FTA and Blank FTA. Blood samples were created by obtaining blood from 2 donors (DJC/VKI) by a phlebotomist as per normal in three 4ml EDTA vials. A small volume of this blood was “clothed” or spotted onto Whatman FTA paper and allowed to dry overnight before sampling. Cells were collected from two donors (VKI/CJA) by using a “lolly-pop” swab to sample the inside of the donor’s cheek for 15 seconds before pressing the swab onto the FTA paper to transfer the DNA. Blank FTA samples were created using clean, unused FTA paper free from any kind of sample or reagent. Using the BSD duet machine, 2 3.2mm spots were punched directly into the Slicprep 96 device in either the checkerboard or zebra stripe pattern (see figure 2 a and 2 b for a representation of the sample arrangement). One checkerboard and one zebra stripe plated was punched per Extraction platform.

a) Checkerboard Pattern



b) Zebra Stripe Pattern



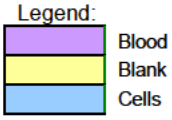


Figure 2. Checkerboard and zebra-stripe patterns utilised in the Extraction contamination check.

The Automated Extraction Setup MP II test program was compiled to replace the current manual Chelex extraction process for blood and cells performed by scientists in the Analytical section; Forensic Biology. The final Test Outline tree structure is presented below as Figure 3. Refer to Figure 4 for the deck layout.

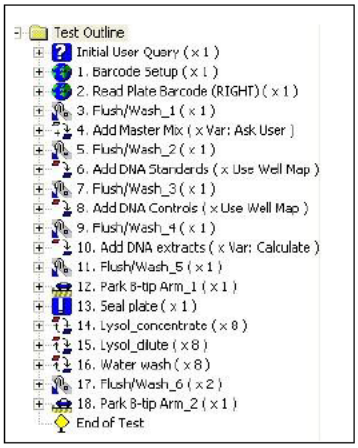


Figure 3. Test Outline for the automated setup of the Extraction program using the Extraction MP II's.

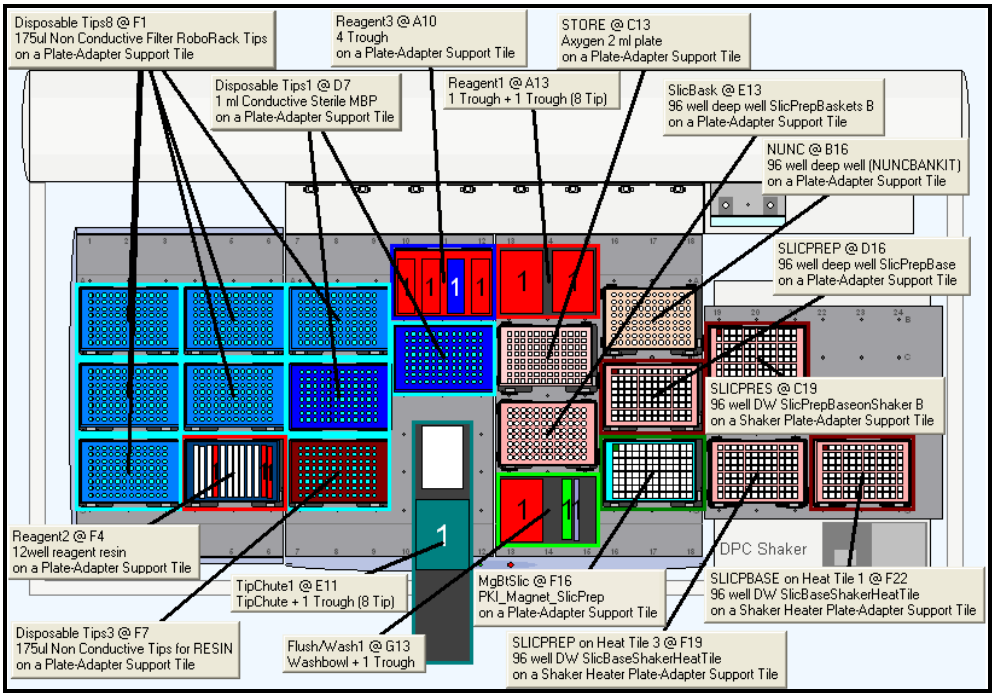


Figure 4. The WinPrep® virtual deck view displaying the necessary labware required to perform automated Extraction test file (Extraction Platform A deck view).

5.3. Verification of the Automated Extraction Chemistry Protocol

Comparisons were made between results generated by the automated and manual methods to verify the performance of the automated Extraction Chemistry setup protocol.

Verification samples consisted of different dilutions of blood and cells spotted in 30ul aliquots onto quartered cotton and rayon swabs. Four blood dilutions of neat, 1/10, 1/100 and 1/1000 and four cell dilutions of neat, 1/5.2, 1/52.2 and 1/522 were used to test the sensitivity of both the manual and automated methods. Dilutions were created using neat sample and 0.9% saline for both sample types. Four replicates of each dilution were made up for each substrate and sample type. The cells used in this verification were collected using a cytobrush method where the donor used a cytobrush for one minute to "swab" the inside of one cheek. Once each cheek was swabbed, the cells on the brush were suspended in 2ml of 0.9% saline. The blood was collected using the same method as in 5.2. 4 separate extractions were performed for the manual set: Blood Rayon, Blood Cotton, Cell Rayon and Cell Cotton. For the automated verification, all sample types were extracted together after being transferred to a Slicprep 96 device for processing on the Extraction Platform B.

6. Results and Discussion

6.1. Gravimetric Evaluation of Pipetting Accuracy and Precision

Pipetting performance of the various tip types and pipetting modes at the highest and lowest volumes tested are outlined in Table 4. The individual Performance Files required for each combination of pipette size and pipetting mode are presented in Table 5.

Table 4. Pipetting performance for 25µL and 175µL Conductive MP II sterile filter RoboRack tips under Blowout and Waste modes.

Tip Type	Volume	Blowout Mode		Waste Mode	
25µL Conductive MP II sterile filter RoboRack	25µL	Mean (µL)	24.96	Not tested	
		CV (%)	0.72		
		Inaccuracy (%)	0.15		
	2µL	Mean (µL)	2.06		
		CV (%)	6.53		
		Inaccuracy (%)	2.91		
175µL Conductive MP II sterile filter RoboRack	175µL	Mean (µL)	174.90	Mean (µL)	175.13
		CV (%)	0.35	CV (%)	1.57
		Inaccuracy (%)	0.06	Inaccuracy (%)	0.07
	15µL	Mean (µL)	15.19	Mean (µL)	15.31
		CV (%)	0.90	CV (%)	5.84
		Inaccuracy (%)	1.25	Inaccuracy (%)	2.08

Table 5. Calibrated slope and offset values for individual pipette types under different pipetting modes.

Tip Size	Pipetting Mode	Performance File	Slope	Offset (Y-intercept)	R^2	Pipetting Range (µL)
25µL Conductive MPII sterile filter RoboRack	Blowout	WaterBlowout 25 ul DT_FW_QHSS_11042006.prf	0.962803	-0.390539	0.999915	1-25
175µL Conductive MPII sterile filter RoboRack	Blowout	WaterBlowout 175 ul DT_FW_QHSS_21032006.prf	0.978905	-1.082636	0.999991	15-175
175µL Conductive MPII sterile filter RoboRack	Waste	WaterWaste 175 ul DT_FW_QHSS_27032006.prf	0.974194	-1.246471	0.999971	15-175

Screenshots of the final Performance Files with pipetting performance sets and calibrated slope and offset values are presented in Figures 5, 6, and 7.

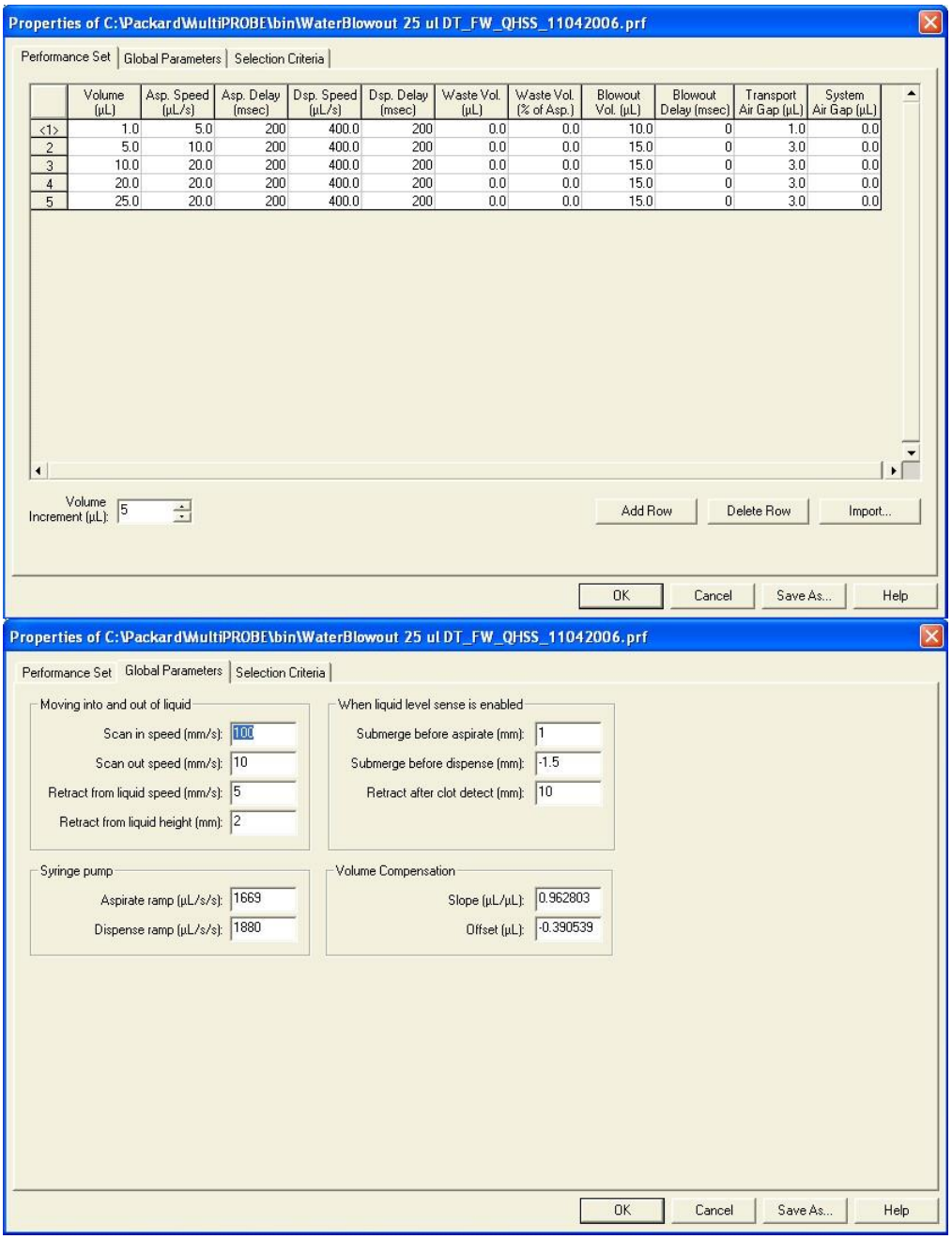


Figure 5. Performance File Global Parameters for the 25µL Conductive MPII sterile filter RoboRack tips under Blowout Mode.

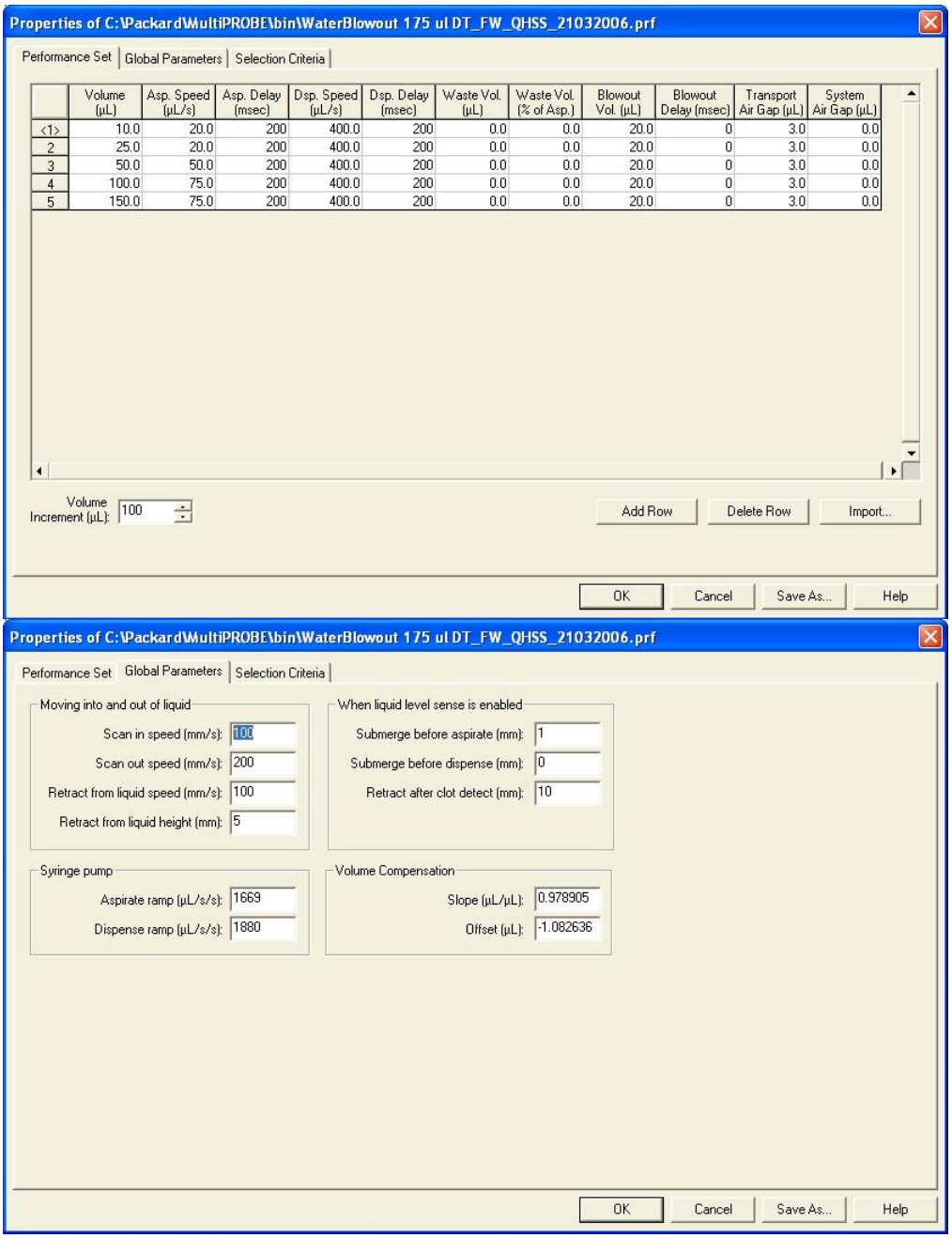


Figure 6. Performance File Global Parameters for the 175µL Conductive MPII sterile filter RoboRack tips under Blowout Mode.

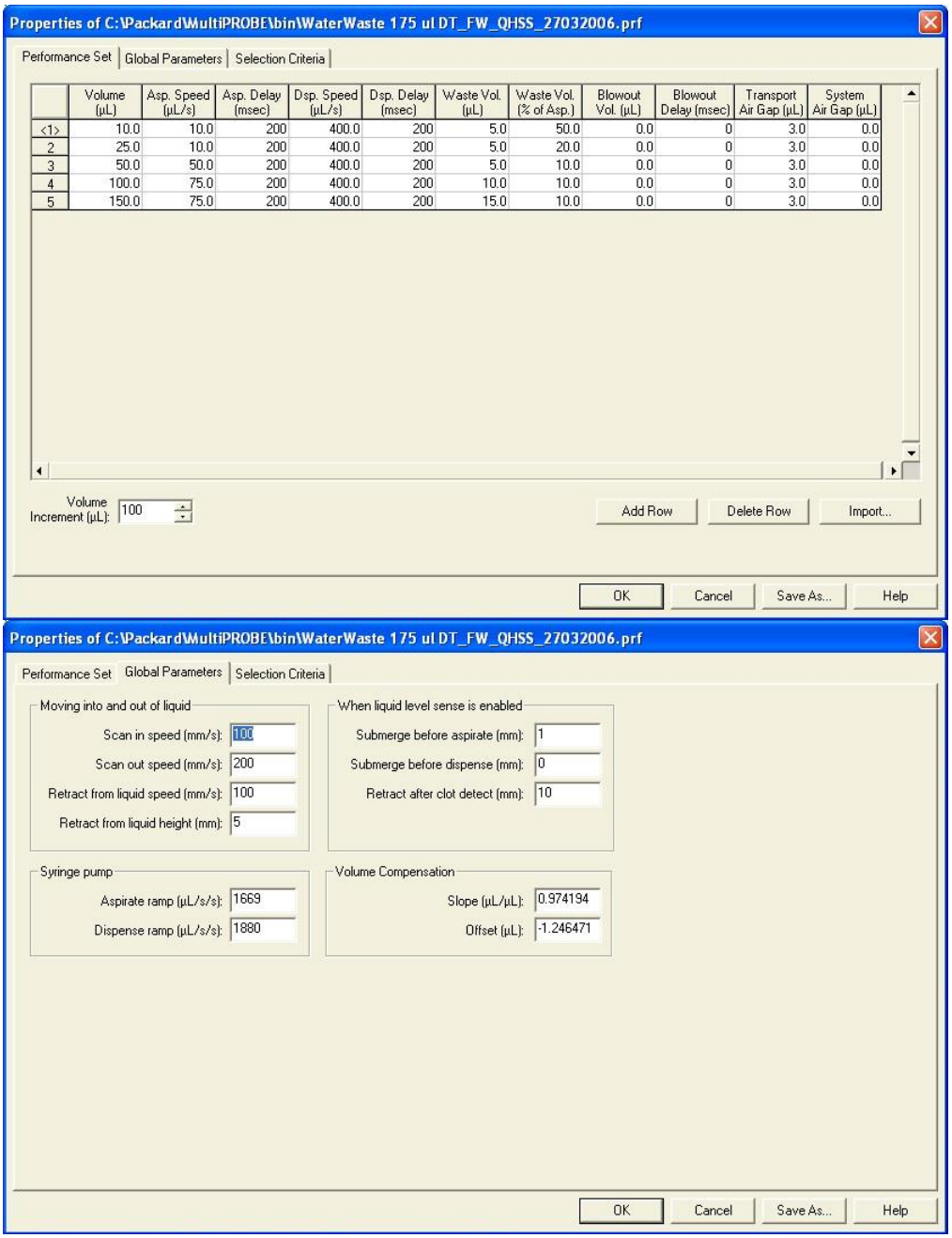


Figure 7. Performance File Global Parameters for the 175µL Conductive MPII sterile filter RoboRack tips under Waste Mode.

Initial pipetting performance at low volumes (1 and 2µL) using the 25µL tips was considered substandard due to the observation of sporadic “zero” volumes, where liquid did not appear to be pipetted and this error was not detected by the Pre-PCR MP II. This observation occurred at a frequency of at least once per 96-well plate. Discussions were

made with colleagues at Forensic Science South Australia (Mark Webster and Chris Hefford). It was put forward that the dispense height is a critical factor for low volume pipetting, and dispensing approximately 1.5mm above the liquid surface allows the expelled liquid to form a drop at the end of the tip which touches the liquid surface, whereby surface tension is then able to pull the drop off the tip. Based on these findings, the "Submerge before dispense (mm)" parameter was changed from 0 to -1.5 (Figure 5). The double negative value results in a dispense height 1.5mm above the liquid surface. This adjustment significantly enhanced pipetting performance at low volumes. To further ensure total delivery of all pipette tip contents, the Performance Set for each Performance File was also adjusted and tested to maximise pipetting accuracy/precision while maintaining minimal drip formation and cross-contamination risks.

6.2. Colorimetric Evaluation of Pipetting

Results for pipetting performance over a range of serial dilutions as determined via colorimetric detection are outlined below in Table 6.

Table 6. Results of colorimetric assays to assess pipetting performance.

Plate ID	Pipetting mode	Absolute		Relative	
607*	Blowout	%CV	13.65	%CV	6.79
		%Inacc	12.12	%Inacc	2.12
		R^2	0.99890		
608*	Blowout	%CV	18.80	%CV	11.73
		%Inacc	2.92	%Inacc	0.27
		R^2	0.99852		
609*	Waste	%CV	10.63	%CV	9.23
		%Inacc	13.18	%Inacc	4.22
		R^2	0.99966		
622†	Blowout	%CV	3.14	%CV	1.63
		%Inacc	4.22	%Inacc	1.92
		R^2	1.00000		
623†	Waste	%CV	5.88	%CV	2.25
		%Inacc	7.51	%Inacc	3.13
		R^2	0.99993		

* Plate consists of ten serial dilutions at 1:2.
† Plate consists of four serial dilutions at 1:2 → 1:5 → 1:5 → 1:5 in tandem.

6.3. Contamination Check via Checkboard and Zebra-stripe Patterns using Quantifiler Assays

The same sets of High and Low female control gDNA, Promega 25ng/µL gDNA, water blanks and DNA standards were used for both plates. The Promega 25ng/µL gDNA was purposely diluted to this concentration as splashback or carryover of 1µL from a well containing Promega 25ng/µL DNA into a well containing water blank would result in detectable contamination at the level of 0.04ng/µL. Carryover of 0.117µL would result in contamination at the lower limit of detection (0.00467ng/µL) as previously established by in-house validation of the Quantifiler system (Hlinka et al., 2006). This value is similar to the

concentration of detectable background readings that are known to occur in Quantifiler reagents (Applied Biosystems, 2005 [letter]).

All wells in the checkerboard pattern exhibited results as expected. Quantitation of Promega 25ng/µL gDNA resulted in concentration values equal to or greater than 25ng/µL (mean 30.65ng/µL, SD 3.65ng/µL). All of the reaction controls showed expected values and amplification was not detected in any of the water blank reactions.

The zebra-stripe layout displayed expected results for all controls and Promega 25ng/µL gDNA. The majority of water blank reactions did not result in amplification, however a small number of reactions yielded low concentration values as presented in Table 7 below.

Table 7. Water blank reactions that displayed amplification in the zebra-stripe pattern plate 1 (run ID: QUACWZebra).

Sample ID	Well	Quantifiler value (ng/µL)
Water_20	D7	0.000233
Water_5	E3	0.000124
Water_29	E9	0.00724
Water_14	F5	0.00192

The whole test was repeated and only the following reaction provided a DNA concentration value:

Table 8. Water blank reactions that displayed amplification in the zebra-stripe pattern plate 2 (run ID: QUACWZebra2).

Sample ID	Well	Quantifiler value (ng/µL)
Water_29	E9	0.00590

Water_29 provided similar concentration values across two different Quantifiler runs. It was suspected that this tube may contain a DNA contaminant. The Water_29 sample (620µL of water) was given a DNA # ID (94355) and concentrated using a Microcon-100 device as per the in-house protocol (QIS 19544). Briefly, 500µL of the sample was transferred into a fresh tube and centrifuged for 6mins at 500g. The remaining 120µL was further transferred to this tube and centrifuged for 6mins at 500g. The Microcon-100 column containing concentrated DNA was inverted into a new tube and centrifuged for 3mins at 1000g. The retentate (approx 260µL) was concentrated in a fresh Microcon-100 filter for 12mins at 500g. The filter was inverted and centrifuged for 3mins at 1000g. The final concentrate (45µL) was quantified in duplicate using the Quantifiler kit (run ID QF#748, setup manually) and resulted in undetermined concentration values (0ng/µL). The concentrated sample was amplified using the AmpFℓSTR® Profiler Plus® kit at 20µL in a 50µL reaction (run ID CW#1184), which resulted in an NSD profile. It appears that the presumed contaminant present in the concentrated Water_29 sample was either too low in concentration and could not result in a detectable Quantifiler result or DNA profile, or the initial detected concentration values were the result of detectable background inherently present in the Quantifiler reagents (Applied Biosystems, 2005 [letter]).

The contamination check in zebra-stripe pattern was repeated using sterile molecular grade Sigma Water (Sigma-Aldrich Corp., St. Louis, MO, USA; Catalogue Number W4502, lot number 83K2357). All controls and Promega 25ng/µL DNA showed expected results. The majority of water blank reactions did not result in amplification, but four samples yielded low concentration values as per Table 9 below.

Table 9. Water blank reactions that displayed amplification in the zebra-stripe pattern plate 3 (run ID: QUACWZebra3).

Sample ID	Well	Quantifiler value (ng/µL)
Sigma Water_25	A9	0.0027
Sigma Water_29	E9	0.000179
Sigma Water_38	G11	0.00211
Sigma Water_8	H3	0.000291

All water samples that previously presented with DNA concentration values were re-quantified in 5 replicates using the Quantifiler kit, which resulted in undetermined concentration values (0.0ng/µL) for all reactions. These results suggest that there was no contamination of the original water samples by the MP II but rather is the result of the inherent detectable background within the Quantifiler reagents.

6.4. Verification of the Automated Quantifiler Protocol

Refer to Table 10 and Figure 8 for results from the comparison of automated versus manual Quantifiler setup.

Table 10. Differences in run results between automated and manual Quantifiler setup methods for 132 DNA samples.

Average CT difference	-0.1293939
Average quant diff (%)	-1.265
Average quant diff (ng/µL)	-0.003
Maximum quant diff (%)	67.391
Maximum quant diff (ng/µL)	0.460
Minimum quant diff (%)	-41.232
minimum quant diff (ng/µL)	-0.420

Quant difference = automated result - manual result
Quant difference % = (quant difference/manual result * 100)

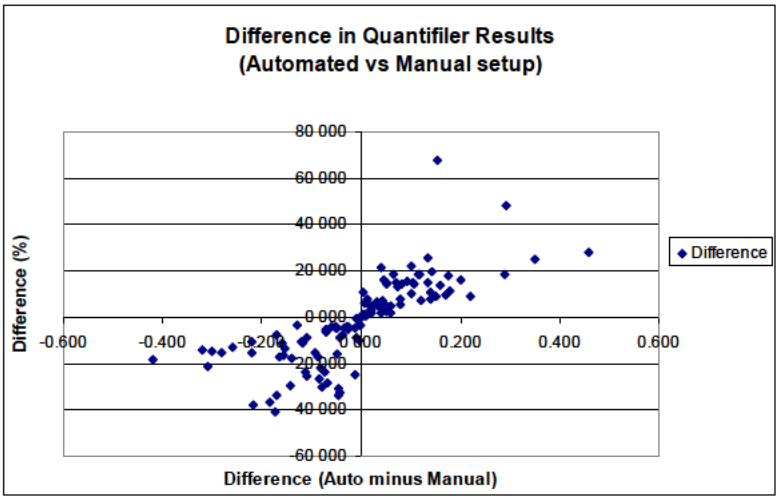


Figure 8. Plot of difference between automated and manual methods for Quantifiler setup. Most samples cluster close to 0, indicating minimal difference between sample results.

The maximum quant value was for sample ID 228675605 (PAC mouth swab), at 3.67ng/µL (auto) and 3.61ng/µL (manual). For both results, this equates to a 3:10 dilution of the original extract before adding 1µL of sample volume in order to give a total of 1ng DNA in the subsequent Profiler Plus® or COfiler® amplification. The lowest quant values obtained

were 0.0374ng/µL (auto) and 0.0455ng/µL (manual), for sample ID 228675715 (DJC mouth swab) and 228675706 (DJC mouth swab) respectively. The corresponding manual or auto results for these samples were 0.0499ng/µL and 0.0409ng/µL respectively, accounting for a difference of 0.0129ng/µL (~25.0%) and 0.0046ng/µL (~10.1%). The low DNA concentration of these samples contribute to stochastic effects during the real-time QPCR DNA quantitation process, reflected in the differences in results between the automated and manual quant setup procedures.

Overall, the maximum quant difference observed was 67.4% for sample ID 129466814 (MMH blood swab), with a maximum quant value of 0.385ng/µL (auto) and a minimum of 0.230ng/µL (manual). The manual result was checked by repeating the quantitation assay manually (DNA # ID 102305, run ID QF#873), to yield a concentration of 0.397ng/µL, yielding a revised difference value of 1.2%. The lower quantitation result obtained previously using the manual Quantifiler setup protocol most likely reflects the occurrence of pipetting error. The minimum quant difference observed was -41.2% for sample ID 228675056 (PAC blood swab), with a maximum quant value of 0.422ng/µL (manual) and a minimum of 0.248ng/µL (auto).

A comparison of standard curve parameter values that are observed after manual or automated setup of Quantifiler assays is outlined in Table 11 below. In both runs, the automated plate displayed Y-intercepts that were closer to the mean of 28.011482. The Y-intercept differences from the mean for the automated plates were 0.13081 and 0.035412 for the first and second runs respectively, compared to the manual values of 0.220831 and 0.362122. The R^2 values for the automated plates were closer to 1 compared to the manual plate results. Although these results suggest that the standard curve parameters from the automated plates appear to out-perform those from the manual plates, a calculation of statistical significance cannot be performed due to limited data.

Table 11. Comparison of standard curve parameter values between manual and automated Quantifiler plates.

Run ID:		**QUACW20060419_01**		**QUACW20060419_02**	
Parameter	**Threshold**	**Manual Result**	**Auto Result**	**Manual Result**	**Auto Result**
Slope	-2.9 to -3.3	-3.037914	-3.176350	-3.190838	-3.168825
Y-Intercept*	27.655034 - 28.367930	28.232313	28.142292	28.373604	28.046894
R^2	≥0.98	0.995921	0.998229	0.992763	0.996018

*Y-intercept range at 2SD.

No amplified product was detected in the reagent blank reactions. Samples that did not contain sufficient DNA were undetermined on both the automated and manual plates, indicating reproducibility and consistency in results. This also indicates that the automated procedure does not introduce contamination into the DNA samples.

7. Summary

Results indicate that the automated setup of the Quantifiler assay for the purposes of quantifying forensic DNA samples is comparable in performance and reproducibility to the current manual setup.

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Project 13: Report on the Verification of an Automated DNA IQ™ Protocol using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform

Iman Muharam, Vojtech Hlinka, Breanna Gallagher, Cecilia Iannuzzi, Generosa Lundie, Thomas Nurthen, Vanessa Ientile
Automation/LIMS Implementation Project, DNA Analysis FSS (April 2008)

1. Abstract

A manual method for extracting DNA from forensic samples using the DNA IQ™ system (Promega Corp., Madison, WI, USA) was validated for routine use in DNA Analysis (FSS). We have verified an automated DNA IQ™ protocol in 96-well format for use on the MultiPROBE® II PLUS HT EX FORENSIC WORKSTATION platforms. Data indicate that results from the automated procedure are comparable to those from the manual procedure. Contamination checks were performed using samples prepared in checkerboard and zebra-stripe format, and results were as expected. We recommend the use of the MultiPROBE® II PLUS platforms to perform automated DNA extraction using the DNA IQ™ system.

2. Introduction

The MultiPROBE® II PLUS HT EX FORENSIC WORKSTATION platforms are equipped to perform automated DNA extractions, as they include a DPC shaker and individual heat controllers to enable on-board lysis and incubation steps. Currently in DNA Analysis, the MultiPROBE® platforms allow walk-away operation of PCR setup protocols for DNA quantitation and amplification.

The DNA IQ™ protocol has been verified or validated by various laboratories for use on the MultiPROBE® II PLUS platform. The laboratories that perform an automated DNA IQ™ protocol include PathWest (Western Australia), Forensic Science South Australia (South Australia) and Centre of Forensic Sciences in Toronto (Ontario). The MultiPROBE® II PLUS instrument comes pre-loaded with an automated DNA IQ™ protocol. Unlike the other laboratories, however, we did not validate the included protocol, but instead validated a manual DNA IQ™ protocol which was based on the CFS automated protocol (PerkinElmer, 2004), followed by verification of an automated protocol.

The verified automated DNA IQ™ protocol is identical to the validated manual protocol used in-house: there are no differences in reagents or volumes. The adopted DNA IQ™ protocol differs slightly, however, from the manufacturer's protocol, as it includes a lysis step in Extraction Buffer (10mM Tris, 1mM EDTA, 100mM NaCl, 20% SDS) in the presence of Proteinase K, before incubating in the DNA IQ™ Lysis Buffer. Furthermore, the lysis incubation conditions were lowered from 70°C to 37°C in order to accommodate extraction of DNA from heat labile materials such as nylon and polyester. In addition, the automated protocol utilises the SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA) for simultaneous processing of samples in a 96-well format.

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3. Aim

To verify an automated DNA IQ™ protocol for use on the MultiPROBE® II PLUS HT EX platforms to allow extraction of DNA from various sample types.

4. Equipment and Materials

- MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (PerkinElmer, Downers Grove, IL, USA)
- Gravimetric Performance Evaluation Option with Mettler SAG285/L balance (Mettler-Toledo, Greifensee, Switzerland)
- DNA IQ™ System (Promega Corp., Madison, WI, USA)
- Extraction Buffer (10mM Tris, 1mM EDTA, 100mM NaCl, 20% SDS)
- SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA)
- Nunc™ Bank-It tubes (Nunc)
- 175µL non-conductive sterile filter RoboRack tips (PerkinElmer, Downers Grove, IL, USA)
- 1000µL Conductive sterile filter Robotix tips (MBP)
- ABI Prism® 7000 SDS (Applied Biosystems, Foster City, CA, USA)
- Quantifiler™ Human DNA Quantification kits (Applied Biosystems, Foster City, CA, USA)
- AmpFℓSTR® Profiler Plus Amplification kits (Applied Biosystems, Foster City, CA, USA)
- GeneAmp® 9700 thermalcycers (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 96-well optical reaction plates (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA)
- GeneScan™ 500 ROX™ Size Standard (Applied Biosystems, Foster City, CA, USA)
- Hi-Di™ Formamide (Applied Biosystems, Foster City, CA, USA)
- 3100 POP-4™ Polymer (Applied Biosystems, Foster City, CA, USA)
- For mock samples:
  - FTA® Classic Card (Whatman Inc., Florham Park, NJ, USA)
  - Sterile cotton swabs (Medical Wire & Equipment, Corsham, Wiltshire, England)
  - Sterile rayon swabs (Copan)

5. Methods

5.1 Automated DNA IQ™ Protocol

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5.2 Contamination Check via Checkboard and Zebra-stripe Patterns using Quantifiler Assays

Three sample types were chosen to create the contamination check plates: Blood FTA, Cell FTA and Blank FTA. Blood samples were created by obtaining blood from 2 donors (DJC/VKI) by a phlebotomist as per normal in three 4ml EDTA vials. A small volume of this blood was “clothed” or spotted onto Whatman FTA paper and allowed to dry overnight before sampling. Cells were collected from two donors (VKI/CJA) by using a “lolly-pop” swab to sample the inside of the donor’s cheek for 15 seconds before pressing the swab onto the FTA paper to transfer the DNA. Blank FTA samples were created using clean, unused FTA paper free from any kind of sample or reagent. Using the BSD duet machine, 2 3.2mm spots were punched directly into the Slicprep 96 device in either the checkerboard or zebra stripe pattern (see figure 2 a and 2 b for a representation of the sample arrangement). One checkerboard and one zebra stripe plated was punched per Extraction platform.

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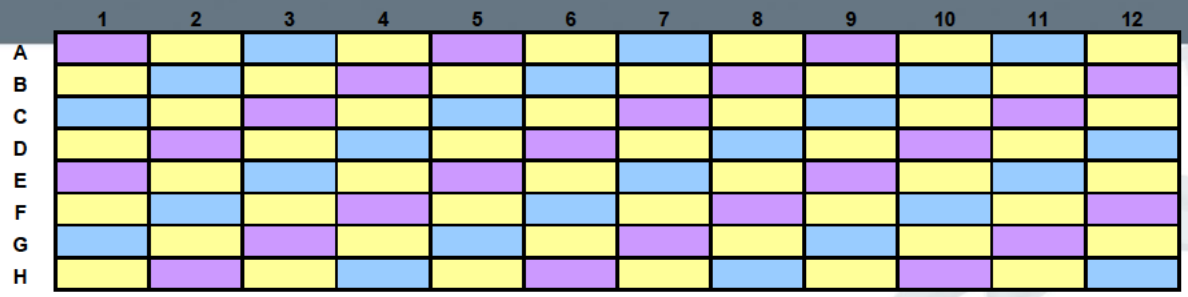
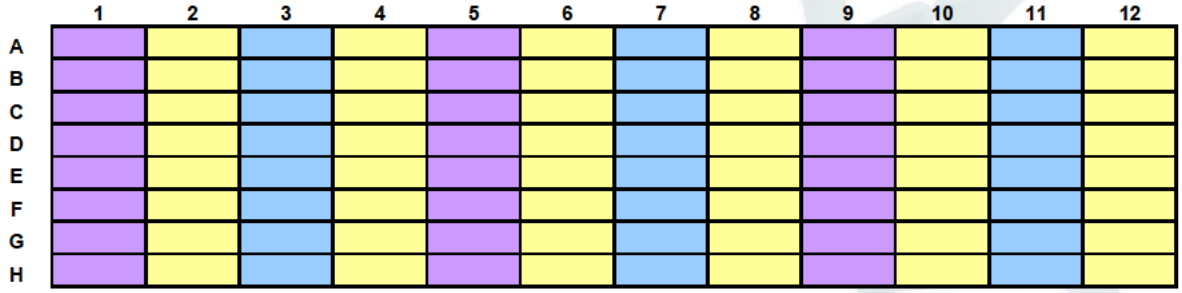


a) Checkerboard Pattern

b) Zebra Stripe Pattern



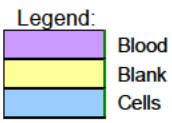


Figure 2. Checkerboard and zebra-stripe patterns utilised in the Extraction contamination check.

The Automated Extraction Setup MP II test program was compiled to replace the current manual Chelex extraction process for blood and cells performed by scientists in the Analytical section; Forensic Biology. The final Test Outline tree structure is presented below as Figure 3. Refer to Figure 4 for the deck layout.

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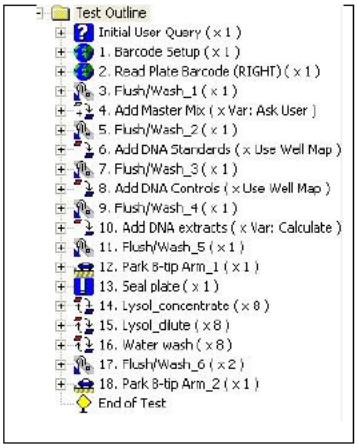


Figure 3. Test Outline for the automated setup of the Extraction program using the Extraction MP II's.

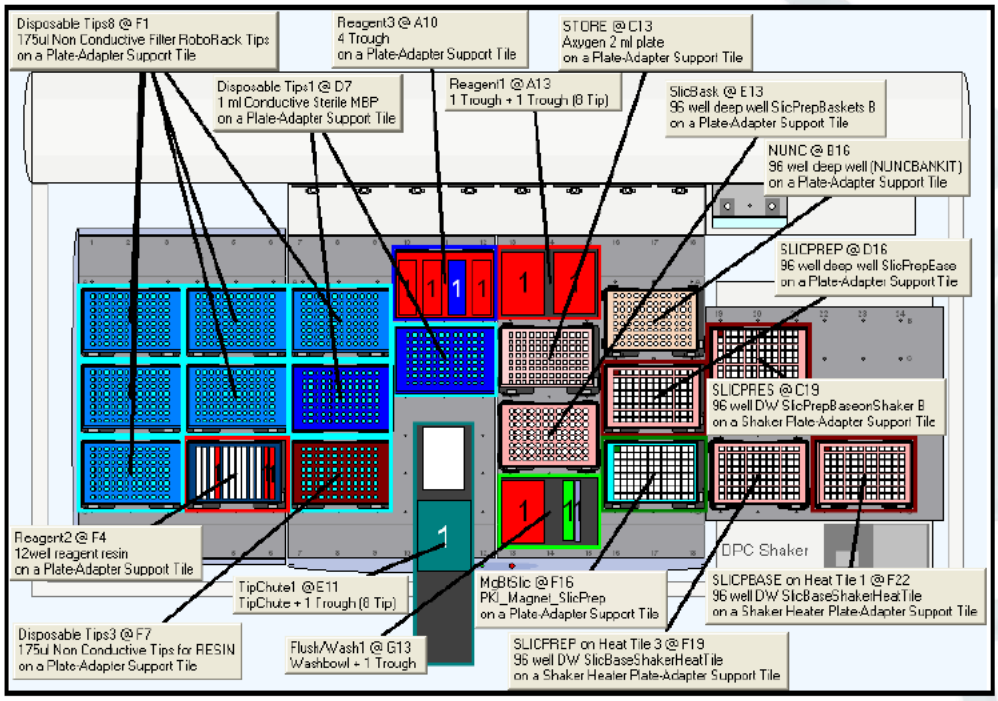


Figure 4. The WinPrep® virtual deck view displaying the necessary labware required to perform automated Extraction test file (Extraction Platform A deck view).

5.3 Verification of the Automated Extraction Chemistry Protocol

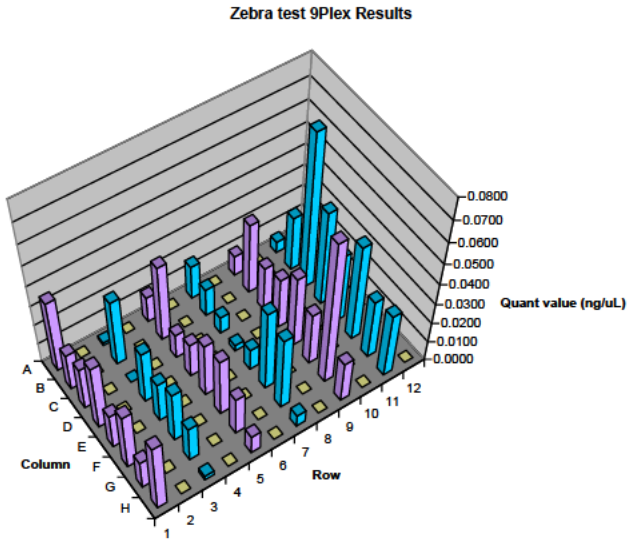
Comparisons were made between results generated by the automated and manual methods to verify the performance of the automated Extraction Chemistry setup protocol.

Verification samples consisted of different dilutions of blood and cells spotted in 30ul aliquots onto quartered cotton and rayon swabs. Four blood dilutions of neat, 1/10, 1/100 and 1/1000 and four cell dilutions of neat, 1/5.2, 1/52.2 and 1/522 were used to test the sensitivity of both the manual and automated methods. Dilutions were created using neat sample and 0.9% saline for both sample types. Four replicates of each dilution were made up for each substrate and sample type. The cells used in this verification were collected using a cytobrush method where the donor used a cytobrush for one minute to "swab" the inside of one cheek. Once each cheek was swabbed, the cells on the brush were suspended in 2ml of 0.9% saline. The blood was collected using the same method as in 5.2. 4 separate extractions were performed for the manual set: Blood Rayon, Blood Cotton, Cell Rayon and Cell Cotton. For the automated verification, all sample types were extracted together after being transferred to a Slicprep 96 device for processing on the Extraction Platform B.

6. Results and Discussion

6.1 Gravimetric Evaluation of Pipetting Accuracy and Precision

6.2 Contamination Check via Checkboard and Zebra-stripe Patterns using



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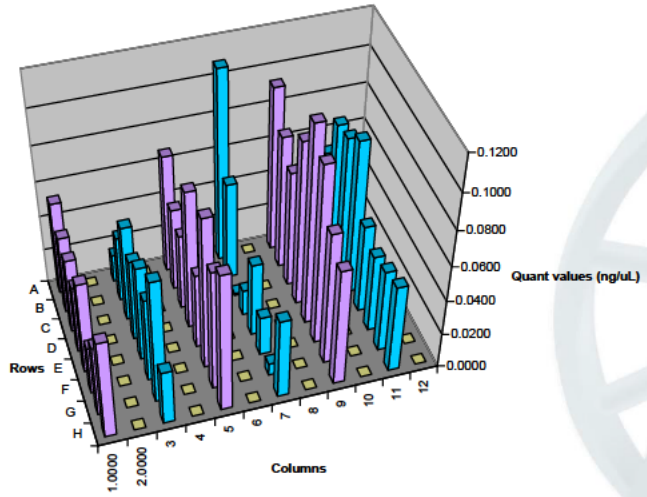
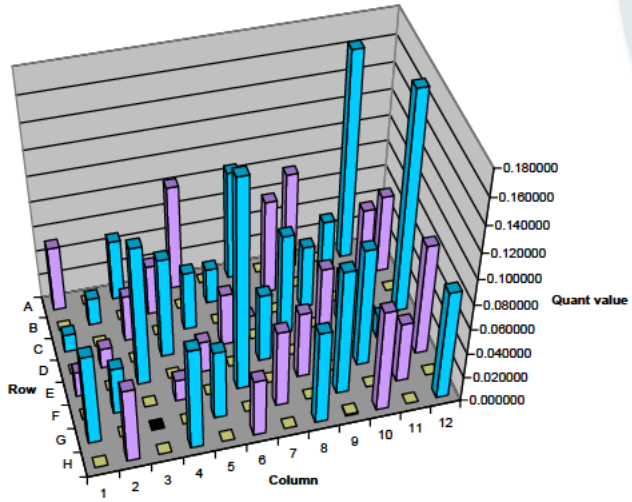
Zebra 9FTAR Results



Checkerboard 9Plex Results



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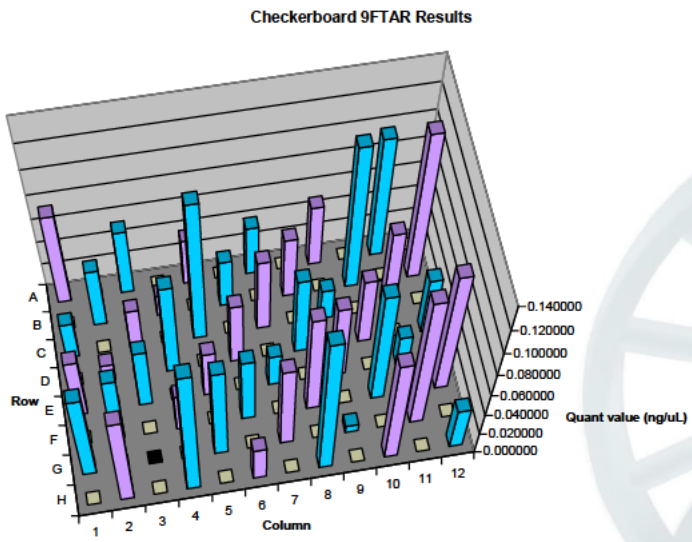

9Plex

	1	2	3	4	5	6	7	8	
A	0.051900	0.000000	0.048300	0.000000	0.083700	0.000000	0.087000	0.000000	0.077
B	0.000000	0.021900	0.000000	0.040000	0.000000	0.028300	0.000000	0.075200	0.000
C	0.014100	0.000000	0.037100	0.000000	0.047100	0.000000	0.000000	0.000000	0.050
D	0.000000	0.015900	0.000000	0.079900	0.000000	0.042100	0.000000	0.080700	0.000
E	0.020200	0.000000	0.111000	0.000000	0.025300	0.000000	0.054600	0.000000	0.066
F	0.000761	0.037500	0.000000	0.017300	0.000000	0.166000	0.000000	0.053000	0.000
G	0.072000	0.000000	Removed	0.000000	0.055200	0.000000	0.061300	0.000000	0.099
H	0.000000	0.059400	0.000000	0.081300	0.000000	0.045800	0.000000	0.074700	0.001

9FTAR

	1	2	3	4	5	6	7	8	
A	0.076300	0.000000	0.054500	0.000000	0.038700	0.000000	0.041100	0.000000	0.050
B	0.000000	0.049700	0.000000	0.018200	0.000000	0.040200	0.000000	0.051000	0.000
C	0.032000	0.000000	0.035200	0.000000	0.118000	0.000000	0.061000	0.000000	0.026
D	0.000000	0.015300	0.000000	0.078200	0.000000	0.052400	0.000000	0.065900	0.000
E	0.052000	0.000000	0.051800	0.000000	0.040500	0.000000	0.027900	0.000000	0.062
F	0.000000	0.058600	0.000000	0.033700	0.000000	0.056600	0.000000	0.085700	0.000
G	0.076300	0.000000	Removed	0.000000	0.081300	0.000000	0.072600	0.000000	0.006
H	0.000000	0.081000	0.000000	0.114000	0.000000	0.030500	0.000000	0.122000	0.000

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	9Plex							
	1	2	3	4	5	6	7	
A	0.0358	0.0000	0.001440	0.000000	0.012800	0.000000	0.016500	0.0000
B	0.0184	0.0000	0.032800	0.000000	0.037700	0.000000	0.013700	0.0000
C	0.0212	0.0000	0.000000	0.000000	0.012100	0.000000	0.008350	0.0000
D	0.0324	0.0000	0.025900	0.000000	0.016900	0.000000	0.003010	0.0000
E	0.0175	0.0000	0.020000	0.000000	0.026700	0.000000	0.010600	0.0000
F	0.0282	0.0000	0.025400	0.000000	0.028700	0.000000	0.039800	0.0000
G	0.0144	0.0000	0.017800	0.000000	0.019100	0.000000	0.036200	0.0000
H	0.0348	0.0000	0.002230	0.000000	0.009310	0.000000	0.006010	0.0000
	9FTAR							
	1	2	3	4	5	6	7	
A	0.0534	0.0000	0.015700	0.000000	0.067500	0.000000	0.109000	0.0000
B	0.0444	0.0000	0.037600	0.000000	0.047300	0.000000	0.055500	0.0000
C	0.0426	0.0000	0.054800	0.000000	0.043100	0.000000	0.001140	0.0000
D	0.0386	0.0000	0.046600	0.000000	0.079200	0.000000	0.014700	0.0000
E	0.0525	0.0000	0.054500	0.000000	0.043200	0.000000	0.042000	0.0000
F	0.0273	0.0000	0.048100	0.000000	0.086800	0.000000	0.022500	0.0000
G	0.0408	0.0000	0.070800	0.000000	0.068900	0.000000	0.006870	0.0000
H	0.0566	0.0000	0.031100	0.000000	0.079300	0.000000	0.045600	0.0000

6.3 Verification of the Automated Extraction Protocol

7. Summary

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Project 13: Report on the Verification of an Automated DNA IQ™ Protocol using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform

Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C.
Automation/LIMS Implementation Project, DNA Analysis FSS (April 2008)

1. Abstract

A manual method for extracting DNA from forensic samples using the DNA IQ™ system (Promega Corp., Madison, WI, USA) was validated for routine use in DNA Analysis (FSS). We have verified an automated DNA IQ™ protocol in 96-well format for use on the MultiPROBE® II PLUS HT EX FORENSIC WORKSTATION platforms. Data indicate that results from the automated procedure are comparable to those from the manual procedure. Contamination checks were performed using samples prepared in checkerboard and zebra-stripe format, and results were as expected. We recommend the use of the MultiPROBE® II PLUS platforms to perform automated DNA extraction using the DNA IQ™ system.

2. Introduction

The MultiPROBE® II PLUS HT EX FORENSIC WORKSTATION platforms are equipped to perform automated DNA extractions, as they include a DPC shaker and individual heat controllers to enable on-board lysis and incubation steps. Currently in DNA Analysis, the MultiPROBE® platforms allow walk-away operation of PCR setup protocols for DNA quantitation and amplification.

The DNA IQ™ protocol has been verified or validated by various laboratories for use on the MultiPROBE® II PLUS platform. The laboratories that perform an automated DNA IQ™ protocol include PathWest (Western Australia), Forensic Science South Australia (South Australia) and Centre of Forensic Sciences in Toronto (Ontario). The MultiPROBE® II PLUS instrument comes pre-loaded with an automated DNA IQ™ protocol. Unlike the other laboratories, however, we did not validate the included protocol, but instead validated a manual DNA IQ™ protocol which was based on the CFS automated protocol (PerkinElmer, 2004), followed by verification of an automated protocol.

The verified automated DNA IQ™ protocol is identical to the validated manual protocol used in-house: there are no differences in reagents or volumes. The adopted DNA IQ™ protocol differs slightly, however, from the manufacturer's protocol, as it includes a lysis step in Extraction Buffer (10mM Tris, 1mM EDTA, 100mM NaCl, 20% SDS) in the presence of Proteinase K, before incubating in the DNA IQ™ Lysis Buffer. Furthermore, the lysis incubation conditions were lowered from 70°C to 37°C in order to accommodate extraction of DNA from heat labile materials such as nylon and polyester. In addition, the automated protocol utilises the SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA) for simultaneous processing of samples in a 96-well format.

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3. Aim

To verify an automated DNA IQ™ protocol for use on the MultiPROBE® II PLUS HT EX platforms to allow extraction of DNA from various sample types.

4. Equipment and Materials

- MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (PerkinElmer, Downers Grove, IL, USA)
- Gravimetric Performance Evaluation Option with Mettler SAG285/L balance (Mettler-Toledo, Greifensee, Switzerland)
- DNA IQ™ System (Promega Corp., Madison, WI, USA)
- Extraction Buffer (10mM Tris, 1mM EDTA, 100mM NaCl, 20% SDS)
- SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA)
- Nunc™ Bank-It tubes (Nunc)
- 175µL non-conductive sterile filter RoboRack tips (PerkinElmer, Downers Grove, IL, USA)
- 1000µL Conductive sterile filter Robotix tips (MBP)
- ABI Prism® 7000 SDS (Applied Biosystems, Foster City, CA, USA)
- Quantifiler™ Human DNA Quantification kits (Applied Biosystems, Foster City, CA, USA)
- AmpFℓSTR® Profiler Plus Amplification kits (Applied Biosystems, Foster City, CA, USA)
- GeneAmp® 9700 thermalcycers (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 96-well optical reaction plates (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA)
- GeneScan™ 500 ROX™ Size Standard (Applied Biosystems, Foster City, CA, USA)
- Hi-Di™ Formamide (Applied Biosystems, Foster City, CA, USA)
- 3100 POP-4™ Polymer (Applied Biosystems, Foster City, CA, USA)
- For mock samples:
  - FTA™® Classic Card (Whatman Inc., Florham Park, NJ, USA)
  - Sterile cotton swabs (Medical Wire & Equipment, Corsham, Wiltshire, England)
  - Sterile rayon swabs (Copan)

5. Methods

5.1 Gravimetric Evaluation of Pipetting Accuracy and Precision

The Gravimetric Performance Evaluation Option with Mettler SAG285/L balance (Mettler-Toledo, Greifensee, Switzerland), hereby referred to as the “gravimetric kit”, was purchased from PerkinElmer (PN 7601823) as an optional accessory upon recommendations made by Forensic Science South Australia staff members. The gravimetric kit consists of a SAG285/L balance and various accessories including a humidified chamber and software for integration with the WinPrep® software. All components were installed by a qualified PerkinElmer service engineer, and subsequently calibrated by a Mettler-Toledo engineer for NATA certification. A NATA certificate for the kit (Report Number Q0737-001-1) was produced on 13 March 2006.

Gravimetric analysis is performed by placing the balance on the platform deck and instructing the MP II to repeatedly pipette certain volumes of system liquid onto the balance pan. Readings are taken automatically by the software and compiled into a results table, which is then used to automatically generate an Excel-based results chart containing mean, %CV and %inaccuracy values. The mean values obtained are used to calculate R^2, slope and Y-intercept (offset) values to calibrate the system’s pipetting.

The pipette tips required for use on the Extraction Platforms include 175µL non-conductive MPII sterile filter RoboRack tips, 1000µL conductive sterile Robotix tips and the Fixed tips of the MPII itself. 175µL tips are used for the purpose of removing reagents and the

transfer of DNA sample. The 1000µL tips were chosen for the purpose of removing supernatant and the fixed tips are used only for the addition of reagents. Pipetting performance was assessed for various volumes using the three different tips in order to calculate appropriate R^2, slope and Y-intercept (offset) values which were then added to the performance file. Values were calculated for both Blowout (single-liquid transfer) and Waste (multidispense) modes for the 1000µL tips, and Blowout mode only for the 175µL and fixed tips. A specialised performance file was created for the addition of resin. The performance file for the 175µL tips uses the already calibrated information of the Blowout mode, however the Blowout volume column values are set to 0, making it preform in a waste like mode. Retesting was performed to confirm accurate and precise pipetting with these settings.

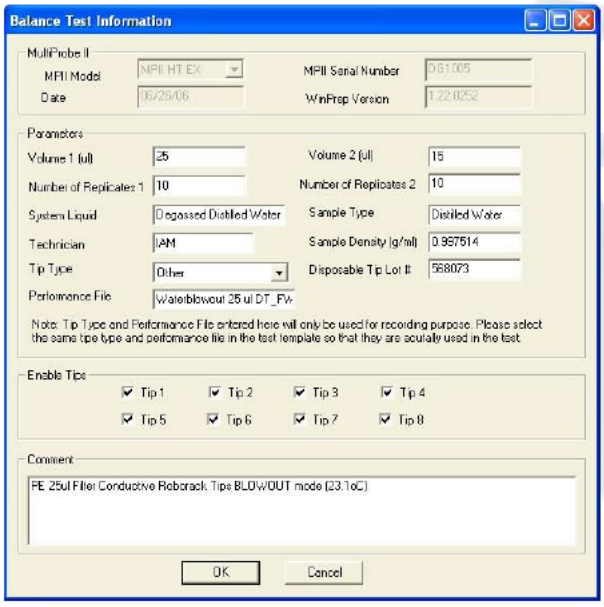

Figure 1 The Balance Test Information Window as present within the Balance Test DT program. All pipetting parameters are entered here and are subsequently transferred to the result output file.

All gravimetric testing was performed using the Balance Test DT test program within WinPrep®. Parameter values that needed to be entered into the Balance Test Information Window (Figure 1) included those as outlined in Table 1.

Table 1 Input values that are required for the various Balance Test Information parameters.

Parameter(s)	Value
Volume 1 and Volume 2	For 175µL tips: 175, 100, 50, 15µL For 1000µL tips: 1000, 700, 400, 100µL For Fixed tips: 1000, 700, 400, 100µL
Number of Replicates	10
System Liquid	Degassed Distilled Water
Sample Type	Distilled Water
Technician	Initials of the operator performing the test
Sample Density (g/ml)	The density of water at environmental temperature*
Tip Type	Other
Disposable Tip Lot #	The lot number of the particular tips in use

Performance File	The appropriate Performance File for the tip (175µL, 1000µL or fixed tips) and pipetting mode (Blowout or Waste) in use
Enable Tips (checkboxes)	Select the actual tips (1 to 8) to be tested
Comments	Free text box to add additional information (eg. Tip type, mode, current environmental room temperature, etc).

* Water density values were obtained from http://www.simetric.co.uk/si_water.htm

Pipetting accuracy and precision were examined at four different volumes for each tip size: 175, 100, 50, 15µL for 175µL tips and 1000, 700, 400, 100µL for the 1000µL and Fixed tips. In order to calculate unbiased values for each set of volumes, the slope and offset values in the relevant Performance File were changed to the default 1 and 0 respectively prior to testing. The mean volumes that were pipetted by each tip (10 replicates per tip) at the four designated volumes were used to generate a standard curve. The slope and offset calculated from this curve was used to calibrate the relevant Performance File. The final Performance File settings were then tested at the highest and lowest volumes to confirm accurate and precise pipetting. In the case of the specialised 175µL Blowout performance file, only high and low volumes are tested as it uses the settings from the original 175µL Blowout performance file.

5.2 Blood Collection

Blood samples were created by obtaining blood from 2 staff donors (DJC/VKI) by a phlebotomist as per normal in three 4mL EDTA vials.

5.3 Cell Collection

The cells used in this verification were collected using a cytobrush method where the donor used a cytobrush for one minute to “swab” the inside of one cheek. Once each cheek was swabbed, the cells on the brush were suspended in 2mL of 0.9% saline.

5.4 FTA cell Collection

Cells were collected from two staff donors (VKI/CJA) by using a “lolly-pop” swab to sample the inside of the donor’s cheek for 15 seconds before pressing the swab onto the FTA™ paper to transfer the DNA.

5.5 Automated DNA IQ™ Protocol

as

5.6 Contamination Check via Checkerboard and Zebra-stripe Patterns

Three sample types were chosen to create the contamination check plates: Blood FTA, Cell FTA and Blank FTA.
A small volume of collected blood was “clothed” (or spotted) onto Whatman FTA™ paper and allowed to dry overnight before sampling. Blank FTA samples were created using clean, unused FTA™ paper free from any kind of sample or reagent. Using the BSD 600 duet machine, two 3.2mm spots were punched directly into the SlicPrep 96 device in either the checkerboard or Zebra-stripe pattern (see figure 2a and 2b for a representation of the sample arrangement). One checkerboard and one Zebra-stripe plate was punched per Extraction platform.

A total of five contamination plates were extracted between both extraction platforms. Three checkerboard and two Zebra-Stripe plates. It was noted during the creation of one of

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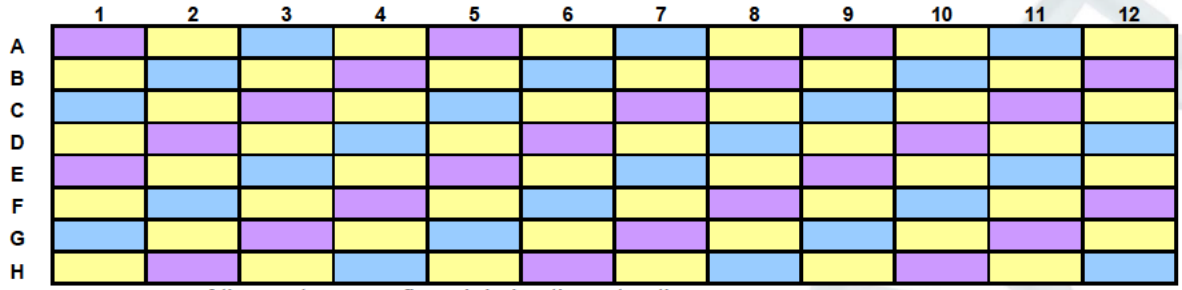
the checkerboard plates that a spot "jumped" into an adjacent well (E3- Cells6). The presence of the spot was confirmed during the extraction.



a) Checkerboard Pattern

b) Zebra Stripe Pattern

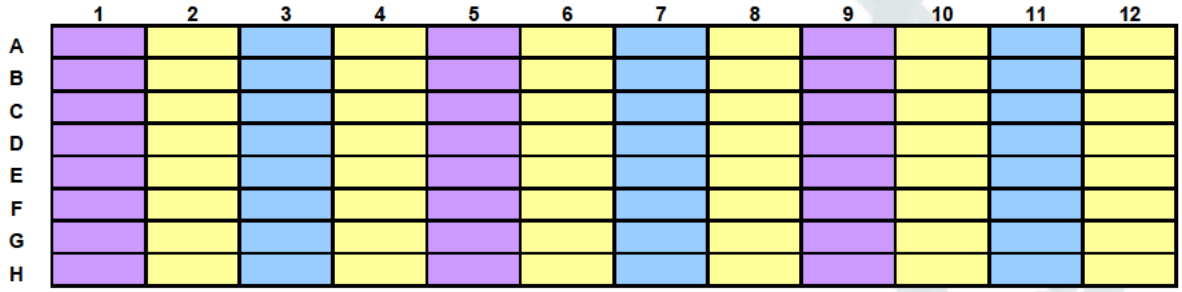


Figure 1. Checkerboard and zebra-stripe patterns utilised in the Extraction contamination check.

The Automated Extraction Setup MP II test program was compiled to replace the current manual Chelex extraction process for blood and cells performed by scientists in the Analytical section; Forensic Biology. The final Test Outline tree structure is presented below as Figure 3. Refer to Figure 4 for the deck layout.

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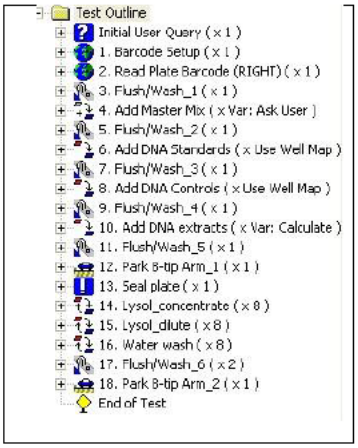

Figure 2 Test Outline for the automated setup of the Extraction program using the Extraction MP II's.

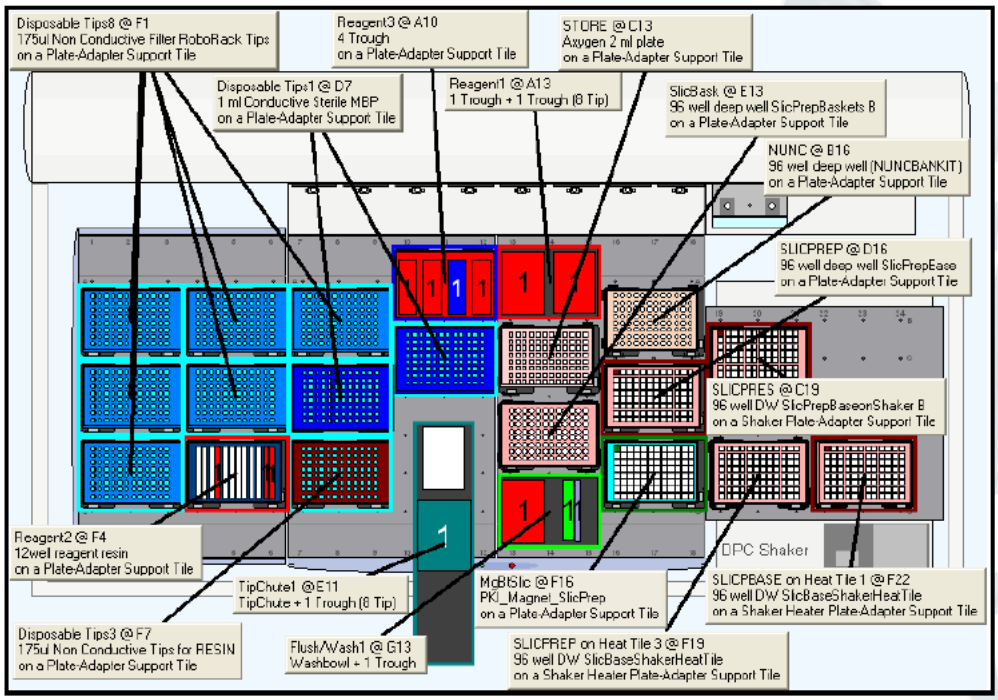

Figure 3 The WinPrep® virtual deck view displaying the necessary labware required to perform automated Extraction test file (Extraction Platform A deck view).

5.7 Heater tile temperature verification

Heat tiles supplied with MPII were modified to accept the SlicPrep 96 device. Temperature

5.8 Verification of the Automated Extraction Chemistry Protocol

Comparisons were made between results generated by the automated and manual methods to verify the performance of the automated Extraction Chemistry setup protocol.

Verification samples consisted of different dilutions of blood and cells spotted in 30µL aliquots onto quartered cotton and rayon swabs. Four blood dilutions of neat, 1/10, 1/100 and 1/1000 and four cell dilutions of neat, 1/5.2, 1/52.2 and 1/522 were used to test the sensitivity of both the manual and automated methods. Dilutions were created using neat sample and 0.9% saline for both sample types. Four replicates of each dilution were made up for each substrate and sample type.

The blood was collected using the same method as in 5.2. 4 separate extractions were performed for the manual set: Blood Rayon, Blood Cotton, Cell Rayon and Cell Cotton. For the automated verification, all sample types were extracted together after being transferred to a Slicprep 96 device for processing on the Extraction Platform B.

5.9 Resin Volume

- 7 µL
- 14 µL

5.10 Extraction volume

- 300 µL
- 350 µL
- 400 µL
- 450 µL
- 500 µL

5.11 Sensitivity

Fbot262

6. Results and Discussion

6.1 Gravimetric Evaluation of Pipetting Accuracy and Precision

6.2 Contamination Check via Checkerboard and Zebra-stripe Patterns results

Table 1 below lists the extraction batch id of the checkerboards and their outcomes.

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Table 2

Type of plate	Extraction batch Id	Extraction Platform	Check passed
Checkerboard-1	VALB20070817_02	Extraction A	Invalidated
Checkerboard-2	VALB20070803_02	Extraction B	Yes
Checkerboard/Zebra	VALB20071022_01	Extraction	Yes
Zebra-Stripe-1	VALB20070803_03	Extraction A	Yes
Zebra-Stripe-2	VALB20070817_03	Extraction B	Yes

Checkerboard1-
Position E3 (Sample Cells 6) was known to have been contaminated prior to start of the extraction. The DNA profile obtained confirmed this mixtures origin was formed from the expected donors. See figure X. Eight of the designated blank samples (Positions D3, A10, F1, H5, C4, E4, B7 and E6); two of the cell samples (A1 and B10) and two of the blood samples (F4 and G7) all exhibited a partial DNA profile previously unknown. This profile did not match any of the positive samples present on the batch. The profile was searched against the Staff database and no matches found.
None of the other blank samples yielded any DNA profile. The rest of the cell and blood samples yielded the correct DNA profile. Although there is no evidence of well to well contamination was observed; the unknown profile obtained has invalidated this plate. A further checkerboard/Zebra-Stripe combination plate was performed to ensure…

Table 3-Unknown profile obtained

Sample description	D3	vWA	FGA	Amel	D8	D21	D18	D5	D13	D7
Blk23-E6	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	11,13
Blk25-B7	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	
Blk15-E4	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	11,13
Blk14-C4	14,17									
Blk20-H5										
Blk3-F1										
Blk10-D3										
Blk37-A10										
Cells19-B10	14,17	14,17	20,21,22,24	X,Y	11,13,16	29,30,	14,15,16	11,15	11,12	11,11
Cells13-A1										
Blood14-G7										
Blood8-F4										

Checkerboard 2 –
None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile, see Figure 4.

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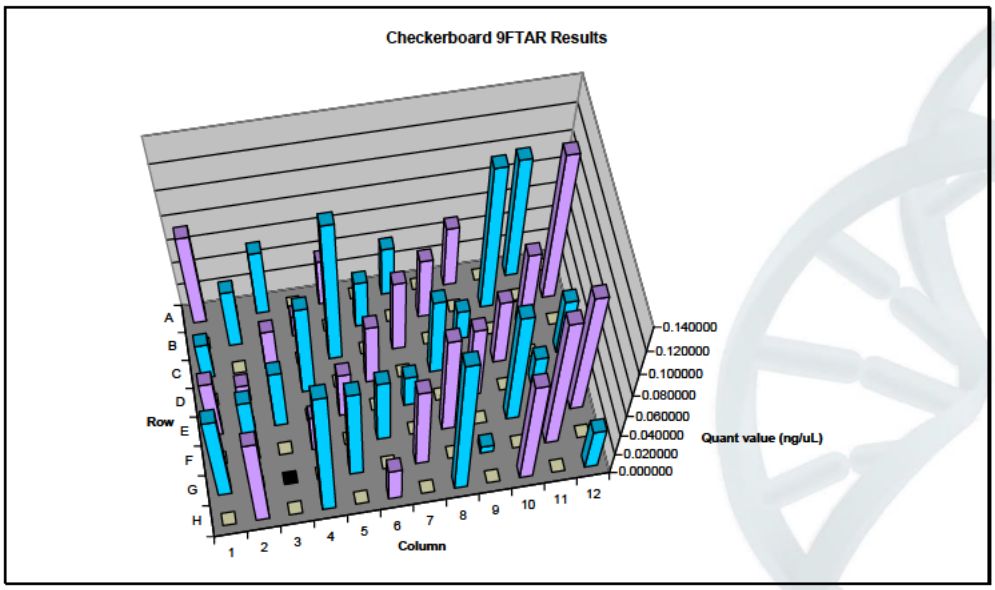

Figure 4

Zebra-Stripe1-
None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile, see Figure 5.

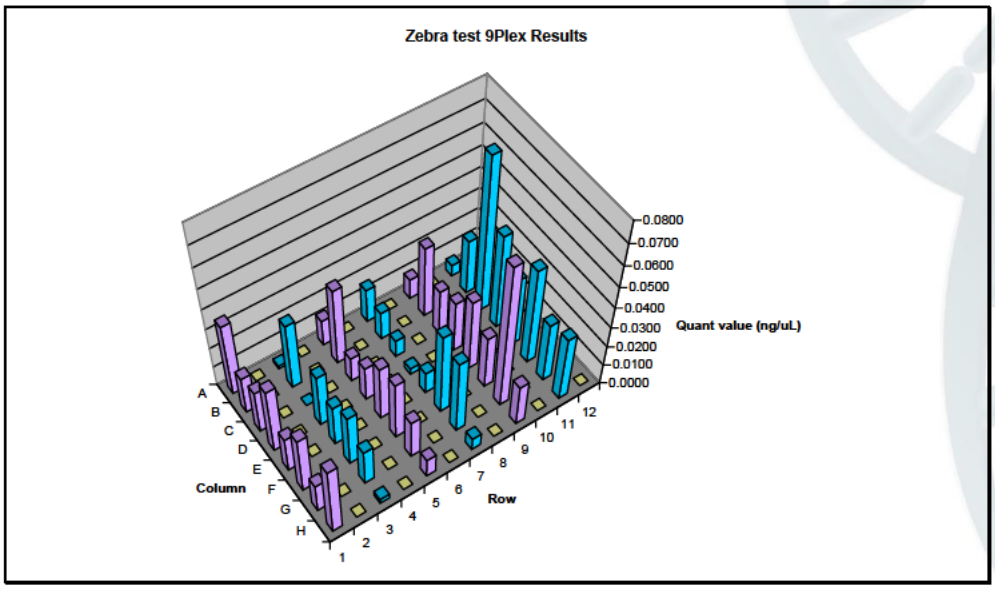

Figure 5

Zebra-Stripe 2-

None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile, see Figure 6.

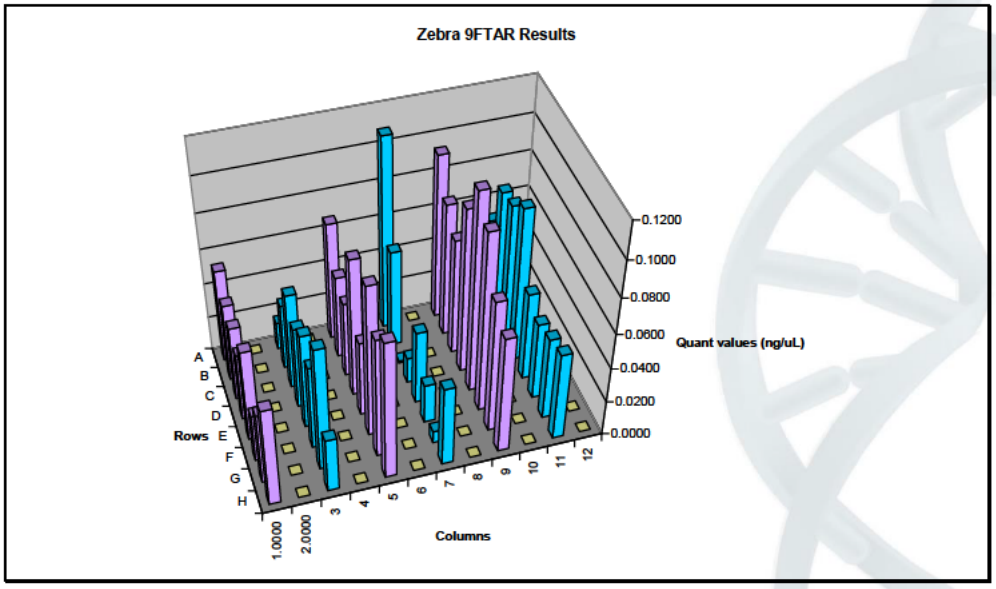

Figure 6

Checkerboard/Zebra -
None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile, see Figure 7.

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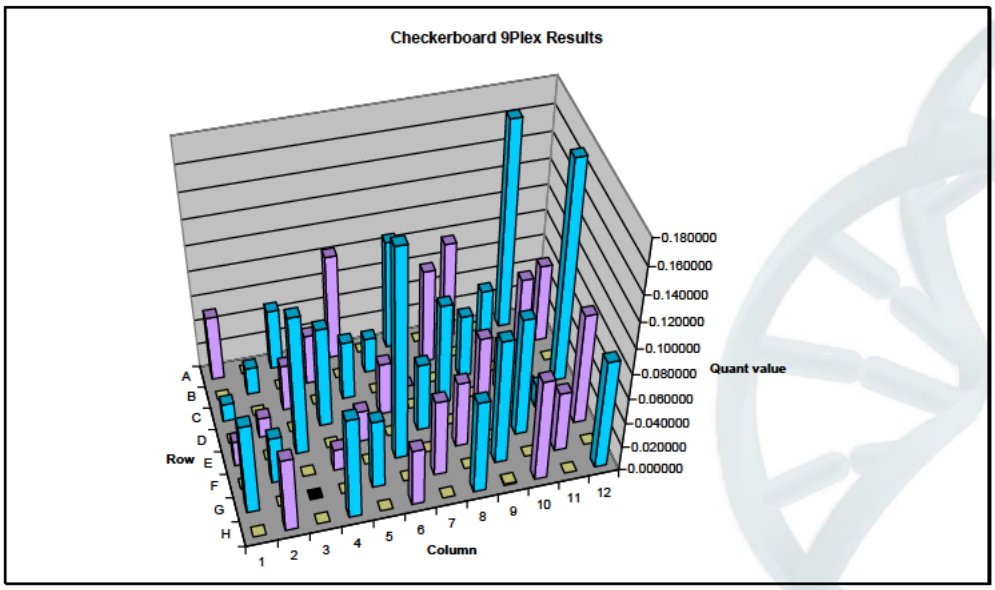

Figure 7

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9Plex

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.051900	0.000000	0.048300	0.000000	0.083700	0.000000	0.087000	0.000000	0.077300	0.000000	0.164000	0.000000
B	0.000000	0.021900	0.000000	0.040000	0.000000	0.028300	0.000000	0.075200	0.000000	0.050100	0.000000	0.062300
C	0.014100	0.000000	0.037100	0.000000	0.047100	0.000000	0.000000	0.000000	0.050400	0.000000	0.071700	0.000000
D	0.000000	0.015900	0.000000	0.079900	0.000000	0.042100	0.000000	0.080700	0.000000	0.039200	0.000000	0.174000
E	0.020200	0.000000	0.111000	0.000000	0.025300	0.000000	0.054600	0.000000	0.066700	0.000000	0.017900	0.000000
F	0.000761	0.037500	0.000000	0.017300	0.000000	0.166000	0.000000	0.053000	0.000000	0.094500	0.000778	0.088700
G	0.072000	0.000000	Removed	0.000000	0.055200	0.000000	0.061300	0.000000	0.099700	0.000000	0.048300	0.000000
H	0.000000	0.059400	0.000000	0.081300	0.000000	0.045800	0.000000	0.074700	0.001210	0.081400	0.000000	0.087200

9FTAR

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.076300	0.000000	0.054500	0.000000	0.038700	0.000000	0.041100	0.000000	0.050500	0.000000	0.098100	0.000000
B	0.000000	0.049700	0.000000	0.018200	0.000000	0.040200	0.000000	0.051000	0.000000	0.118000	0.001560	0.120000
C	0.032000	0.000000	0.035200	0.000000	0.118000	0.000000	0.061000	0.000000	0.026000	0.000000	0.067900	0.000000
D	0.000000	0.015300	0.000000	0.078200	0.000000	0.052400	0.000000	0.065900	0.000000	0.056400	0.000000	0.048000
E	0.052000	0.000000	0.051800	0.000000	0.040500	0.000000	0.027900	0.000000	0.062900	0.000000	0.026300	0.000000
F	0.000000	0.058600	0.000000	0.033700	0.000000	0.056600	0.000000	0.085700	0.000000	0.096500	0.000000	0.104000
G	0.076300	0.000000	Removed	0.000000	0.081300	0.000000	0.072600	0.000000	0.006630	0.000000	0.114000	0.000000
H	0.000000	0.081000	0.000000	0.114000	0.000000	0.030500	0.000000	0.122000	0.000000	0.092400	0.000000	0.037500

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	9Plex											
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0358	0.0000	0.001440	0.000000	0.012800	0.000000	0.016500	0.000000	0.009840	0.000000	0.005540	0.000000
B	0.0184	0.0000	0.032800	0.000000	0.037700	0.000000	0.013700	0.000000	0.034600	0.000000	0.026500	0.000000
C	0.0212	0.0000	0.000000	0.000000	0.012100	0.000000	0.008350	0.000000	0.021700	0.000000	0.074200	0.000000
D	0.0324	0.0000	0.025900	0.000000	0.016900	0.000000	0.003010	0.000000	0.024800	0.000000	0.046000	0.000000
E	0.0175	0.0000	0.020000	0.000000	0.026700	0.000000	0.010600	0.000000	0.034700	0.000000	0.033300	0.000000
F	0.0282	0.0000	0.025400	0.000000	0.028700	0.000000	0.039800	0.000000	0.025700	0.000000	0.047100	0.000000
G	0.0144	0.0000	0.017800	0.000000	0.019100	0.000000	0.036200	0.000000	0.069900	0.000000	0.029200	0.000000
H	0.0348	0.0000	0.002230	0.000000	0.009310	0.000000	0.006010	0.000000	0.020200	0.000000	0.031600	0.000000
	9FTAR											
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0534	0.0000	0.015700	0.000000	0.067500	0.000000	0.109000	0.000000	0.093500	0.000000	0.051000	0.009600
B	0.0444	0.0000	0.037600	0.000000	0.047300	0.000000	0.055500	0.000000	0.076100	0.000000	0.076800	0.000000
C	0.0426	0.0000	0.054800	0.000000	0.043100	0.000000	0.001140	0.000000	0.066200	0.000000	0.079300	0.000000
D	0.0386	0.0000	0.046600	0.000000	0.079200	0.000000	0.014700	0.000000	0.093700	0.000000	0.087900	0.000000
E	0.0525	0.0000	0.054500	0.000000	0.043200	0.000000	0.042000	0.000000	0.113000	0.000000	0.050500	0.000000
F	0.0273	0.0000	0.048100	0.000000	0.086800	0.000000	0.022500	0.000000	0.102000	0.000000	0.044300	0.000000
G	0.0408	0.0000	0.070800	0.000000	0.068900	0.000000	0.006870	0.000000	0.075700	0.000000	0.047300	0.000000
H	0.0566	0.0000	0.031100	0.000000	0.079300	0.000000	0.045600	0.000000	0.066900	0.000000	0.050300	0.000000

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6.3 Verification of the Automated Extraction Protocol

7. Summary and Recommendations

We recommend the following:

- Use of MPII for automated extraction of reference samples
- Use of MPII for automated extraction of casework samples
- Ongoing development of the automated extraction program to increase the efficiency of the extraction

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Project 13. Report on the Verification of an Automated DNA IQ™ Protocol using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform

Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C., Ientile, V.
Automation/LIMS Implementation Project, DNA Analysis FSS (August 2008)

1. Abstract

A manual method for extracting DNA from forensic samples using the DNA IQ™ system (Promega Corp., Madison, WI, USA) was validated for routine use in DNA Analysis (FSS). We have verified an automated DNA IQ™ protocol in 96-well format for use on the MultiPROBE® II PLUS HT EX FORENSIC WORKSTATION platforms (PerkinElmer, Downers Grove, IL, USA). Data indicate that results from the automated procedure are comparable to those from the manual procedure. Contamination checks were performed using samples prepared in checkerboard and zebra-stripe format, and results were as expected. We recommend the use of the MultiPROBE® II PLUS HT EX platforms to perform automated DNA extraction using the DNA IQ™ system.

2. Introduction

The MultiPROBE® II PLUS HT EX FORENSIC WORKSTATION platforms (PerkinElmer, Downers Grove, IL, USA) are equipped to perform automated DNA extractions, as they include a DPC shaker and individual heat controllers to enable on-board lysis and incubation steps. Currently in DNA Analysis, the MultiPROBE® platforms allow walk-away operation of PCR setup protocols for DNA quantitation and amplification.

The DNA IQ™ protocol has been verified or validated by various laboratories for use on the MultiPROBE® II PLUS platform. The laboratories that perform an automated DNA IQ™ protocol include PathWest (Western Australia), Forensic Science South Australia (South Australia) and Centre of Forensic Sciences in Toronto (Ontario). The MultiPROBE® II PLUS instrument comes pre-loaded with an automated DNA IQ™ protocol. Unlike the other laboratories, however, we did not validate the included protocol, but instead validated a manual DNA IQ™ protocol which was based on the CFS automated protocol (PerkinElmer, 2004), followed by verification of an automated protocol based on the validated manual method.

The verified automated DNA IQ™ protocol is identical to the validated manual protocol used in-house: there are no differences in reagents or volumes. The adopted DNA IQ™ protocol differs slightly, however, from the manufacturer's protocol, as it includes a lysis step using Extraction Buffer (10mM Tris, 1mM EDTA, 100mM NaCl, 20% SDS) in the presence of Proteinase K, before incubating in the DNA IQ™ Lysis Buffer. Furthermore, the lysis incubation conditions were lowered from 70°C to 37°C in order to accommodate extraction of DNA from heat labile materials such as nylon and polyester. In addition, the automated protocol utilises the SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA) for simultaneous processing of samples in a 96-well format.

3. Aim

To verify an automated DNA IQ™ protocol for use on the MultiPROBE® II PLUS HT EX platforms to allow extraction of DNA from various sample types.

4. Equipment and Materials

- MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (PerkinElmer, Downers Grove, IL, USA)
- Gravimetric Performance Evaluation Option with Mettler SAG285/L balance (Mettler-Toledo, Greifensee, Switzerland)
- DNA IQ™ System (Promega Corp., Madison, WI, USA)
- Extraction Buffer (10mM Tris, 1mM EDTA, 100mM NaCl, 20% SDS)
- SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA)
- Nunc™ Bank-It tubes (Nunc)
- 175µL non-conductive sterile filter RoboRack tips (PerkinElmer, Downers Grove, IL, USA)
- 1000µL Conductive sterile filter Robotix tips (MBP)
- ABI Prism® 7000 SDS (Applied Biosystems, Foster City, CA, USA)
- Quantifiler™ Human DNA Quantification kits (Applied Biosystems, Foster City, CA, USA)
- AmpFℓSTR® Profiler Plus Amplification kits (Applied Biosystems, Foster City, CA, USA)
- GeneAmp® 9700 thermalcycers (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 96-well optical reaction plates (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA)
- GeneScan™ 500 ROX™ Size Standard (Applied Biosystems, Foster City, CA, USA)
- Hi-Di™ Formamide (Applied Biosystems, Foster City, CA, USA)
- 3100 POP-4™ Polymer (Applied Biosystems, Foster City, CA, USA)
- For mock samples:
  - FTA™® Classic Card (Whatman Inc., Florham Park, NJ, USA)
  - Sterile cotton swabs (Medical Wire & Equipment, Corsham, Wiltshire, England)
  - Sterile rayon swabs (Copan)

5. Methods

5.1 Gravimetric Evaluation of Pipetting Accuracy and Precision

The Gravimetric Performance Evaluation Option with Mettler SAG285/L balance (Mettler-Toledo, Greifensee, Switzerland), hereby referred to as the “gravimetric kit”, was purchased from PerkinElmer (PN 7601823) as an optional accessory upon recommendations made by Forensic Science South Australia staff members. The gravimetric kit consists of a SAG285/L balance and various accessories including a humidified chamber and software for integration with the WinPrep® software. All components were installed by a qualified PerkinElmer service engineer, and subsequently calibrated by a Mettler-Toledo engineer for NATA certification. A NATA certificate for the kit (Report Number Q0737-001-1) was produced on 13 March 2006.

Gravimetric analysis is performed by placing the balance on the platform deck and instructing the MP II to repeatedly pipette certain volumes of system liquid onto the balance pan. Readings are taken automatically by the software and compiled into a results table, which is then used to automatically generate an Excel-based results chart containing mean, %CV and %inaccuracy values. The mean values obtained are used to calculate R^2, slope and Y-intercept (offset) values to calibrate the system’s pipetting.

The pipette tips required for use on the Extraction Platforms include 175µL non-conductive MPII sterile filter RoboRack tips, 1000µL conductive sterile Robotix tips and the fixed tips of the MPII itself. The 175µL tips are used for the purpose of removing reagents and the

transfer of DNA sample. The 1000µL tips were chosen for the purpose of removing supernatant and the fixed tips are used only for the addition of reagents. Pipetting performance was assessed for various volumes using the three different tips in order to calculate appropriate R^2, slope and Y-intercept (offset) values which were then added to the performance file. Values were calculated for both Blowout (single-liquid transfer) and Waste (multidispense) modes for the 1000µL tips, and Blowout mode only for the 175µL and fixed tips. A specialised performance file was created for the addition of resin. The performance file for the 175µL tips uses the already calibrated information of the Blowout mode, however the Blowout volume column values are set to 0, making it preform in a waste like mode. Retesting was performed to confirm accurate and precise pipetting with these settings.

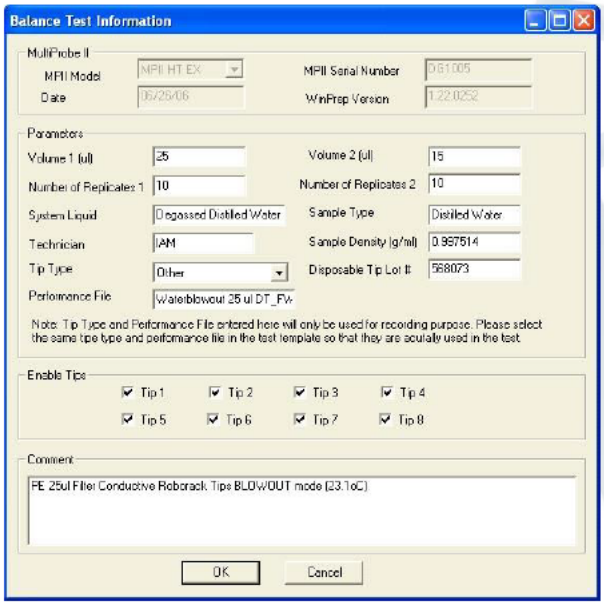


Figure 1 The Balance Test Information Window as present within the Balance Test DT program. All pipetting parameters are entered here and are subsequently transferred to the result output file.

All gravimetric testing was performed using the Balance Test DT test program within WinPrep®. Parameter values that needed to be entered into the Balance Test Information Window (Figure 1) included those as outlined in Table 1.

Table 1 Input values that are required for the various Balance Test Information parameters.

Parameter(s)	Value
Volume 1 and Volume 2	For 175µL tips: 175, 100, 50, 15µL For 1000µL tips: 1000, 700, 400, 100µL For Fixed tips: 1000, 700, 400, 100µL
Number of Replicates	10
System Liquid	Degassed Distilled Water
Sample Type	Distilled Water
Technician	Initials of the operator performing the test
Sample Density (g/ml)	The density of water at environmental temperature*
Tip Type	Other
Disposable Tip Lot #	The lot number of the particular tips in use

Performance File	The appropriate Performance File for the tip (175µL, 1000µL or fixed tips) and pipetting mode (Blowout or Waste) in use
Enable Tips (checkboxes)	Select the actual tips (1 to 8) to be tested
Comments	Free text box to add additional information (eg. Tip type, mode, current environmental room temperature, etc).

* Water density values were obtained from http://www.simetric.co.uk/si_water.htm

Pipetting accuracy and precision were examined at four different volumes for each tip size: 175, 100, 50, 15µL for 175µL tips and 1000, 700, 400, 100µL for the 1000µL and Fixed tips. In order to calculate unbiased values for each set of volumes, the slope and offset values in the relevant Performance File were changed to the default 1 and 0 respectively prior to testing. The mean volumes that were pipetted by each tip (10 replicates per tip) at the four designated volumes were used to generate a standard curve. The slope and offset calculated from this curve was used to calibrate the relevant Performance File. The final Performance File settings were then tested at the highest and lowest volumes (as per Table 1) to confirm accurate and precise pipetting. In the case of the specialised 175µL Blowout performance file, only high and low volumes are tested as it uses the settings from the original 175µL Blowout performance file.

5.2 Blood Collection

Blood samples were created by obtaining blood from 2 staff donors (DJC/VKI) by a phlebotomist as per normal in three 4mL EDTA vials.

5.3 Cell Collection

The cells used in this verification were collected using a cytobrush method where the donor used a cytobrush for one minute to “swab” the inside of one cheek. Once each cheek was swabbed, the cells on the brush were suspended in 2mL of 0.9% saline.

5.4 FTA cell Collection

Cells were collected from two staff donors (VKI/CJA) by using a “lolly-pop” swab to sample the inside of the donor’s cheek for 15 seconds before pressing the swab onto the FTA™ paper to transfer the DNA.

5.5 Automated DNA IQ™ Protocol

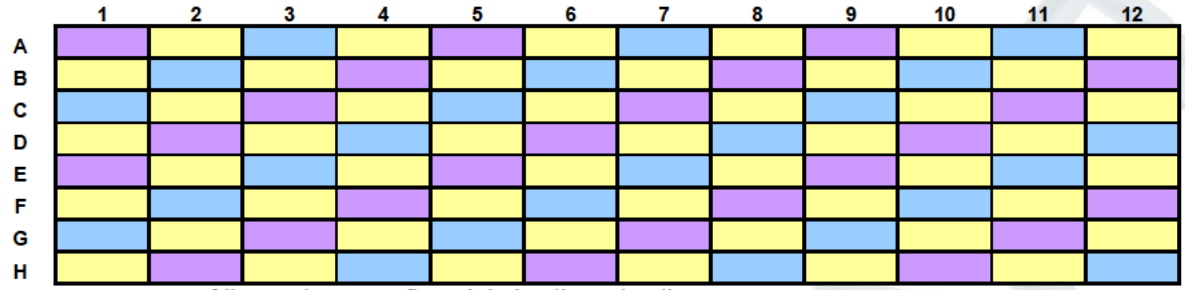
as

5.6 Contamination Check via Checkerboard and Zebra-stripe Patterns

Three sample types were chosen to create the contamination check plates: Blood FTA, Cell FTA and Blank FTA.
A small volume of collected blood was “clothed” (or spotted) onto Whatman FTA™ paper and allowed to dry overnight before sampling. Blank FTA samples were created using clean, unused FTA™ paper free from any kind of sample or reagent. Using the BSD 600 duet machine, two 3.2mm spots were punched directly into the SlicPrep 96 device in either the checkerboard or Zebra-stripe pattern (see figure 2a and 2b for a representation of the sample arrangement). One checkerboard and one Zebra-stripe plate was punched per Extraction platform.

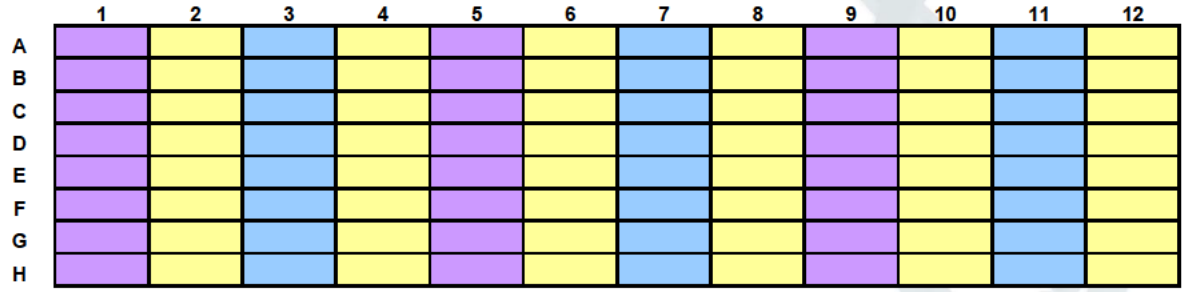
A total of five contamination plates were extracted between both extraction platforms. Three checkerboard and two Zebra-Stripe plates. It was noted during the creation of one of

the checkerboard plates that a spot "jumped" into an adjacent well (E3- Cells6). The presence of the spot was confirmed during the extraction.



a) Checkerboard Pattern

b) Zebra Stripe Pattern



Legend:

Figure 1. Checkerboard and zebra-stripe patterns utilised in the Extraction contamination check.

The Automated Extraction Setup MP II test program was compiled to replace the current manual Chelex extraction process for blood and cells performed by scientists in the Analytical section; Forensic Biology. The final Test Outline tree structure is presented below as Figure 3. Refer to Figure 4 for the deck layout.

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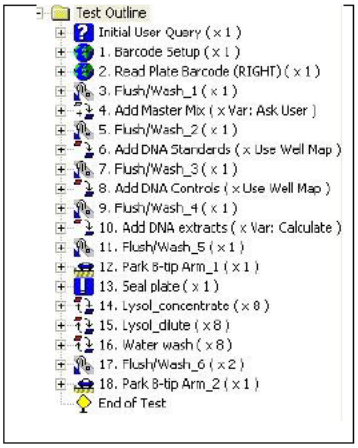

Figure 2 Test Outline for the automated setup of the Extraction program using the Extraction MP II's.

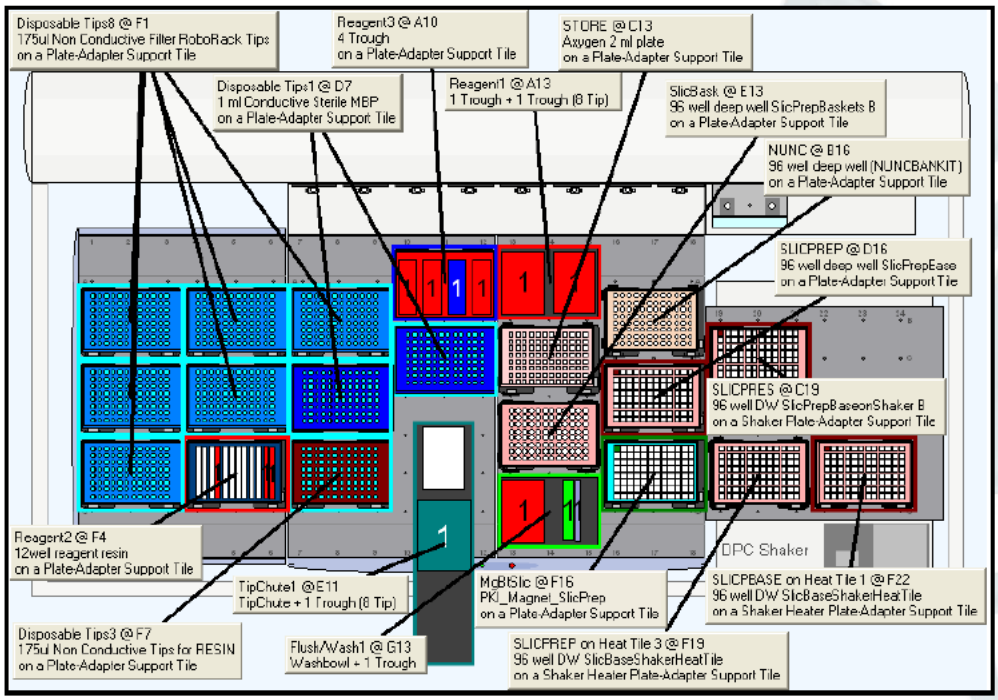

Figure 3 The WinPrep® virtual deck view displaying the necessary labware required to perform automated Extraction test file (Extraction Platform A deck view).

5.7 Heater tile temperature verification

Heat tiles supplied with MPII were modified to accept the SlicPrep 96 device. Temperature

5.8 Verification of the Automated Extraction Chemistry Protocol

Comparisons were made between results generated by the automated and manual methods to verify the performance of the automated Extraction Chemistry setup protocol.

Verification samples consisted of different dilutions of blood and cells spotted in 30µL aliquots onto quartered cotton and rayon swabs. Four blood dilutions of neat, 1/10, 1/100 and 1/1000 and four cell dilutions of neat, 1/5.2, 1/52.2 and 1/522 were used to test the sensitivity of both the manual and automated methods. Dilutions were created using neat sample and 0.9% saline for both sample types. Four replicates of each dilution were made up for each substrate and sample type.

The blood was collected using the same method as in 5.2. 4 separate extractions were performed for the manual set: Blood Rayon, Blood Cotton, Cell Rayon and Cell Cotton. For the automated verification, all sample types were extracted together after being transferred to a Slicprep 96 device for processing on the Extraction Platform B.

5.9 Resin Volume

- 7 µL
- 14 µL

5.10 Extraction volume

- 300 µL
- 350 µL
- 400 µL
- 450 µL
- 500 µL

5.11 Sensitivity

Fbot262

6. **Results and Discussion**

6.1 **Gravimetric Evaluation of Pipetting Accuracy and Precision**

6.2 **Contamination Check via Checkerboard and Zebra-stripe Patterns results**

Table 1 below lists the extraction batch id of the checkerboards and their outcomes.

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Table 2

Type of plate	Extraction batch Id	Extraction Platform	Check passed
Checkerboard-1	VALB20070817_02	Extraction A	Invalidated
Checkerboard-2	VALB20070803_02	Extraction B	Yes
Checkerboard/Zebra	VALB20071022_01	Extraction	Yes
Zebra-Stripe-1	VALB20070803_03	Extraction A	Yes
Zebra-Stripe-2	VALB20070817_03	Extraction B	Yes

Checkerboard1-
Position E3 (Sample Cells 6) was known to have been contaminated prior to start of the extraction. The DNA profile obtained confirmed this mixtures origin was formed from the expected donors. See figure X. Eight of the designated blank samples (Positions D3, A10, F1, H5, C4, E4, B7 and E6); two of the cell samples (A1 and B10) and two of the blood samples (F4 and G7) all exhibited a partial DNA profile previously unknown. This profile did not match any of the positive samples present on the batch. The profile was searched against the Staff database and no matches found.
None of the other blank samples yielded any DNA profile. The rest of the cell and blood samples yielded the correct DNA profile. Although there is no evidence of well to well contamination was observed; the unknown profile obtained has invalidated this plate. A further checkerboard/Zebra-Stripe combination plate was performed to ensure…

Table 3-Unknown profile obtained

Sample description	D3	vWA	FGA	Amel	D8	D21	D18	D5	D13	D7
Blk23-E6	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	11,13
Blk25-B7	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	
Blk15-E4	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	11,13
Blk14-C4	14,17									
Blk20-H5										
Blk3-F1										
Blk10-D3										
Blk37-A10										
Cells19-B10	14,17	14,17	20,21,22,24	X,Y	11,13,16	29,30,	14,15,16	11,15	11,12	11,11
Cells13-A1										
Blood14-G7										
Blood8-F4										

Checkerboard 2 –
None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile, see Figure 4.

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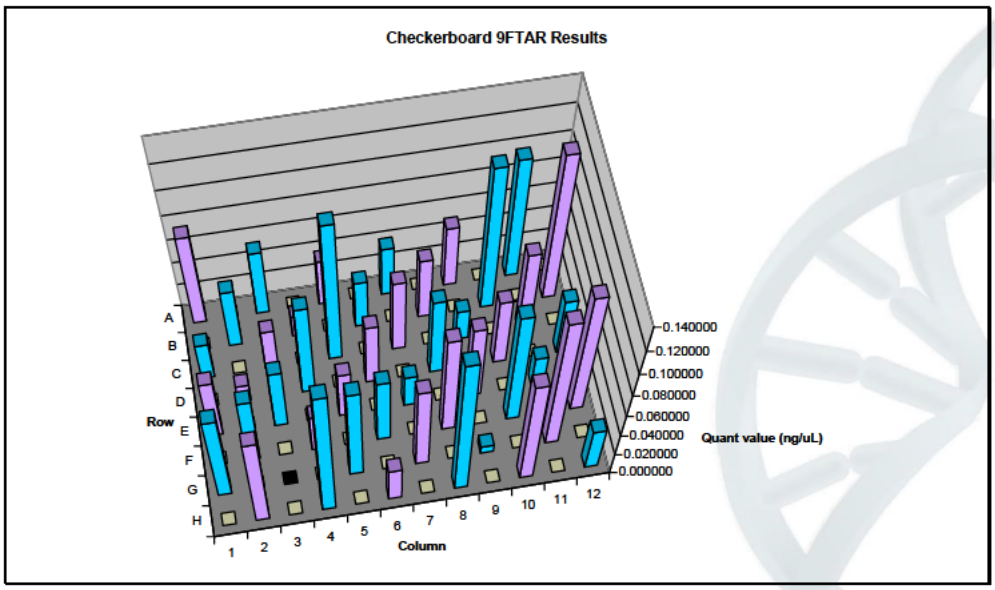

Figure 4

Zebra-Stripe1-
None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile, see Figure 5.

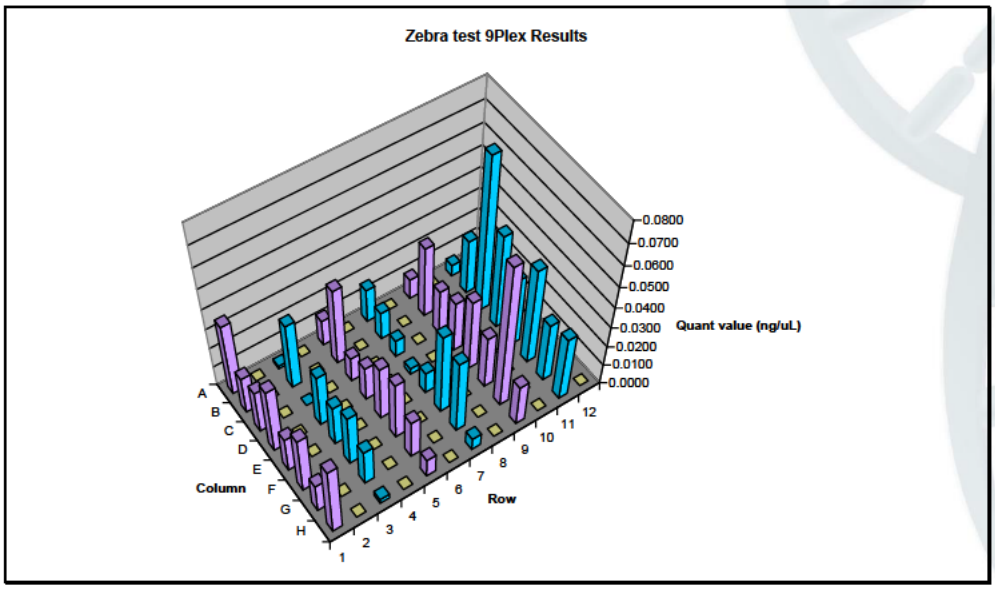

Figure 5

Zebra-Stripe 2-

None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile, see Figure 6.

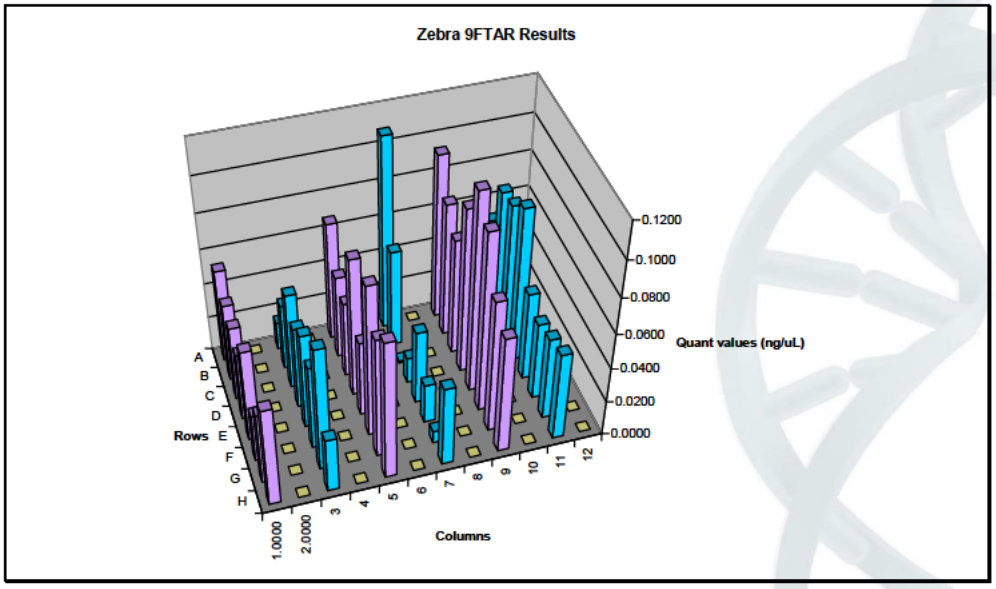

Figure 6

Checkerboard/Zebra -
None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile, see Figure 7.

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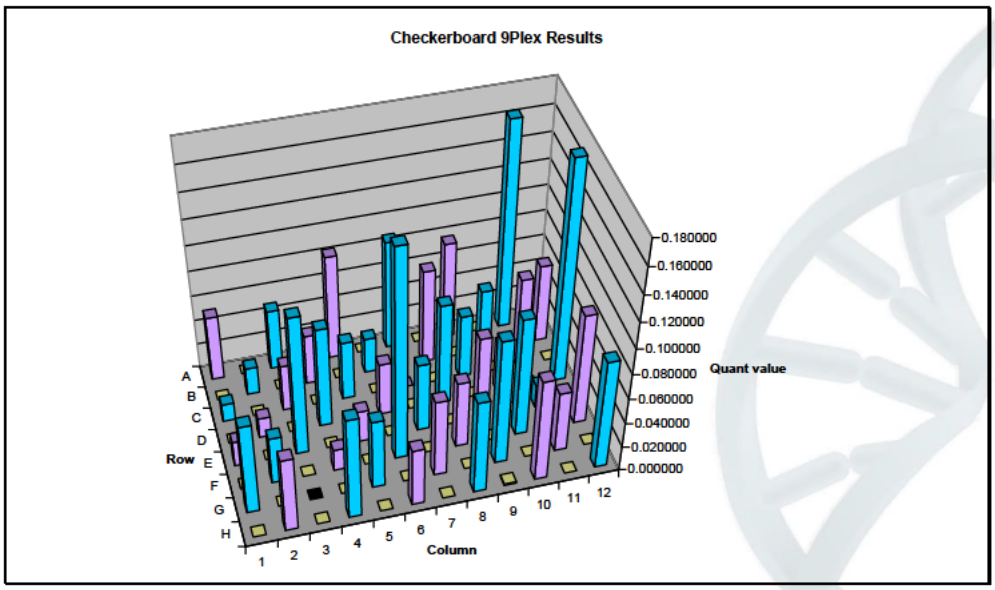

Figure 7

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9Plex

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.051900	0.000000	0.048300	0.000000	0.083700	0.000000	0.087000	0.000000	0.077300	0.000000	0.164000	0.000000
B	0.000000	0.021900	0.000000	0.040000	0.000000	0.028300	0.000000	0.075200	0.000000	0.050100	0.000000	0.062300
C	0.014100	0.000000	0.037100	0.000000	0.047100	0.000000	0.000000	0.000000	0.050400	0.000000	0.071700	0.000000
D	0.000000	0.015900	0.000000	0.079900	0.000000	0.042100	0.000000	0.080700	0.000000	0.039200	0.000000	0.174000
E	0.020200	0.000000	0.111000	0.000000	0.025300	0.000000	0.054600	0.000000	0.066700	0.000000	0.017900	0.000000
F	0.000761	0.037500	0.000000	0.017300	0.000000	0.166000	0.000000	0.053000	0.000000	0.094500	0.000778	0.088700
G	0.072000	0.000000	Removed	0.000000	0.055200	0.000000	0.061300	0.000000	0.099700	0.000000	0.048300	0.000000
H	0.000000	0.059400	0.000000	0.081300	0.000000	0.045800	0.000000	0.074700	0.001210	0.081400	0.000000	0.087200

9FTAR

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.076300	0.000000	0.054500	0.000000	0.038700	0.000000	0.041100	0.000000	0.050500	0.000000	0.098100	0.000000
B	0.000000	0.049700	0.000000	0.018200	0.000000	0.040200	0.000000	0.051000	0.000000	0.118000	0.001560	0.120000
C	0.032000	0.000000	0.035200	0.000000	0.118000	0.000000	0.061000	0.000000	0.026000	0.000000	0.067900	0.000000
D	0.000000	0.015300	0.000000	0.078200	0.000000	0.052400	0.000000	0.065900	0.000000	0.056400	0.000000	0.048000
E	0.052000	0.000000	0.051800	0.000000	0.040500	0.000000	0.027900	0.000000	0.062900	0.000000	0.026300	0.000000
F	0.000000	0.058600	0.000000	0.033700	0.000000	0.056600	0.000000	0.085700	0.000000	0.096500	0.000000	0.104000
G	0.076300	0.000000	Removed	0.000000	0.081300	0.000000	0.072600	0.000000	0.006630	0.000000	0.114000	0.000000
H	0.000000	0.081000	0.000000	0.114000	0.000000	0.030500	0.000000	0.122000	0.000000	0.092400	0.000000	0.037500

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	9Plex											
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0358	0.0000	0.001440	0.000000	0.012800	0.000000	0.016500	0.000000	0.009840	0.000000	0.005540	0.000000
B	0.0184	0.0000	0.032800	0.000000	0.037700	0.000000	0.013700	0.000000	0.034600	0.000000	0.026500	0.000000
C	0.0212	0.0000	0.000000	0.000000	0.012100	0.000000	0.008350	0.000000	0.021700	0.000000	0.074200	0.000000
D	0.0324	0.0000	0.025900	0.000000	0.016900	0.000000	0.003010	0.000000	0.024800	0.000000	0.046000	0.000000
E	0.0175	0.0000	0.020000	0.000000	0.026700	0.000000	0.010600	0.000000	0.034700	0.000000	0.033300	0.000000
F	0.0282	0.0000	0.025400	0.000000	0.028700	0.000000	0.039800	0.000000	0.025700	0.000000	0.047100	0.000000
G	0.0144	0.0000	0.017800	0.000000	0.019100	0.000000	0.036200	0.000000	0.069900	0.000000	0.029200	0.000000
H	0.0348	0.0000	0.002230	0.000000	0.009310	0.000000	0.006010	0.000000	0.020200	0.000000	0.031600	0.000000
	9FTAR											
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0534	0.0000	0.015700	0.000000	0.067500	0.000000	0.109000	0.000000	0.093500	0.000000	0.051000	0.009600
B	0.0444	0.0000	0.037600	0.000000	0.047300	0.000000	0.055500	0.000000	0.076100	0.000000	0.076800	0.000000
C	0.0426	0.0000	0.054800	0.000000	0.043100	0.000000	0.001140	0.000000	0.066200	0.000000	0.079300	0.000000
D	0.0386	0.0000	0.046600	0.000000	0.079200	0.000000	0.014700	0.000000	0.093700	0.000000	0.087900	0.000000
E	0.0525	0.0000	0.054500	0.000000	0.043200	0.000000	0.042000	0.000000	0.113000	0.000000	0.050500	0.000000
F	0.0273	0.0000	0.048100	0.000000	0.086800	0.000000	0.022500	0.000000	0.102000	0.000000	0.044300	0.000000
G	0.0408	0.0000	0.070800	0.000000	0.068900	0.000000	0.006870	0.000000	0.075700	0.000000	0.047300	0.000000
H	0.0566	0.0000	0.031100	0.000000	0.079300	0.000000	0.045600	0.000000	0.066900	0.000000	0.050300	0.000000

6.3 **Verification of the Automated Extraction Protocol**

7. Summary and Recommendations

We recommend the following:

- Use of MPII for automated extraction of reference samples
- Use of MPII for automated extraction of casework samples
- Ongoing development of the automated extraction program to increase the efficiency of the extraction

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Project 13. Report on the Verification of an Automated DNA IQ™ Protocol using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform

Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C., Ientile, V.
Automation/LIMS Implementation Project, DNA Analysis FSS (August 2008)

1. Abstract

A manual method for extracting DNA from forensic samples using the DNA IQ™ system (Promega Corp., Madison, WI, USA) was validated for routine use in DNA Analysis (FSS). We have verified an automated DNA IQ™ protocol in 96-well format for use on the MultiPROBE® II PLUS HT EX FORENSIC WORKSTATION platforms (PerkinElmer, Downers Grove, IL, USA). Data indicate that results from the automated procedure are comparable to those from the manual procedure. Contamination checks were performed using samples prepared in checkerboard and zebra-stripe format, and results were as expected. We recommend the use of the MultiPROBE® II PLUS HT EX platforms to perform automated DNA extraction using the DNA IQ™ system.

2. Introduction

The MultiPROBE® II PLUS HT EX FORENSIC WORKSTATION platforms (PerkinElmer, Downers Grove, IL, USA) are equipped to perform automated DNA extractions, as they include a DPC shaker and individual heat controllers to enable on-board lysis and incubation steps. Currently in DNA Analysis, the MultiPROBE® platforms allow walk-away operation of PCR setup protocols for DNA quantitation and amplification.

The DNA IQ™ protocol has been verified or validated by various laboratories for use on the MultiPROBE® II PLUS platform. The laboratories that perform an automated DNA IQ™ protocol include PathWest (Western Australia), Forensic Science South Australia (South Australia) and Centre of Forensic Sciences in Toronto (Ontario). The MultiPROBE® II PLUS instrument comes pre-loaded with an automated DNA IQ™ protocol. Unlike the other laboratories, however, we did not validate the included protocol, but instead validated a manual DNA IQ™ protocol which was based on the CFS automated protocol (PerkinElmer, 2004), followed by verification of an automated protocol based on the validated manual method.

The verified automated DNA IQ™ protocol is identical to the validated manual protocol used in-house: there are no differences in reagents or volumes. The adopted DNA IQ™ protocol differs slightly, however, from the manufacturer's protocol, as it includes a lysis step using Extraction Buffer (10mM Tris, 1mM EDTA, 100mM NaCl, 20% SDS) in the presence of Proteinase K, before incubating in the DNA IQ™ Lysis Buffer. Furthermore, the lysis incubation conditions were lowered from 70°C to 37°C in order to accommodate extraction of DNA from heat labile materials such as nylon and polyester. In addition, the automated protocol utilises the SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA) for simultaneous processing of samples in a 96-well format.

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3. Aim

To verify an automated DNA IQ™ protocol for use on the MultiPROBE® II PLUS HT EX platforms to allow extraction of DNA from various sample types.

4. Equipment and Materials

- MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (PerkinElmer, Downers Grove, IL, USA)
- Gravimetric Performance Evaluation Option with Mettler SAG285/L balance (Mettler-Toledo, Greifensee, Switzerland)
- DNA IQ™ System (Promega Corp., Madison, WI, USA)
- Extraction Buffer (10mM Tris, 1mM EDTA, 100mM NaCl, 20% SDS)
- SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA)
- Nunc™ Bank-It tubes (Nunc A/S, Roskilde, Denmark)
- 175µL non-conductive sterile filter RoboRack tips (PerkinElmer, Downers Grove, IL, USA)
- 1000µL Conductive sterile filter Robotix tips (Molecular BioProducts, San Diego, CA, USA)
- ABI Prism® 7000 SDS (Applied Biosystems, Foster City, CA, USA)
- Quantifiler™ Human DNA Quantification kits (Applied Biosystems, Foster City, CA, USA)
- AmpFℓSTR® Profiler Plus Amplification kits (Applied Biosystems, Foster City, CA, USA)
- GeneAmp® 9700 thermalcycers (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 96-well optical reaction plates (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA)
- GeneScan™ 500 ROX™ Size Standard (Applied Biosystems, Foster City, CA, USA)
- Hi-Di™ Formamide (Applied Biosystems, Foster City, CA, USA)
- 3100 POP-4™ Polymer (Applied Biosystems, Foster City, CA, USA)
- For mock samples:
  - FTA™® Classic Card (Whatman Inc., Florham Park, NJ, USA)
  - Sterile cotton swabs (Medical Wire & Equipment, Corsham, Wiltshire, England)
  - Sterile rayon swabs (Copan Italia SPA, Brescia, Italy)

5. Methods

5.1 Gravimetric Evaluation of Pipetting Accuracy and Precision

The Gravimetric Performance Evaluation Option with Mettler SAG285/L balance (Mettler-Toledo, Greifensee, Switzerland), hereby referred to as the “gravimetric kit”, was purchased from PerkinElmer (PN 7601823) as an optional accessory upon recommendations made by Forensic Science South Australia staff members. The gravimetric kit consists of a SAG285/L balance and various accessories including a humidified chamber and software for integration with the WinPrep® software. All components were installed by a qualified PerkinElmer service engineer, and subsequently calibrated by a Mettler-Toledo engineer for NATA certification. A NATA certificate for the kit (Report Number Q0737-001-1) was produced on 13 March 2006.

Gravimetric analysis is performed by placing the balance on the platform deck and instructing the MP II to repeatedly pipette certain volumes of system liquid onto the balance pan. Readings are taken automatically by the software and compiled into a results table, which is then used to automatically generate an Excel-based results chart containing mean, %CV and %inaccuracy values. The mean values obtained are used to calculate R^2, slope and Y-intercept (offset) values to calibrate the system’s pipetting.

The pipette tips required for use on the Extraction Platforms include 175µL non-conductive MPII sterile filter RoboRack tips, 1000µL conductive sterile Robotix tips and the fixed tips of the MPII itself. The 175µL tips are used for the purpose of removing reagents and the

transfer of DNA sample. The 1000µL tips were chosen for the purpose of removing supernatant and the fixed tips are used only for the addition of reagents. Pipetting performance was assessed for various volumes using the three different tips in order to calculate appropriate R^2, slope and Y-intercept (offset) values which were then added to the performance file. Values were calculated for both Blowout (single-liquid transfer) and Waste (multidispense) modes for the 1000µL tips, and Blowout mode only for the 175µL and fixed tips.

For the addition of resin, a specialised performance file was created based on the performance file for 175µL tips in blowout mode, except the "Blowout Volume" column values are set to 0 to allow pipetting similar to waste mode. Retesting was performed to confirm accurate and precise pipetting with these settings.

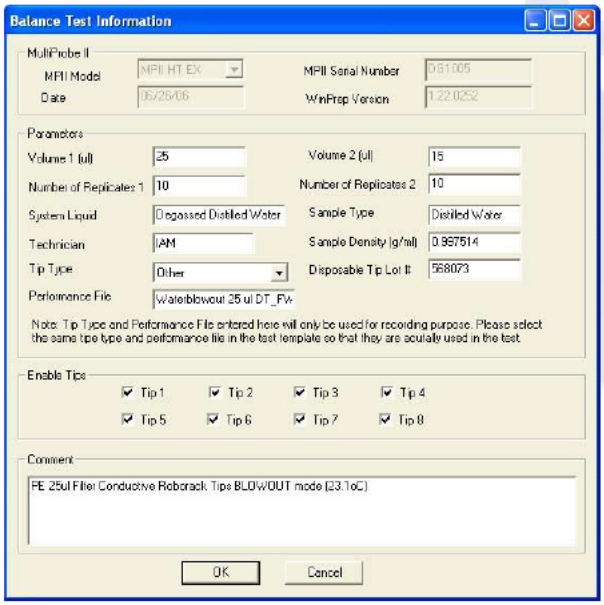

Figure 1. The Balance Test Information Window as present within the Balance Test DT program. All pipetting parameters are entered here and are subsequently transferred to the result output file.

All gravimetric testing was performed using the Balance Test DT test program within WinPrep®. Parameter values that needed to be entered into the Balance Test Information Window (Figure 1) included those as outlined in Table 1.

Table 1 Input values that are required for the various Balance Test Information parameters.

Parameter(s)	Value
Volume 1 and Volume 2	For 175µL tips: 175, 100, 50, 15µL For 1000µL tips: 1000, 700, 400, 100µL

	For Fixed tips: 1000, 700, 400, 100µL
Number of Replicates	10
System Liquid	Degassed Distilled Water
Sample Type	Distilled Water
Technician	Initials of the operator performing the test
Sample Density (g/ml)	The density of water at environmental temperature*
Tip Type	Other
Disposable Tip Lot #	The lot number of the particular tips in use
Performance File	The appropriate Performance File for the tip (175µL, 1000µL or fixed tips) and pipetting mode (Blowout or Waste) in use
Enable Tips (checkboxes)	Select the actual tips (1 to 8) to be tested
Comments	Free text box to add additional information (eg. Tip type, mode, current environmental room temperature, etc).

* Water density values were obtained from http://www.simetric.co.uk/si_water.htm

Pipetting accuracy and precision were examined at four different volumes for each tip size: 175, 100, 50, 15µL for 175µL tips and 1000, 700, 400, 100µL for the 1000µL and Fixed tips. In order to calculate unbiased values for each set of volumes, the slope and offset values in the relevant Performance File were changed to the default 1 and 0 respectively prior to testing. The mean volumes that were pipetted by each tip (10 replicates per tip) at the four designated volumes were used to generate a standard curve. The slope and offset calculated from this curve was used to calibrate the relevant Performance File. The final Performance File settings were then tested at the highest and lowest volumes (as per Table 1) to confirm accurate and precise pipetting. In the case of the specialised 175µL Blowout performance file, only high and low volumes are tested as it uses the settings from the original 175µL Blowout performance file.

5.2 Blood Collection

Blood samples were created by obtaining blood from 2 staff donors (DJC/VKI) by a phlebotomist as per normal in three 4mL EDTA vials.

5.3 Cell Collection

The cells used in this verification were collected using a cytobrush method where the donor used a cytobrush for one minute to “swab” the inside of one cheek. Once each cheek was swabbed, the cells on the brush were suspended in 2mL of 0.9% saline.

5.4 FTA cell Collection

Cells were collected from two staff donors (VKI/CJA) by using a “lolly-pop” swab to sample the inside of the donor’s cheek for 15 seconds before pressing the swab onto the FTA™ paper to transfer the DNA.

5.5 Automated DNA IQ™ Protocol

The automated DNA IQ™ protocol, based on the validated manual method (refer to Project 11), was programmed in WinPrep™ software. The final, optimised protocol was named “DNA IQ Extraction_Ver1.1.mpt”. A screenshot of the Test Outline window for this protocol is depicted below in Figure 2. The deck layout is illustrated in Figure 3.

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Figure 2. The Test Outline window displaying individual nodes within the DNA IQ Extraction_Ver1.1.mpt program test file.

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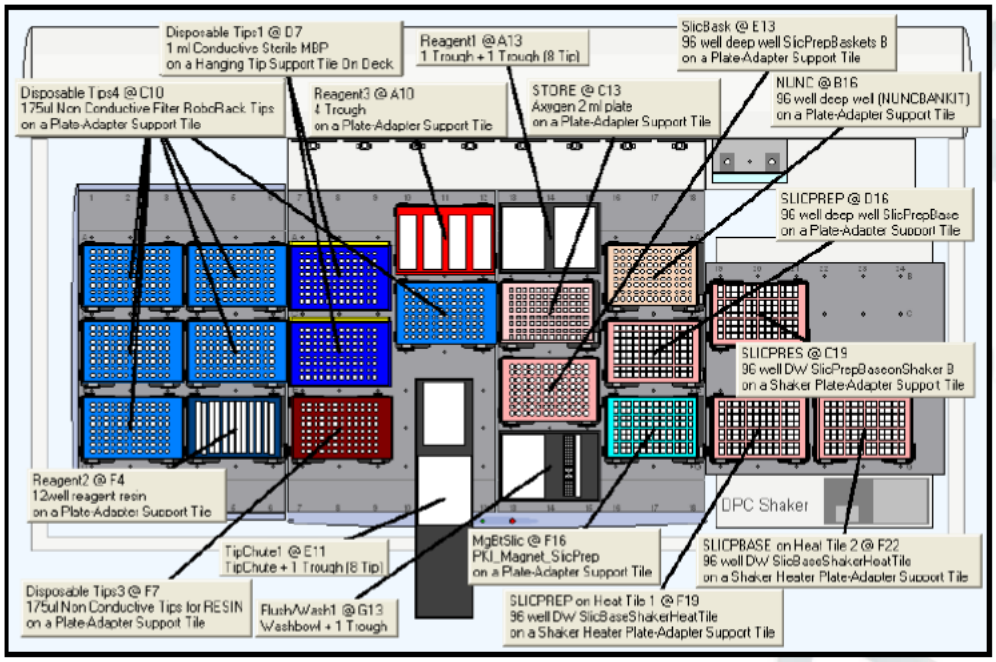

Figure 3. The deck layout for DNA IQ Extraction_Ver1.1.mpt, displaying the required labware on the platform deck.

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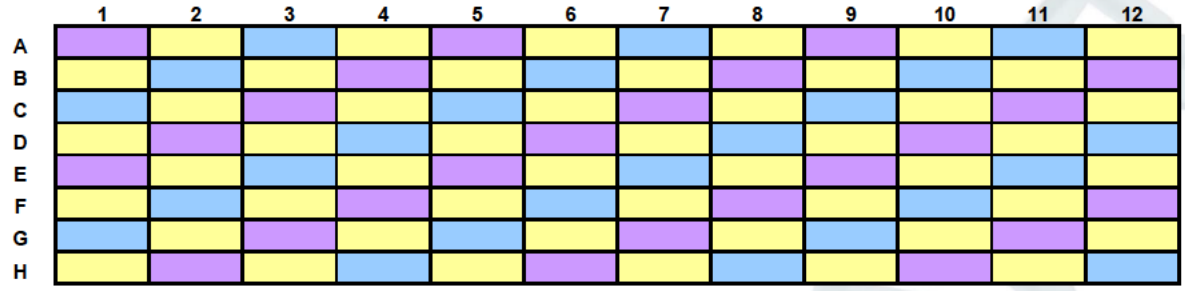
5.6 Contamination Check via Checkerboard and Zebra-stripe Patterns

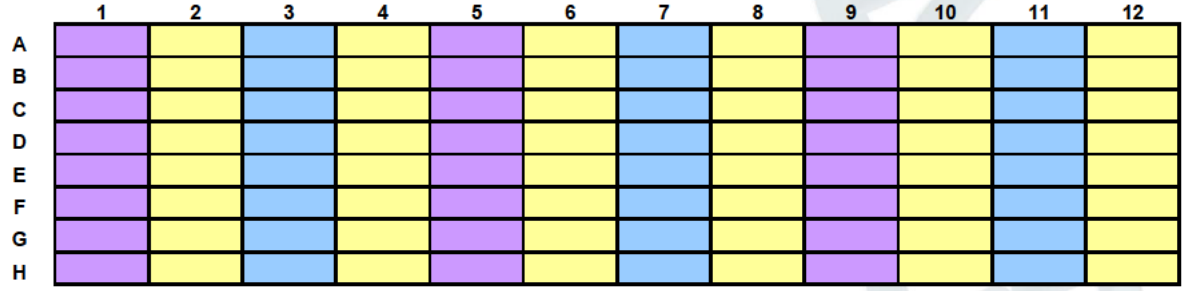
Three sample types were chosen to create the contamination check plates: Blood FTA, Cell FTA and Blank FTA.
A small volume of collected blood was “clothed” (or spotted) onto Whatman FTA™ paper and allowed to dry overnight before sampling. Blank FTA samples were created using clean, unused FTA™ paper free from any kind of sample or reagent. Using the BSD 600 duet machine, two 3.2mm spots were punched directly into the SlicPrep 96 device in either the checkerboard or Zebra-stripe pattern (see figure 2a and 2b for a representation of the sample arrangement). One checkerboard and one Zebra-stripe plate was punched per Extraction platform.

A total of five contamination plates were extracted between both extraction platforms. Three checkerboard and two Zebra-Stripe plates. It was noted during the creation of one of the checkerboard plates that a spot “jumped” into an adjacent well (E3- Cells6). The presence of the spot was confirmed during the extraction.

a) Checkerboard Pattern



b) Zebra Stripe Pattern



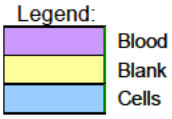


Figure 1. Checkerboard and zebra-stripe patterns utilised in the Extraction contamination check.

The Automated Extraction Setup MP II test program was compiled to replace the current manual Chelex extraction process for blood and cells performed by scientists in the Analytical section; Forensic Biology. The final Test Outline tree structure is presented below as Figure 3. Refer to Figure 4 for the deck layout.

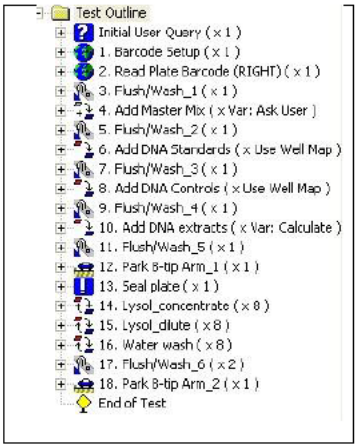


Figure 2 Test Outline for the automated setup of the Extraction program using the Extraction MP II's.

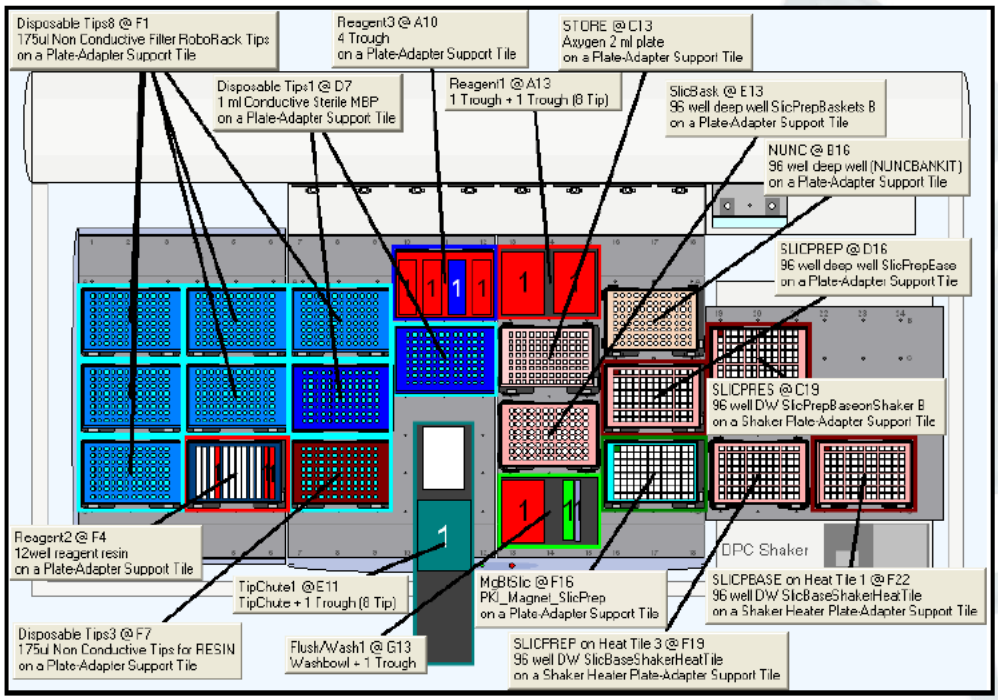


Figure 3 The WinPrep® virtual deck view displaying the necessary labware required to perform automated Extraction test file (Extraction Platform A deck view).

5.7 Heater tile temperature verification

Heat tiles supplied with MPII were modified to accept the SlicPrep 96 device. Temperature

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5.8 Verification of the Automated Extraction Chemistry Protocol

Comparisons were made between results generated by the automated and manual methods to verify the performance of the automated Extraction Chemistry setup protocol.

Verification samples consisted of different dilutions of blood and cells spotted in 30µL aliquots onto quartered cotton and rayon swabs. Four blood dilutions of neat, 1/10, 1/100 and 1/1000 and four cell dilutions of neat, 1/5.2, 1/52.2 and 1/522 were used to test the sensitivity of both the manual and automated methods. Dilutions were created using neat sample and 0.9% saline for both sample types. Four replicates of each dilution were made up for each substrate and sample type.

The blood was collected using the same method as in 5.2. 4 separate extractions were performed for the manual set: Blood Rayon, Blood Cotton, Cell Rayon and Cell Cotton. For the automated verification, all sample types were extracted together after being transferred to a Slicprep 96 device for processing on the Extraction Platform B.

5.9 Resin Volume

- 7 µL
- 14 µL

5.10 Extraction volume

- 300 µL
- 350 µL
- 400 µL
- 450 µL
- 500 µL

5.11 Sensitivity

Fbot262

6. Results and Discussion

6.1 Gravimetric Evaluation of Pipetting Accuracy and Precision

EXTN A:
WaterBlowoutFixedTips_08112007.prf
WaterBlowout 175 ul DT_FW 13112007RESIN.prf
WaterWasteFT_1 ml_27082007.prf
WaterBlowout 1ml DT _QHSS09112007.prf
WaterBlowout 175 ul DT_FW QHSS 13112007.prf
WaterWaste 1 ml DT_FW QHSS12112007.prf

EXTN B:
WaterBlowout 1ml DT_QHSS23102007.prf
WaterBlowoutFixed Tips_1ml_26102007.prf
WaterWaste_1ml_27082007.prf

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WaterBlowout 175ul DT_FW 25102007.prf
WaterWaste 1ml DT_FW 24102007.prf
WaterBlowout 175 ul DT_FW 25102007RESIN.prf
WaterBlowout 25ul DT_FW_QHSS_060907.prf

6.2 **Contamination Check via Checkerboard and Zebra-stripe Patterns results**

Table 1 below lists the extraction batch id of the checkerboards and their outcomes.

Table 2

Type of plate	Extraction batch Id	Extraction Platform	Check passed
Checkerboard-1	VALB20070817_02	Extraction A	Invalidated
Checkerboard-2	VALB20070803_02	Extraction B	Yes
Checkerboard/Zebra	VALB20071022_01	Extraction	Yes
Zebra-Stripe-1	VALB20070803_03	Extraction A	Yes
Zebra-Stripe-2	VALB20070817_03	Extraction B	Yes

Checkerboard1-
Position E3 (Sample Cells 6) was known to have been contaminated prior to start of the extraction. The DNA profile obtained confirmed this mixtures origin was formed from the expected donors. See figure X. Eight of the designated blank samples (Positions D3, A10, F1, H5, C4, E4, B7 and E6); two of the cell samples (A1 and B10) and two of the blood samples (F4 and G7) all exhibited a partial DNA profile previously unknown. This profile did not match any of the positive samples present on the batch. The profile was searched against the Staff database and no matches found.
None of the other blank samples yielded any DNA profile. The rest of the cell and blood samples yielded the correct DNA profile. Although there is no evidence of well to well contamination was observed; the unknown profile obtained has invalidated this plate. A further checkerboard/Zebra-Stripe combination plate was performed to ensure…

Table 3-Unknown profile obtained

Sample description	D3	vWA	FGA	Amel	D8	D21	D18	D5	D13	D7
Blk23-E6	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	11,13
Blk25-B7	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	
Blk15-E4	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	11,13
Blk14-C4	14,17									
Blk20-H5										
Blk3-F1										
Blk10-D3										
Blk37-A10										
Cells19-B10	14,17	14,17	20,21,22,24	X,Y	11,13,16	29,30,	14,15,16	11,15	11,12	11,11
Cells13-A1										
Blood14-G7										
Blood8-F4										

Checkerboard 2 –

None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile, see Figure 4.

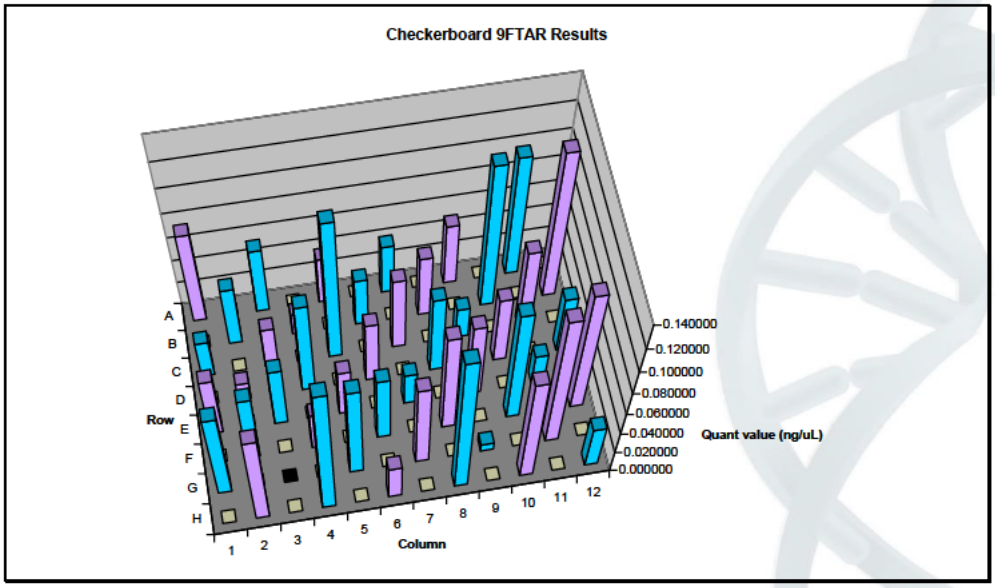

Figure 4

Zebra-Stripe1-
None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile, see Figure 5.

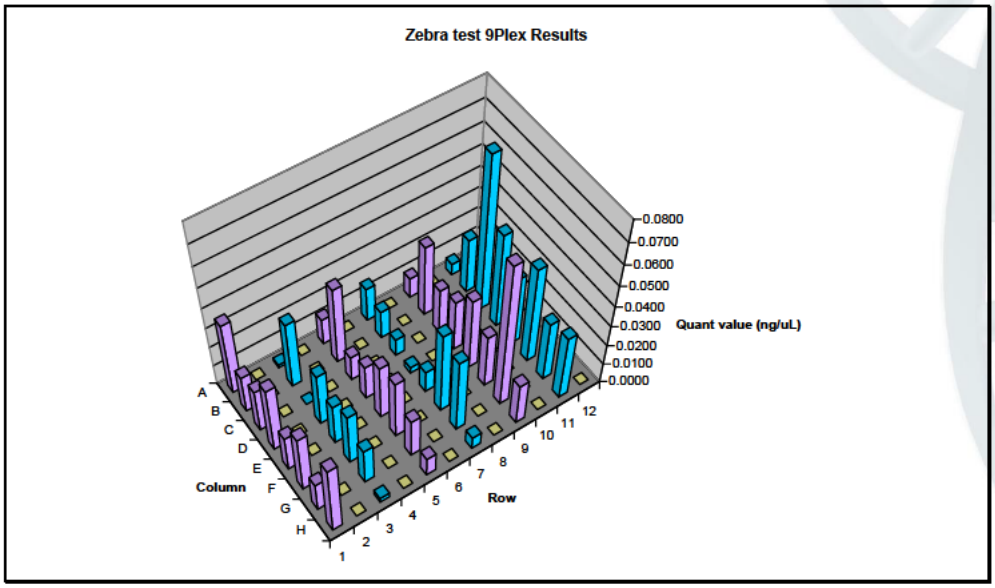

Figure 5

Zebra-Stripe 2-
None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile, see Figure 6.

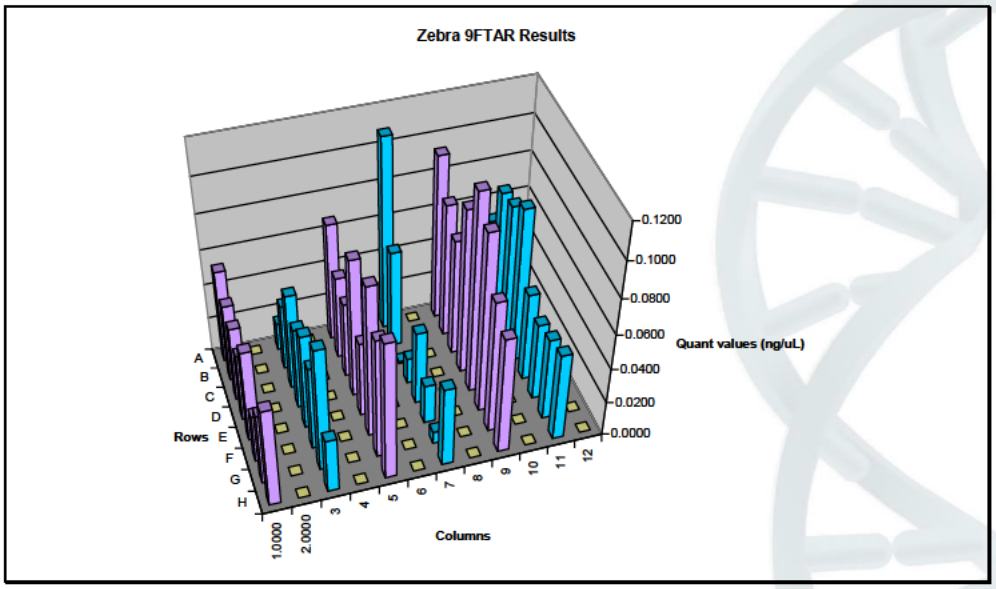

Figure 6

Checkerboard/Zebra -
None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile, see Figure 7.

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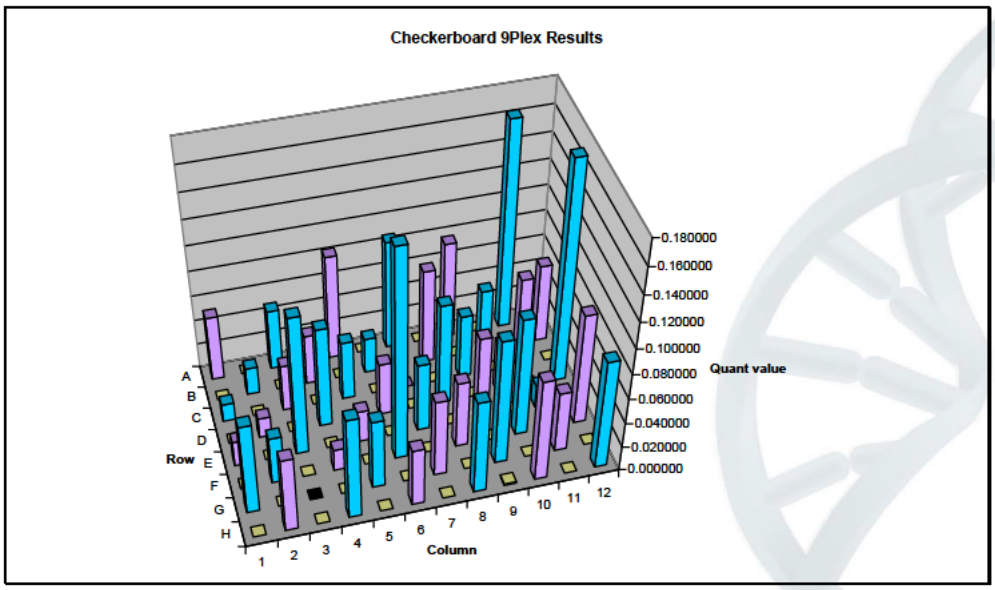

Figure 7

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9Plex

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.051900	0.000000	0.048300	0.000000	0.083700	0.000000	0.087000	0.000000	0.077300	0.000000	0.164000	0.000000
B	0.000000	0.021900	0.000000	0.040000	0.000000	0.028300	0.000000	0.075200	0.000000	0.050100	0.000000	0.062300
C	0.014100	0.000000	0.037100	0.000000	0.047100	0.000000	0.000000	0.000000	0.050400	0.000000	0.071700	0.000000
D	0.000000	0.015900	0.000000	0.079900	0.000000	0.042100	0.000000	0.080700	0.000000	0.039200	0.000000	0.174000
E	0.020200	0.000000	0.111000	0.000000	0.025300	0.000000	0.054600	0.000000	0.066700	0.000000	0.017900	0.000000
F	0.000761	0.037500	0.000000	0.017300	0.000000	0.166000	0.000000	0.053000	0.000000	0.094500	0.000778	0.088700
G	0.072000	0.000000	Removed	0.000000	0.055200	0.000000	0.061300	0.000000	0.099700	0.000000	0.048300	0.000000
H	0.000000	0.059400	0.000000	0.081300	0.000000	0.045800	0.000000	0.074700	0.001210	0.081400	0.000000	0.087200

9FTAR

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.076300	0.000000	0.054500	0.000000	0.038700	0.000000	0.041100	0.000000	0.050500	0.000000	0.098100	0.000000
B	0.000000	0.049700	0.000000	0.018200	0.000000	0.040200	0.000000	0.051000	0.000000	0.118000	0.001560	0.120000
C	0.032000	0.000000	0.035200	0.000000	0.118000	0.000000	0.061000	0.000000	0.026000	0.000000	0.067900	0.000000
D	0.000000	0.015300	0.000000	0.078200	0.000000	0.052400	0.000000	0.065900	0.000000	0.056400	0.000000	0.048000
E	0.052000	0.000000	0.051800	0.000000	0.040500	0.000000	0.027900	0.000000	0.062900	0.000000	0.026300	0.000000
F	0.000000	0.058600	0.000000	0.033700	0.000000	0.056600	0.000000	0.085700	0.000000	0.096500	0.000000	0.104000
G	0.076300	0.000000	Removed	0.000000	0.081300	0.000000	0.072600	0.000000	0.006630	0.000000	0.114000	0.000000
H	0.000000	0.081000	0.000000	0.114000	0.000000	0.030500	0.000000	0.122000	0.000000	0.092400	0.000000	0.037500

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	9Plex											
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0358	0.0000	0.001440	0.000000	0.012800	0.000000	0.016500	0.000000	0.009840	0.000000	0.005540	0.000000
B	0.0184	0.0000	0.032800	0.000000	0.037700	0.000000	0.013700	0.000000	0.034600	0.000000	0.026500	0.000000
C	0.0212	0.0000	0.000000	0.000000	0.012100	0.000000	0.008350	0.000000	0.021700	0.000000	0.074200	0.000000
D	0.0324	0.0000	0.025900	0.000000	0.016900	0.000000	0.003010	0.000000	0.024800	0.000000	0.046000	0.000000
E	0.0175	0.0000	0.020000	0.000000	0.026700	0.000000	0.010600	0.000000	0.034700	0.000000	0.033300	0.000000
F	0.0282	0.0000	0.025400	0.000000	0.028700	0.000000	0.039800	0.000000	0.025700	0.000000	0.047100	0.000000
G	0.0144	0.0000	0.017800	0.000000	0.019100	0.000000	0.036200	0.000000	0.069900	0.000000	0.029200	0.000000
H	0.0348	0.0000	0.002230	0.000000	0.009310	0.000000	0.006010	0.000000	0.020200	0.000000	0.031600	0.000000
	9FTAR											
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0534	0.0000	0.015700	0.000000	0.067500	0.000000	0.109000	0.000000	0.093500	0.000000	0.051000	0.009600
B	0.0444	0.0000	0.037600	0.000000	0.047300	0.000000	0.055500	0.000000	0.076100	0.000000	0.076800	0.000000
C	0.0426	0.0000	0.054800	0.000000	0.043100	0.000000	0.001140	0.000000	0.066200	0.000000	0.079300	0.000000
D	0.0386	0.0000	0.046600	0.000000	0.079200	0.000000	0.014700	0.000000	0.093700	0.000000	0.087900	0.000000
E	0.0525	0.0000	0.054500	0.000000	0.043200	0.000000	0.042000	0.000000	0.113000	0.000000	0.050500	0.000000
F	0.0273	0.0000	0.048100	0.000000	0.086800	0.000000	0.022500	0.000000	0.102000	0.000000	0.044300	0.000000
G	0.0408	0.0000	0.070800	0.000000	0.068900	0.000000	0.006870	0.000000	0.075700	0.000000	0.047300	0.000000
H	0.0566	0.0000	0.031100	0.000000	0.079300	0.000000	0.045600	0.000000	0.066900	0.000000	0.050300	0.000000

6.3 **Verification of the Automated Extraction Protocol**

7. Summary and Recommendations

We recommend the following:

- Use of MPII for automated extraction of reference samples
- Use of MPII for automated extraction of casework samples
- Ongoing development of the automated extraction program to increase the efficiency of the extraction

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Project 13. Report on the Verification of an Automated DNA IQ™ Protocol using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform

Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C., Ientile, V.
Automation/LIMS Implementation Project, DNA Analysis FSS (August 2008)

1. Abstract

A manual method for extracting DNA from forensic samples using the DNA IQ™ system (Promega Corp., Madison, WI, USA) was validated for routine use in DNA Analysis (FSS). We have verified an automated DNA IQ™ protocol in 96-well format for use on the MultiPROBE® II PLUS HT EX FORENSIC WORKSTATION platforms (PerkinElmer, Downers Grove, IL, USA). Data indicate that results from the automated procedure are comparable to those from the manual procedure. Contamination checks were performed using samples prepared in checkerboard and zebra-stripe format, and results were as expected. We recommend the use of the MultiPROBE® II PLUS HT EX platforms to perform automated DNA extraction using the DNA IQ™ system.

2. Introduction

The MultiPROBE® II PLUS HT EX FORENSIC WORKSTATION platforms (PerkinElmer, Downers Grove, IL, USA) are equipped to perform automated DNA extractions, as they include a DPC shaker and individual heat controllers to enable on-board lysis and incubation steps. Currently in DNA Analysis, the MultiPROBE® platforms allow walk-away operation of PCR setup protocols for DNA quantitation and amplification.

The DNA IQ™ protocol has been verified or validated by various laboratories for use on the MultiPROBE® II PLUS platform. The laboratories that perform an automated DNA IQ™ protocol include PathWest (Western Australia), Forensic Science South Australia (South Australia) and Centre of Forensic Sciences in Toronto (Ontario). The MultiPROBE® II PLUS instrument comes pre-loaded with an automated DNA IQ™ protocol. Unlike the other laboratories, however, we did not validate the included protocol, but instead validated a manual DNA IQ™ protocol which was based on the CFS automated protocol (PerkinElmer, 2004), followed by verification of an automated protocol based on the validated manual method.

The verified automated DNA IQ™ protocol is identical to the validated manual protocol used in-house: there are no differences in reagents or volumes. The adopted DNA IQ™ protocol differs slightly, however, from the manufacturer's protocol, as it includes a lysis step using Extraction Buffer (10mM Tris, 1mM EDTA, 100mM NaCl, 20% SDS) in the presence of Proteinase K, before incubating in the DNA IQ™ Lysis Buffer. Furthermore, the lysis incubation conditions were lowered from 70°C to 37°C in order to accommodate extraction of DNA from heat labile materials such as nylon and polyester. In addition, the automated protocol utilises the SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA) for simultaneous processing of samples in a 96-well format.

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3. Aim

To verify an automated DNA IQ™ protocol for use on the MultiPROBE® II PLUS HT EX platforms to allow extraction of DNA from various sample types.

4. Equipment and Materials

- MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (PerkinElmer, Downers Grove, IL, USA)
- Gravimetric Performance Evaluation Option with Mettler SAG285/L balance (Mettler-Toledo, Greifensee, Switzerland)
- DNA IQ™ System (Promega Corp., Madison, WI, USA)
- Extraction Buffer (10mM Tris, 1mM EDTA, 100mM NaCl, 20% SDS)
- SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA)
- Nunc™ Bank-It tubes (Nunc A/S, Roskilde, Denmark)
- 175µL non-conductive sterile filter RoboRack tips (PerkinElmer, Downers Grove, IL, USA)
- 1000µL Conductive sterile filter Robotix tips (Molecular BioProducts, San Diego, CA, USA)
- ABI Prism® 7000 SDS (Applied Biosystems, Foster City, CA, USA)
- Quantifiler™ Human DNA Quantification kits (Applied Biosystems, Foster City, CA, USA)
- AmpFℓSTR® Profiler Plus Amplification kits (Applied Biosystems, Foster City, CA, USA)
- GeneAmp® 9700 thermalcycers (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 96-well optical reaction plates (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA)
- GeneScan™ 500 ROX™ Size Standard (Applied Biosystems, Foster City, CA, USA)
- Hi-Di™ Formamide (Applied Biosystems, Foster City, CA, USA)
- 3100 POP-4™ Polymer (Applied Biosystems, Foster City, CA, USA)
- Cytobrush® Plus Cell Collector (Cooper Surgical, Inc., Trumbull, CT, USA)
- 0.9% saline solution (Baxter Healthcare, Old Toongabbie, NSW, Australia)
- For mock samples:
  - FTA™® Classic Card (Whatman Inc., Florham Park, NJ, USA)
  - Sterile cotton swabs (Medical Wire & Equipment, Corsham, Wiltshire, England)
  - Sterile rayon swabs (Copan Italia SPA, Brescia, Italy)

5. Methods

5.1 Gravimetric Evaluation of Pipetting Accuracy and Precision

Gravimetric analysis was performed by placing the SAG285/L balance on the platform deck and instructing the MP II to repeatedly pipette certain volumes of system liquid onto the balance pan. Readings were taken automatically by the software and compiled into a results table, which was then used to automatically generate an Excel-based results chart containing mean, %CV and %inaccuracy values. The mean values obtained were used to calculate R^2, slope and Y-intercept (offset) values to calibrate the system's pipetting.

Pipetting performance was assessed for various volumes using the three different tips in order to calculate appropriate R^2, slope and Y-intercept (offset) values which were then added to the performance file. Values were calculated for both Blowout (single-liquid transfer) and Waste (multidispense) modes for the 1000µL tips, and Blowout mode only for the 175µL and fixed tips.

For the addition of resin, a specialised performance file was created based on the performance file for 175µL tips in blowout mode, except the "Blowout Volume" column values were set to 0 to allow pipetting similar to waste mode. Retesting was performed to confirm accurate and precise pipetting with these settings.

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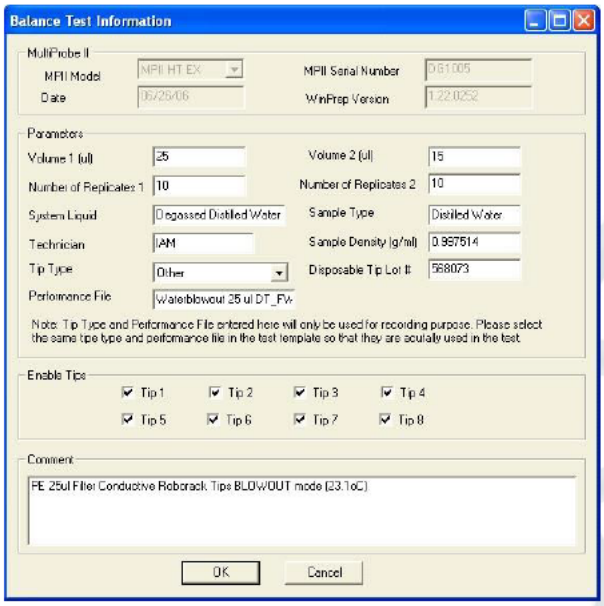

Figure 1. The Balance Test Information Window as present within the Balance Test DT program. All pipetting parameters are entered here and are subsequently transferred to the result output file.

All gravimetric testing was performed using the Balance Test DT test program within WinPrep®. Parameter values that needed to be entered into the Balance Test Information Window (Figure 1) included those as outlined in Table 1.

Table 1 Input values that are required for the various Balance Test Information parameters.

Parameter(s)	Value
Volume 1 and Volume 2	For 175µL tips: 175, 100, 50, 15µL For 1000µL tips: 1000, 700, 400, 100µL For Fixed tips: 1000, 700, 400, 100µL
Number of Replicates	10
System Liquid	Degassed Nanopure Water
Sample Type	Nanopure Water
Technician	Initials of the operator performing the test
Sample Density (g/ml)	The density of water at environmental temperature*
Tip Type	Other
Disposable Tip Lot #	The lot number of the particular tips in use
Performance File	The appropriate Performance File for the tip (175µL, 1000µL or fixed tips) and pipetting mode (Blowout or Waste) in use
Enable Tips (checkboxes)	Select the actual tips (1 to 8) to be tested
Comments	Free text box to add additional information (eg. Tip type, mode, current environmental room temperature, etc).

* Water density values were obtained from http://www.simetric.co.uk/si_water.htm

Pipetting accuracy and precision were examined at four different volumes for each tip size: 175, 100, 50, 15µL for 175µL tips and 1000, 700, 400, 100µL for the 1000µL and Fixed tips. In order to calculate unbiased values for each set of volumes, the slope and offset values in the relevant Performance File were changed to the default 1 and 0 respectively prior to

testing. The mean volumes that were pipetted by each tip (10 replicates per tip) at the four designated volumes were used to generate a standard curve. The slope and offset calculated from this curve was used to calibrate the relevant Performance File. The final Performance File settings were then tested at the highest and lowest volumes (as per Table 1) to confirm accurate and precise pipetting.

5.2 Blood Collection

Blood samples were collected from 2 staff donors (DJC/VKI) by a phlebotomist as per normal in three 4mL EDTA vials. Blood samples were stored at 4ºC.

5.3 Cell Collection

Buccal cells were collected using a modified Cytobrush® protocol (Mulot *et al*., 2005; Satia-Abouta *et al*., 2002). The donor was instructed to brush the inside of one cheek for one minute using a Cytobrush®. Then, with another Cytobrush®, the other cheek was also sampled. Once each cheek was swabbed, the cells on the brush were suspended in 2mL of 0.9% saline solution. Buccal cell samples were stored at 4ºC.

5.4 FTA cell Collection

Cells were collected from two staff donors (VKI/CJA) by using a “lolly-pop” swab to sample the inside of the donor’s cheek for 15 seconds before pressing the swab onto the FTA™ paper to transfer the DNA. FTA™ cards were stored at room temperature.

5.5 Automated DNA IQ™ Protocol

The automated DNA IQ™ protocol, based on the validated manual method (refer to Project 11), was programmed in WinPrep™ software. The final, optimised protocol was named “DNA IQ Extraction_Ver1.1.mpt”. A screenshot of the Test Outline window for this protocol is depicted below in Figure 2. The deck layout is illustrated in Figure 3.

The automated DNA IQ™ protocol was designed to mimic the validated manual method, with minor modifications. Briefly, the changes include:

- Increasing the volume of Extraction Buffer to 500µL;
- A SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA) was used for sample lysis;
- Incubation steps and any shaking steps were performed on the DPC shaker;
- CRS toroid magnet (P/N 5083175) was used for isolating the DNA IQ™ resin.

Reagents used in the automated protocol were as per the manual method.

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Figure 2. The Test Outline window displaying individual nodes within the DNA IQ Extraction_Ver1.1.mpt program test file.

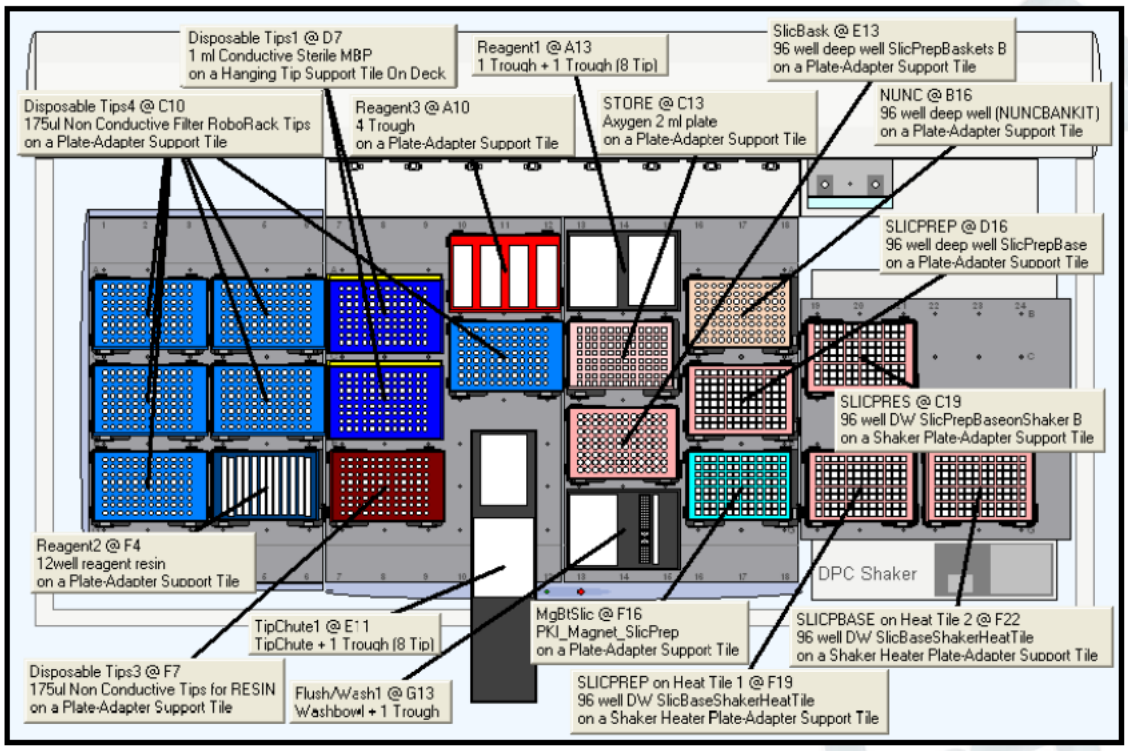

Figure 3. The deck layout for DNA IQ Extraction_Ver1.1.mpt, displaying the required labware on the platform deck.

5.6 Contamination Check via Checkerboard and Zebra-stripe Patterns

Samples consisting of two 3.2mm FTA® discs (containing blood, buccal cells, or blank cards) were arranged in a checkboard and zebra-stripe pattern in SlicPrep™ plates using the BSD Duet 600 instrument (BSD Robotics, Brisbane, QLD, Australia) and extracted on the MultiPROBE® II PLUS HT EX platforms using the automated DNA IQ™ protocol. One checkerboard and one zebra-stripe plate was processed on each platform.

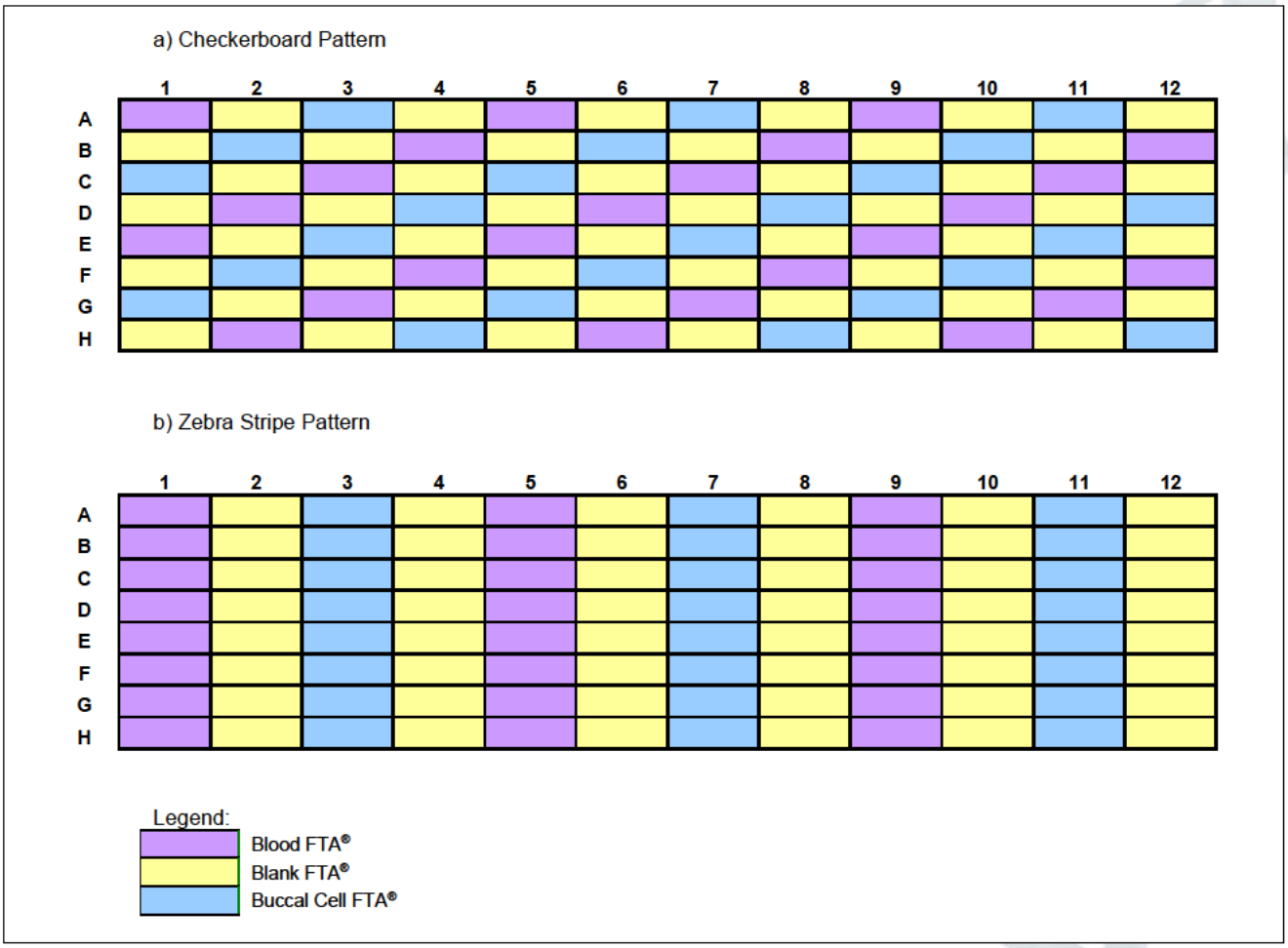

Figure 4. Checkerboard and zebra-stripe patterns utilised in the Extraction contamination check.

5.7 Heater tile temperature verification

Heat tiles supplied with MPII were modified to accept the SlicPrep 96 device. Temperature

5.8 Verification of the Automated Extraction Chemistry Protocol

Comparisons were made between results generated by the automated and manual methods to verify the performance of the automated Extraction Chemistry setup protocol.

Verification samples consisted of different dilutions of blood and cells spotted in 30µL aliquots onto quartered cotton and rayon swabs. Four blood dilutions of neat, 1/10, 1/100 and 1/1000 and four cell dilutions of neat, 1/5.2, 1/52.2 and 1/522 were used to test the

sensitivity of both the manual and automated methods. Dilutions were created using neat sample and 0.9% saline for both sample types. Four replicates of each dilution were made up for each substrate and sample type.

The blood was collected using the same method as in 5.2. 4 separate extractions were performed for the manual set: Blood Rayon, Blood Cotton, Cell Rayon and Cell Cotton. For the automated verification, all sample types were extracted together after being transferred to a Slicprep 96 device for processing on the Extraction Platform B.

5.9 Resin Volume

- 7 µL
- 14 µL

5.10 Extraction volume

- 300 µL
- 350 µL
- 400 µL
- 450 µL
- 500 µL

5.11 Sensitivity

Fbot262

6. Results and Discussion

6.1 Gravimetric Evaluation of Pipetting Accuracy and Precision

EXTN A:
WaterBlowoutFixedTips_08112007.prf
WaterBlowout 175 ul DT_FW 13112007RESIN.prf
WaterWasteFT_1 ml_27082007.prf
WaterBlowout 1ml DT _QHSS09112007.prf
WaterBlowout 175 ul DT_FW QHSS 13112007.prf
WaterWaste 1 ml DT_FW QHSS12112007.prf

EXTN B:
WaterBlowout 1ml DT_QHSS23102007.prf
WaterBlowoutFixed Tips_1ml_26102007.prf
WaterWaste_1ml_27082007.prf
WaterBlowout 175ul DT_FW 25102007.prf
WaterWaste 1ml DT_FW 24102007.prf
WaterBlowout 175 ul DT_FW 25102007RESIN.prf
WaterBlowout 25ul DT_FW_QHSS_060907.prf

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6.2 **Contamination Check via Checkerboard and Zebra-stripe Patterns results**

Table 1 below lists the extraction batch id of the checkerboards and their outcomes.

Table 2

Type of plate	Extraction batch Id	Extraction Platform	Check passed
Checkerboard-1	VALB20070817_02	Extraction A	Invalidated
Checkerboard-2	VALB20070803_02	Extraction B	Yes
Checkerboard/Zebra	VALB20071022_01	Extraction	Yes
Zebra-Stripe-1	VALB20070803_03	Extraction A	Yes
Zebra-Stripe-2	VALB20070817_03	Extraction B	Yes

Checkerboard1-

Position E3 (Sample Cells 6) was known to have been contaminated prior to start of the extraction. The DNA profile obtained confirmed this mixtures origin was formed from the expected donors. See figure X. Eight of the designated blank samples (Positions D3, A10, F1, H5, C4, E4, B7 and E6); two of the cell samples (A1 and B10) and two of the blood samples (F4 and G7) all exhibited a partial DNA profile previously unknown. This profile did not match any of the positive samples present on the batch. The profile was searched against the Staff database and no matches found.

None of the other blank samples yielded any DNA profile. The rest of the cell and blood samples yielded the correct DNA profile. Although there is no evidence of well to well contamination was observed; the unknown profile obtained has invalidated this plate. A further checkerboard/Zebra-Stripe combination plate was performed to ensure…

Table 3-Unknown profile obtained

Sample description	D3	vWA	FGA	Amel	D8	D21	D18	D5	D13	D7
Blk23-E6	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	11,13
Blk25-B7	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	
Blk15-E4	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	11,13
Blk14-C4	14,17									
Blk20-H5										
Blk3-F1										
Blk10-D3										
Blk37-A10										
Cells19-B10	14,17	14,17	20,21,22,24	X,Y	11,13,16	29,30,	14,15,16	11,15	11,12	11,11
Cells13-A1										
Blood14-G7										
Blood8-F4										

Checkerboard 2 –

None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile, see Figure 4.

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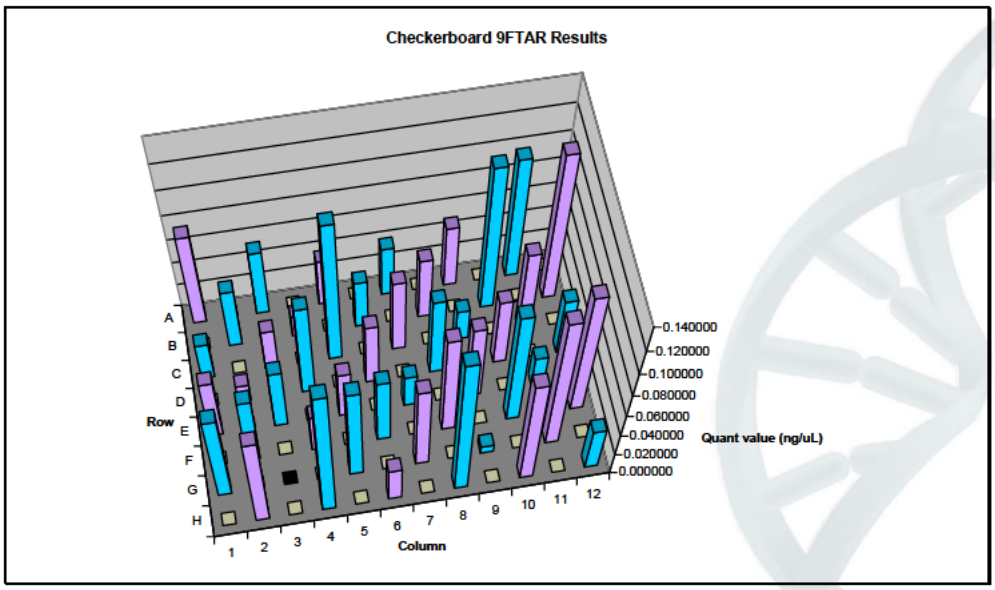

Figure 2

Zebra-Stripe1-
None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile, see Figure 5.

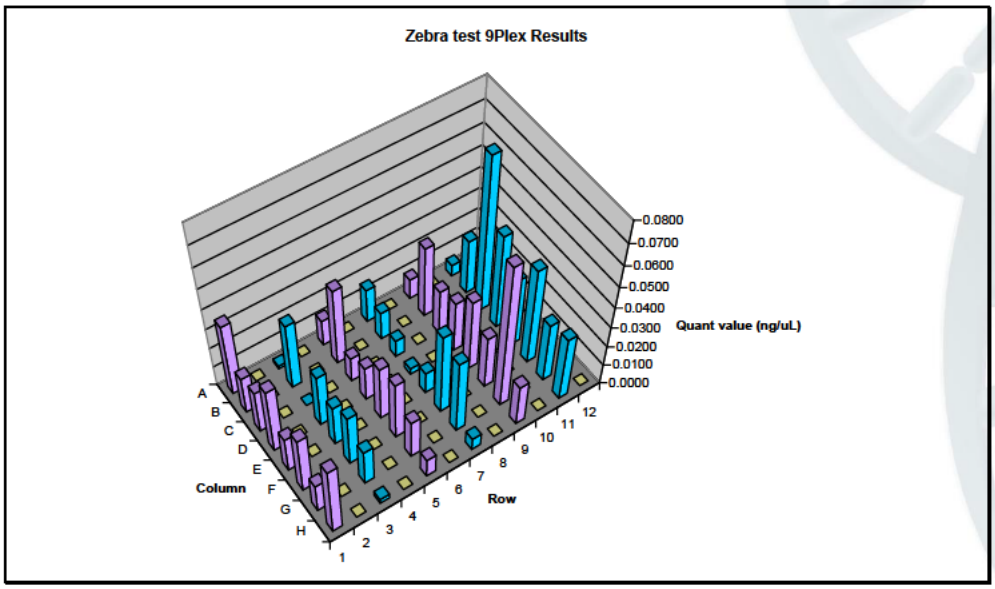

Figure 3

Zebra-Stripe 2-

None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile, see Figure 6.

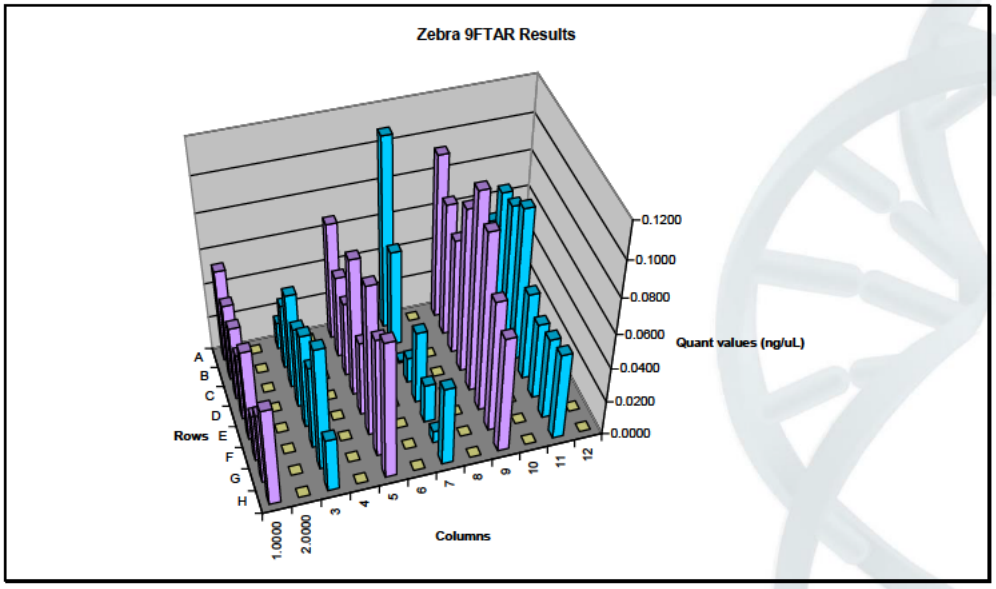

Figure 4

Checkerboard/Zebra -
None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile, see Figure 7.

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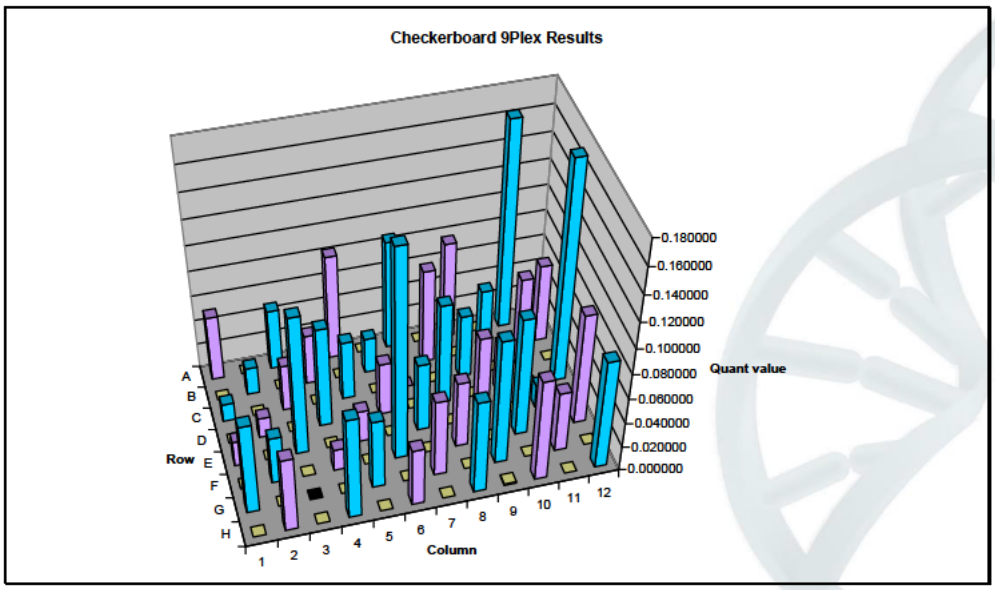

Figure 5

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9Plex

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.051900	0.000000	0.048300	0.000000	0.083700	0.000000	0.087000	0.000000	0.077300	0.000000	0.164000	0.000000
B	0.000000	0.021900	0.000000	0.040000	0.000000	0.028300	0.000000	0.075200	0.000000	0.050100	0.000000	0.062300
C	0.014100	0.000000	0.037100	0.000000	0.047100	0.000000	0.000000	0.000000	0.050400	0.000000	0.071700	0.000000
D	0.000000	0.015900	0.000000	0.079900	0.000000	0.042100	0.000000	0.080700	0.000000	0.039200	0.000000	0.174000
E	0.020200	0.000000	0.111000	0.000000	0.025300	0.000000	0.054600	0.000000	0.066700	0.000000	0.017900	0.000000
F	0.000761	0.037500	0.000000	0.017300	0.000000	0.166000	0.000000	0.053000	0.000000	0.094500	0.000778	0.088700
G	0.072000	0.000000	Removed	0.000000	0.055200	0.000000	0.061300	0.000000	0.099700	0.000000	0.048300	0.000000
H	0.000000	0.059400	0.000000	0.081300	0.000000	0.045800	0.000000	0.074700	0.001210	0.081400	0.000000	0.087200

9FTAR

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.076300	0.000000	0.054500	0.000000	0.038700	0.000000	0.041100	0.000000	0.050500	0.000000	0.098100	0.000000
B	0.000000	0.049700	0.000000	0.018200	0.000000	0.040200	0.000000	0.051000	0.000000	0.118000	0.001560	0.120000
C	0.032000	0.000000	0.035200	0.000000	0.118000	0.000000	0.061000	0.000000	0.026000	0.000000	0.067900	0.000000
D	0.000000	0.015300	0.000000	0.078200	0.000000	0.052400	0.000000	0.065900	0.000000	0.056400	0.000000	0.048000
E	0.052000	0.000000	0.051800	0.000000	0.040500	0.000000	0.027900	0.000000	0.062900	0.000000	0.026300	0.000000
F	0.000000	0.058600	0.000000	0.033700	0.000000	0.056600	0.000000	0.085700	0.000000	0.096500	0.000000	0.104000
G	0.076300	0.000000	Removed	0.000000	0.081300	0.000000	0.072600	0.000000	0.006630	0.000000	0.114000	0.000000
H	0.000000	0.081000	0.000000	0.114000	0.000000	0.030500	0.000000	0.122000	0.000000	0.092400	0.000000	0.037500

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	9Plex											
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0358	0.0000	0.001440	0.000000	0.012800	0.000000	0.016500	0.000000	0.009840	0.000000	0.005540	0.000000
B	0.0184	0.0000	0.032800	0.000000	0.037700	0.000000	0.013700	0.000000	0.034600	0.000000	0.026500	0.000000
C	0.0212	0.0000	0.000000	0.000000	0.012100	0.000000	0.008350	0.000000	0.021700	0.000000	0.074200	0.000000
D	0.0324	0.0000	0.025900	0.000000	0.016900	0.000000	0.003010	0.000000	0.024800	0.000000	0.046000	0.000000
E	0.0175	0.0000	0.020000	0.000000	0.026700	0.000000	0.010600	0.000000	0.034700	0.000000	0.033300	0.000000
F	0.0282	0.0000	0.025400	0.000000	0.028700	0.000000	0.039800	0.000000	0.025700	0.000000	0.047100	0.000000
G	0.0144	0.0000	0.017800	0.000000	0.019100	0.000000	0.036200	0.000000	0.069900	0.000000	0.029200	0.000000
H	0.0348	0.0000	0.002230	0.000000	0.009310	0.000000	0.006010	0.000000	0.020200	0.000000	0.031600	0.000000
	9FTAR											
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0534	0.0000	0.015700	0.000000	0.067500	0.000000	0.109000	0.000000	0.093500	0.000000	0.051000	0.009600
B	0.0444	0.0000	0.037600	0.000000	0.047300	0.000000	0.055500	0.000000	0.076100	0.000000	0.076800	0.000000
C	0.0426	0.0000	0.054800	0.000000	0.043100	0.000000	0.001140	0.000000	0.066200	0.000000	0.079300	0.000000
D	0.0386	0.0000	0.046600	0.000000	0.079200	0.000000	0.014700	0.000000	0.093700	0.000000	0.087900	0.000000
E	0.0525	0.0000	0.054500	0.000000	0.043200	0.000000	0.042000	0.000000	0.113000	0.000000	0.050500	0.000000
F	0.0273	0.0000	0.048100	0.000000	0.086800	0.000000	0.022500	0.000000	0.102000	0.000000	0.044300	0.000000
G	0.0408	0.0000	0.070800	0.000000	0.068900	0.000000	0.006870	0.000000	0.075700	0.000000	0.047300	0.000000
H	0.0566	0.0000	0.031100	0.000000	0.079300	0.000000	0.045600	0.000000	0.066900	0.000000	0.050300	0.000000

6.3 **Verification of the Automated Extraction Protocol**

7. Summary and Recommendations

We recommend the following:

- Use of MPII for automated extraction of reference samples
- Use of MPII for automated extraction of casework samples
- Ongoing development of the automated extraction program to increase the efficiency of the extraction

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Project 13. Report on the Verification of an Automated DNA IQ™ Protocol using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform

Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C., Ientile, V.
Automation/LIMS Implementation Project, DNA Analysis FSS (August 2008)

1. Abstract

A manual method for extracting DNA from forensic samples using the DNA IQ™ system (Promega Corp., Madison, WI, USA) was validated for routine use in DNA Analysis (FSS). We have verified an automated DNA IQ™ protocol in 96-well format for use on the MultiPROBE® II PLUS HT EX Forensic Workstation platforms (PerkinElmer, Downers Grove, IL, USA). Data indicate that results from the automated procedure are comparable to those from the manual procedure. Contamination checks were performed using samples prepared in checkerboard and zebra-stripe format, and results were as expected. We recommend the use of the MultiPROBE® II PLUS HT EX platforms to perform automated DNA extraction using the DNA IQ™ system.

2. Introduction

The MultiPROBE® II PLUS HT EX FORENSIC WORKSTATION platforms (PerkinElmer, Downers Grove, IL, USA) are equipped to perform automated DNA extractions, as they include a DPC shaker and individual heat controllers to enable on-board lysis and incubation steps. Currently in DNA Analysis, the MultiPROBE® platforms allow walk-away operation of PCR setup protocols for DNA quantitation and amplification.

The DNA IQ™ protocol has been verified or validated by various laboratories for use on the MultiPROBE® II PLUS platform. The laboratories that perform an automated DNA IQ™ protocol include PathWest (Western Australia), Forensic Science South Australia (South Australia) and Centre of Forensic Sciences in Toronto (Ontario). The MultiPROBE® II PLUS instrument comes pre-loaded with an automated DNA IQ™ protocol. Unlike the other laboratories, however, we did not validate the included protocol, but instead validated a manual DNA IQ™ protocol which was based on the CFS automated protocol (PerkinElmer, 2004), followed by verification of an automated protocol based on the validated manual method.

The verified automated DNA IQ™ protocol is identical to the validated manual protocol used in-house: there are no differences in reagents or volumes. The adopted DNA IQ™ protocol differs slightly, however, from the manufacturer's protocol, as it includes a lysis step using Extraction Buffer (10mM Tris, 1mM EDTA, 100mM NaCl, 20% SDS) in the presence of Proteinase K, before incubating in the DNA IQ™ Lysis Buffer. Furthermore, the lysis incubation conditions were lowered from 70°C to 37°C in order to accommodate extraction of DNA from heat labile materials such as nylon and polyester. In addition, the automated protocol utilises the SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA) for simultaneous processing of samples in a 96-well format.

3. Aim

To verify an automated DNA IQ™ protocol for use on the MultiPROBE® II PLUS HT EX platforms to allow extraction of DNA from various sample types.

4. Equipment and Materials

- MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (PerkinElmer, Downers Grove, IL, USA)
- Gravimetric Performance Evaluation Option with Mettler SAG285/L balance (Mettler-Toledo, Greifensee, Switzerland)
- DNA IQ™ System (Promega Corp., Madison, WI, USA)
- Extraction Buffer (10mM Tris, 1mM EDTA, 100mM NaCl, 20% SDS)
- SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA)
- Nunc™ Bank-It tubes (Nunc A/S, Roskilde, Denmark)
- 175µL non-conductive sterile filter RoboRack tips (PerkinElmer, Downers Grove, IL, USA)
- 1000µL Conductive sterile filter Robotix tips (Molecular BioProducts, San Diego, CA, USA)
- ABI Prism® 7000 SDS (Applied Biosystems, Foster City, CA, USA)
- Quantifiler™ Human DNA Quantification kits (Applied Biosystems, Foster City, CA, USA)
- AmpFℓSTR® Profiler Plus Amplification kits (Applied Biosystems, Foster City, CA, USA)
- GeneAmp® 9700 thermalcycers (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 96-well optical reaction plates (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA)
- GeneScan™ 500 ROX™ Size Standard (Applied Biosystems, Foster City, CA, USA)
- Hi-Di™ Formamide (Applied Biosystems, Foster City, CA, USA)
- 3100 POP-4™ Polymer (Applied Biosystems, Foster City, CA, USA)
- Cytobrush® Plus Cell Collector (Cooper Surgical, Inc., Trumbull, CT, USA)
- 0.9% saline solution (Baxter Healthcare, Old Toongabbie, NSW, Australia)
- For mock samples:
  - FTA™® Classic Card (Whatman Inc., Florham Park, NJ, USA)
  - Sterile cotton swabs (Medical Wire & Equipment, Corsham, Wiltshire, England)
  - Sterile rayon swabs (Copan Italia SPA, Brescia, Italy)

5. Methods

5.1 Gravimetric Evaluation of Pipetting Accuracy and Precision

Gravimetric analysis was performed by placing the SAG285/L balance on the platform deck and instructing the MP II to repeatedly pipette certain volumes of system liquid onto the balance pan. Readings were taken automatically by the software and compiled into a results table, which was then used to automatically generate an Excel-based results chart containing mean, %CV and %inaccuracy values. The mean values obtained were used to calculate R^2, slope and Y-intercept (offset) values to calibrate the system's pipetting.

Pipetting performance was assessed for various volumes using three different tips in order to calculate appropriate R^2, slope and Y-intercept (offset) values which were then added to the performance file. Values were calculated for both Blowout (single-liquid transfer) and Waste (multidispense) modes for the 1000µL conductive tips, and Blowout mode only for the 175µL non-conductive tips and fixed tips.

For the addition of resin, a specialised performance file was created based on the performance file for 175µL tips in blowout mode, except the "Blowout Volume" column values were set to 0 to allow pipetting performance that is similar to waste mode. Retesting was performed to confirm accurate and precise pipetting with these settings.

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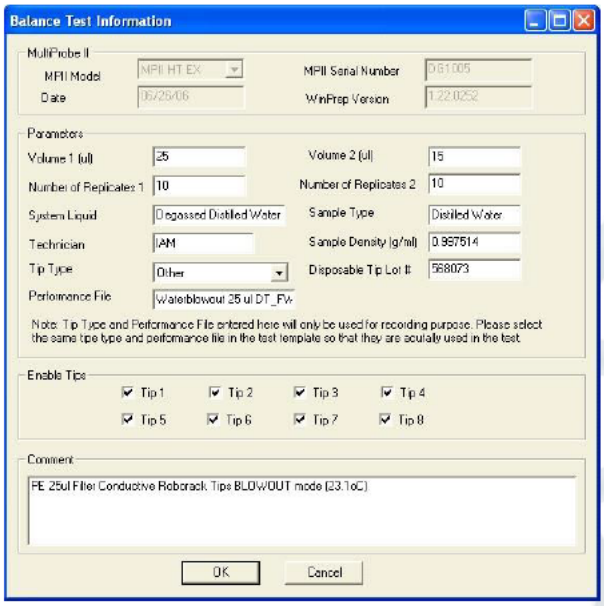

Figure 1. The Balance Test Information Window as present within the Balance Test DT program. All pipetting parameters are entered here and are subsequently transferred to the result output file.

All gravimetric testing was performed using the Balance Test DT test program within WinPrep®. Parameter values that needed to be entered into the Balance Test Information Window (Figure 1) included those as outlined in Table 1.

Table 1. Input values that are required for the various Balance Test Information parameters.

Parameter(s)	Value
Volume 1 and Volume 2	For 175µL tips: 175, 100, 50, 15µL For 1000µL tips: 1000, 700, 400, 100µL For fixed tips: 1000, 700, 400, 100µL
Number of Replicates	10
System Liquid	Degassed Nanopure Water
Sample Type	Nanopure Water
Technician	Initials of the operator performing the test
Sample Density (g/ml)	The density of water at environmental temperature*
Tip Type	Other
Disposable Tip Lot #	The lot number of the particular tips in use
Performance File	The appropriate Performance File for the tip (175µL, 1000µL or fixed tips) and pipetting mode (Blowout or Waste) in use
Enable Tips (checkboxes)	Select the actual tips (1 to 8) to be tested
Comments	Free text box to add additional information (eg. Tip type, mode, current environmental room temperature, etc).

* Water density values were obtained from http://www.simetric.co.uk/si_water.htm

Pipetting accuracy and precision were examined at four different volumes for each tip size: 175, 100, 50, 15µL for 175µL tips and 1000, 700, 400, 100µL for the 1000µL and fixed tips. In order to calculate unbiased values for each set of volumes, the slope and offset values in the relevant Performance File were changed to the default 1 and 0 respectively prior to testing. The mean volumes that were pipetted by each tip (10 replicates per tip) at the four

designated volumes were used to generate a standard curve. The slope and offset calculated from this curve was used to calibrate the relevant Performance File. The final Performance File settings were then tested at the highest and lowest volumes (as per Table 1) to confirm accurate and precise pipetting.

5.2 Blood Collection

Blood samples were collected from 2 staff donors (DJC/VKI) by a phlebotomist as per normal in three 4mL EDTA vials. Blood samples were stored at 4ºC.

5.3 Cell Collection

Buccal cells were collected using a modified Cytobrush® protocol (Mulot *et al*., 2005; Satia-Abouta *et al*., 2002). The donor was instructed to brush the inside of one cheek for one minute using a Cytobrush®. Then, with another Cytobrush®, the other cheek was also sampled. Once each cheek was swabbed, the cells on the brush were suspended in 2mL of 0.9% saline solution. Buccal cell samples were stored at 4ºC.

5.4 FTA cell Collection

Cells were collected from two staff donors (VKI/CJA) by using a “lolly-pop” swab to sample the inside of the donor’s cheek for 15 seconds before pressing the swab onto the FTA™ paper to transfer the DNA. FTA™ cards were stored at room temperature.

5.5 Heater tile temperature verification

Heat tiles supplied with the MultiPROBE® II PLUS HT EX platforms were modified to accept the SlicPrep™ 96 Device. For testing, 1mL of nanopure water (at room temperature) was added to each well. The plate was then placed on a heater tile (controlled by the MP II heater controller) and allowed to reach temperature. The temperatures tested were 37ºC and 65ºC. Temperature readings for specific outer and inner wells (i.e. A1, A6, A12, D1, D6, D12, H1, H6, H12) were taken at regular intervals up to 45 minutes, using calibrated thermometer probes. The data were collated and means calculated to determine the distribution of heat over the tile.

5.6 Verification of automated DNA IQ™ Protocol

The automated DNA IQ™ protocol, based on the validated manual method (refer to Project 11), was programmed in WinPrep™ software. The final, optimised protocol was named “DNA IQ Extraction_Ver1.1.mpt”. A screenshot of the Test Outline window for this protocol is depicted below in Figure 2. The deck layout is illustrated in Figure 3.

The automated DNA IQ™ protocol was designed to mimic the validated manual method, with minor modifications. Briefly, the changes include:

- Increasing the volume of Extraction Buffer to 500µL;
- A SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA) was used for sample lysis;
- Incubation steps and any shaking steps were performed on the integrated DPC shaker;
- CRS toroid magnet (P/N 5083175) was used for isolating the DNA IQ™ resin.

Reagents used in the automated protocol were as per the manual method.

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Figure 2. The Test Outline window displaying individual nodes within the DNA IQ Extraction_Ver1.1.mpt program test file.

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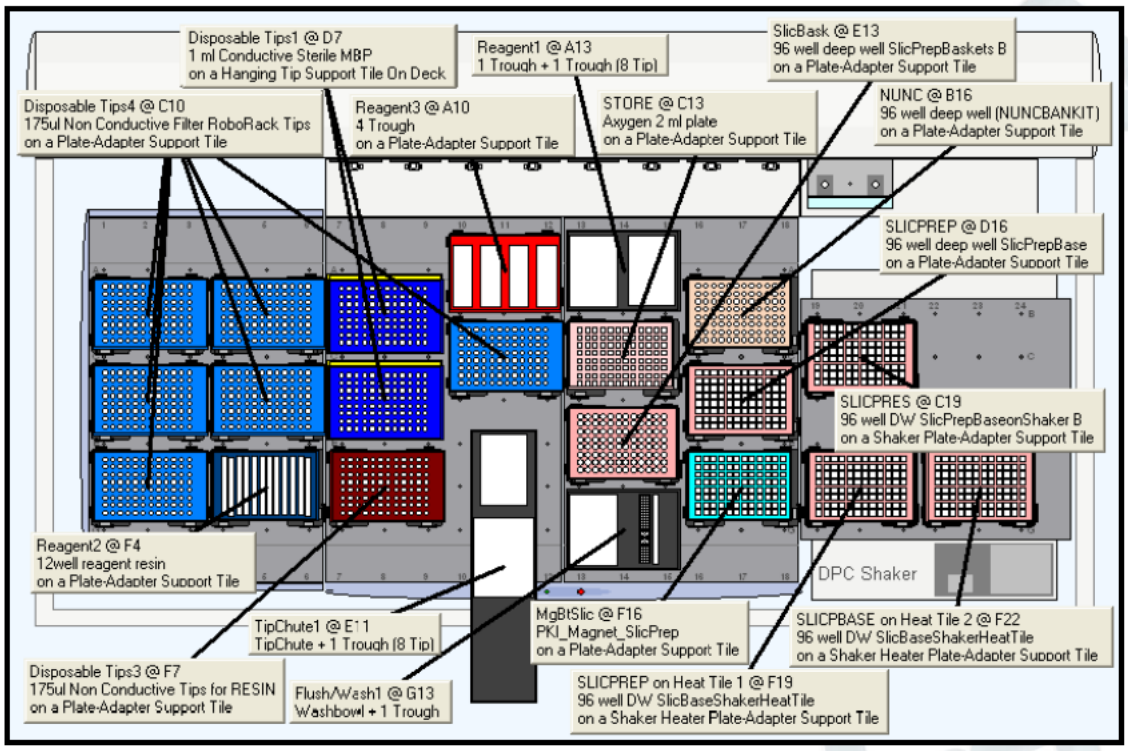

Figure 3. The deck layout for DNA IQ Extraction_Ver1.1.mpt, displaying the required labware on the platform deck.

The automated DNA IQ™ protocol was used to perform the following tests.

5.6.1. Contamination Check via Checkerboard and Zebra-stripe Patterns

Samples consisting of two 3.2mm FTA® discs (containing blood, buccal cells, or blank cards) were arranged in a checkboard and zebra-stripe pattern (Figure 4) in SlicPrep™ plates using the BSD Duet 600 instrument (BSD Robotics, Brisbane, QLD, Australia) and extracted on the MultiPROBE® II PLUS HT EX platforms using the automated DNA IQ™ protocol. One checkerboard and one zebra-stripe plate was processed on each platform.

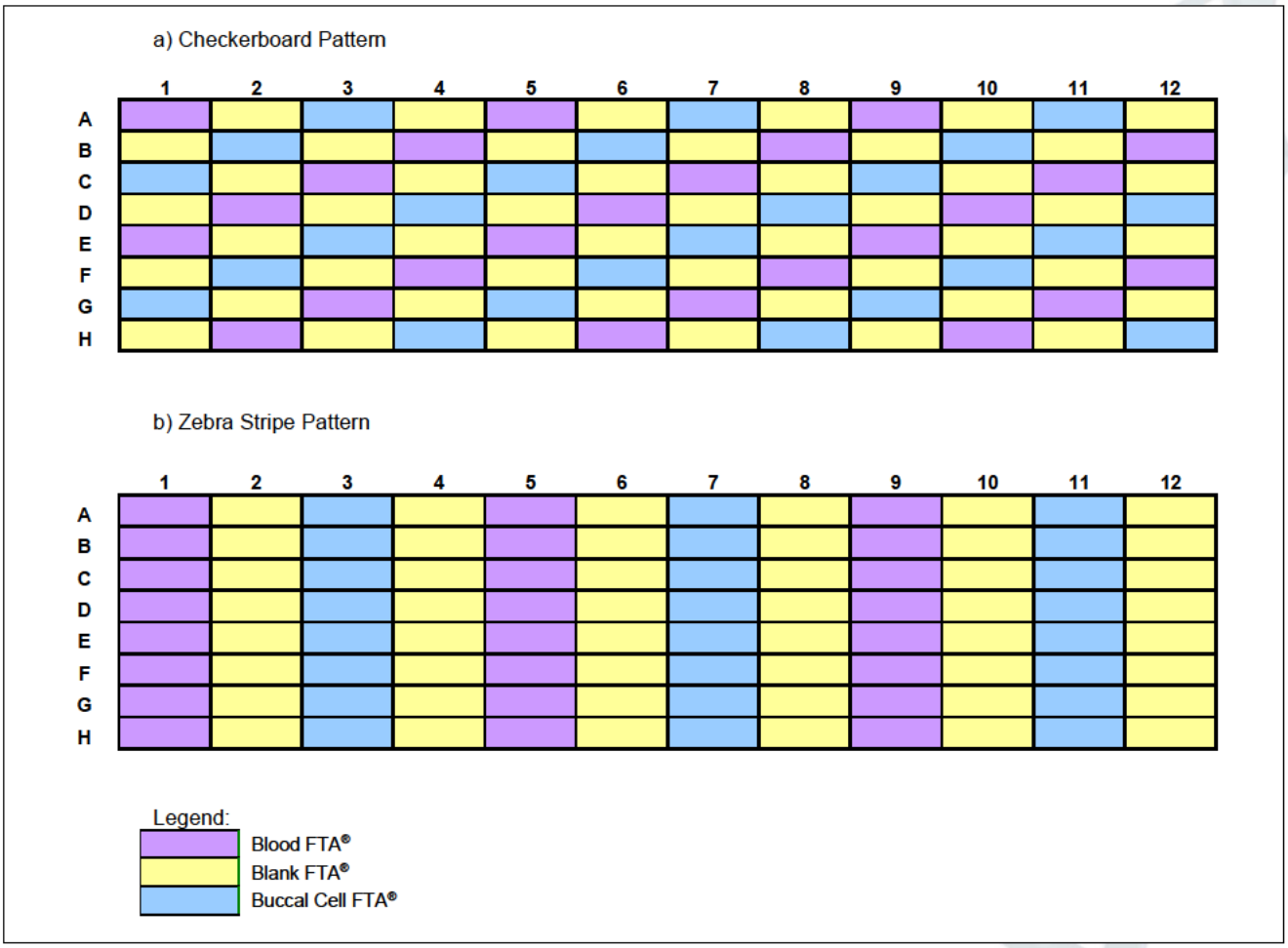

Figure 4. Checkerboard and zebra-stripe patterns utilised in the contamination check.

5.6.2. Comparisons with the manual DNA IQ™ method

Comparisons were made between results generated by the automated and manual methods to verify the performance of the automated DNA IQ™ protocol.

Verification samples consisted of different dilutions of blood and cells spotted in 30µL aliquots onto quartered cotton and rayon swabs. Four blood dilutions of neat, 1/10, 1/100 and 1/1000 and four cell dilutions of neat, 1/5.2, 1/52.2 and 1/522 were used to test the sensitivity of both the manual and automated methods. Dilutions were created using 0.9% saline solution for both sample types. Four replicates of each dilution were made up for each substrate and sample type.

The blood was collected using the same method as in 5.2. Four separate extractions were performed for the manual set based on the combination of sample type and swab type: Blood Rayon, Blood Cotton, Cell Rayon and Cell Cotton. For the automated verification, all

sample types were extracted together after being transferred to a SlicPrep™ 96 Device to allow automated processing.

5.6.3. Resin volume

The performance of the automated DNA IQ™ protocol was assessed when either 7µL or 14µL of DNA IQ™ resin was used in the protocol.

5.6.4. Modifying extraction volumes

The performance of the automated DNA IQ™ protocol was assessed for varying volumes of extraction buffer at 300, 350, 400, 450 and 500µL. In each case, the volume of DNA IQ™ Lysis Buffer was kept at 2x the volume of extraction buffer. Samples extracted were blood swabs, prepared as per ???.

5.6.5. Sensitivity of the automated DNA IQ™ protocol

The sensitivity of the automated DNA IQ™ protocol was assessed using dilutions of whole blood at neat, 1:10, 1:50, 1:100 and 1:1000.

6. Results and Discussion

6.1 Gravimetric Evaluation of Pipetting Accuracy and Precision

EXTN A:
WaterBlowoutFixedTips_08112007.prf
WaterBlowout 175 ul DT_FW 13112007RESIN.prf
WaterWasteFT_1 ml_27082007.prf
WaterBlowout 1ml DT _QHSS09112007.prf
WaterBlowout 175 ul DT_FW QHSS 13112007.prf
WaterWaste 1 ml DT_FW QHSS12112007.prf

EXTN B:
WaterBlowout 1ml DT_QHSS23102007.prf
WaterBlowoutFixed Tips_1ml_26102007.prf
WaterWaste_1ml_27082007.prf
WaterBlowout 175ul DT_FW 25102007.prf
WaterWaste 1ml DT_FW 24102007.prf
WaterBlowout 175 ul DT_FW 25102007RESIN.prf
WaterBlowout 25ul DT_FW_QHSS_060907.prf

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6.2 Contamination Check via Checkerboard and Zebra-stripe Patterns

Table 2 below lists the Extraction Batch ID's of the contamination checks.

Table 2. Extraction Batch ID's for the various contamination check plates that were processed on the MP II platforms using the automated DNA IQ™ protocol.

Type of plate	Extraction batch Id	Extraction Platform	Check passed
Checkerboard 1	VALB20070817_02	Extraction A	Invalidated
Checkerboard 2	VALB20070803_02	Extraction B	Yes
Zebra-Stripe 1	VALB20070803_03	Extraction A	Yes
Zebra-Stripe 2	VALB20070817_03	Extraction B	Yes
Checkerboard/Zebra	VALB20071022_01	Extraction A	Yes

Checkerboard 1

Position E3 (Sample Cells 6) was known to have been contaminated prior to the start of the extraction, during the STORstar process (???). The result showed a mixed DNA profile, with contributing alleles originating from the expected wells. See figure X. In addition to this contamination event, eight of the designated blank samples (positions D3, A10, F1, H5, C4, E4, B7 and E6), two of the cell samples (A1 and B10) and two of the blood samples (F4 and G7) all exhibited a partial DNA profile that was previously unknown (Table 3). This profile did not match any of the positive control samples present on the batch. The DNA profile was searched against the Staff Database and no matches were found. The source of this contaminating DNA profile could not be identified.

None of the other blank samples yielded any DNA profile. The rest of the cell and blood samples yielded the correct DNA profile. Although there is no evidence of well-to-well contamination, the unknown DNA profile obtained has invalidated this plate. A further checkerboard/Zebra-Stripe combination plate was performed to ensure…

Table 3. The DNA profile of the unknown contaminant that was observed in Checkboard-1.

Sample description	D3	vWA	FGA	Amel	D8	D21	D18	D5	D13	D7
Blk23-E6	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	11,13
Blk25-B7	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	
Blk15-E4	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	11,13
Blk14-C4	14,17									
Blk20-H5										
Blk3-F1										
Blk10-D3										
Blk37-A10										
Cells19-B10	14,17	14,17	20,21,22,24	X,Y	11,13,16	29,30,	14,15,16	11,15	11,12	11,11
Cells13-A1										
Blood14-G7										
Blood8-F4										

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Checkerboard 2
None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile. Figure 5 illustrates the DNA quantitation results from this plate. DNA was not detected in any of the blank samples.

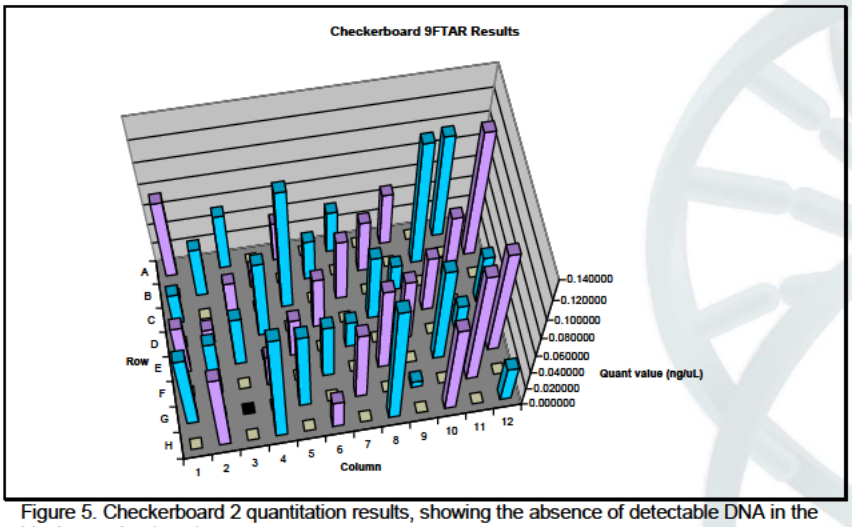

Figure 5. Checkerboard 2 quantitation results, showing the absence of detectable DNA in the blank samples (grey).

Zebra-Stripe 1
None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile. Figure 6 illustrates the absence of detectable DNA in the blank samples.

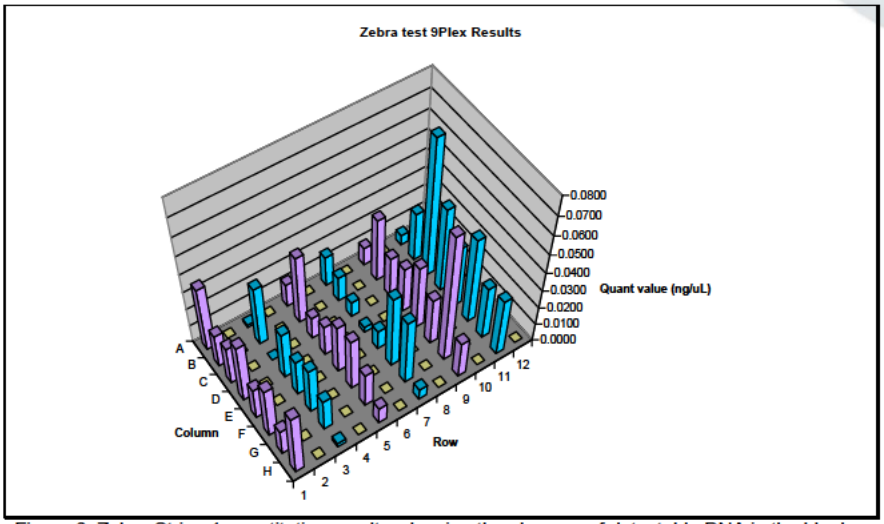

Figure 6. Zebra-Stripe 1 quantitation results, showing the absence of detectable DNA in the blank samples (grey).

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Zebra-Stripe 2
None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile. Figure 7 shows the absence of detectable DNA in the blank samples.

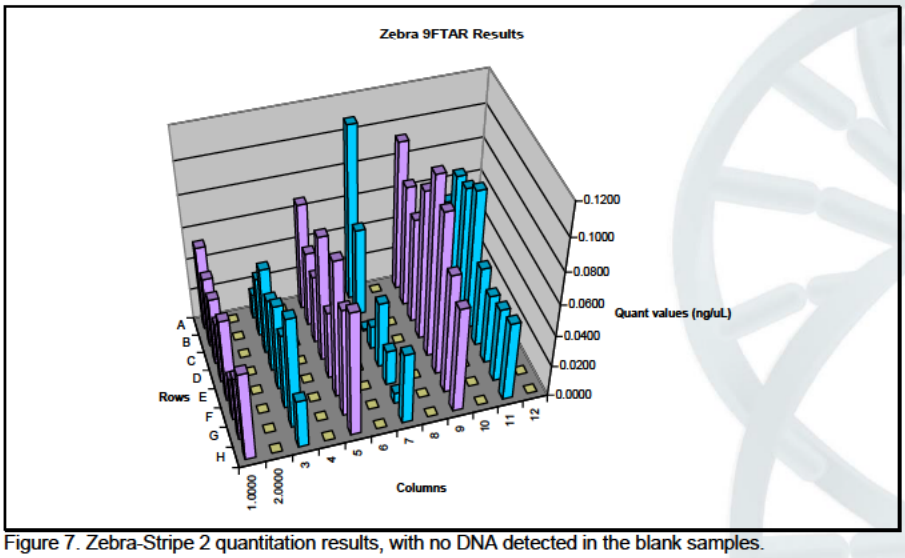

Figure 7. Zebra-Stripe 2 quantitation results, with no DNA detected in the blank samples.

Checkerboard/Zebra
None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile. DNA was undetected in the blank samples (Figure 8).

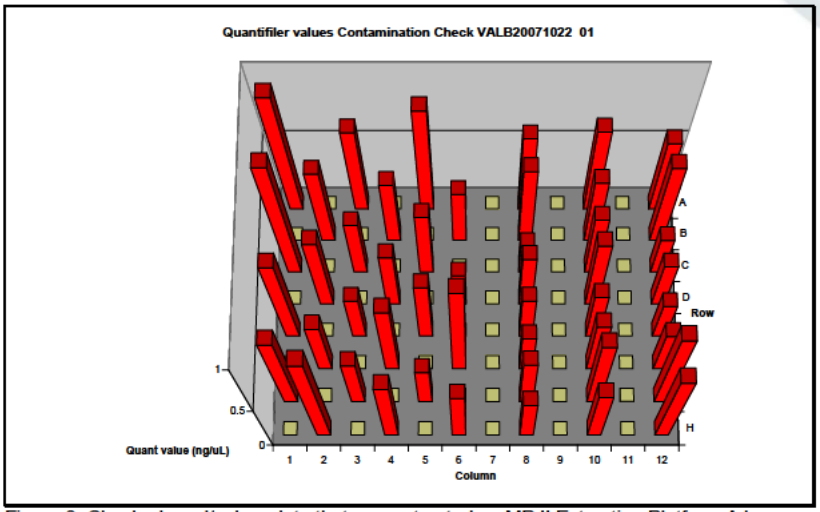

Figure 8. Checkerboard/zebra plate that was extracted on MP II Extraction Platform A because the previous plate was invalidated. DNA was not detected in the blank samples (grey).

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9Plex

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.051900	0.000000	0.048300	0.000000	0.083700	0.000000	0.087000	0.000000	0.077300	0.000000	0.164000	0.000000
B	0.000000	0.021900	0.000000	0.040000	0.000000	0.028300	0.000000	0.075200	0.000000	0.050100	0.000000	0.062300
C	0.014100	0.000000	0.037100	0.000000	0.047100	0.000000	0.000000	0.000000	0.050400	0.000000	0.071700	0.000000
D	0.000000	0.015900	0.000000	0.079900	0.000000	0.042100	0.000000	0.080700	0.000000	0.039200	0.000000	0.174000
E	0.020200	0.000000	0.111000	0.000000	0.025300	0.000000	0.054600	0.000000	0.066700	0.000000	0.017900	0.000000
F	0.000761	0.037500	0.000000	0.017300	0.000000	0.166000	0.000000	0.053000	0.000000	0.094500	0.000778	0.088700
G	0.072000	0.000000	Removed	0.000000	0.055200	0.000000	0.061300	0.000000	0.099700	0.000000	0.048300	0.000000
H	0.000000	0.059400	0.000000	0.081300	0.000000	0.045800	0.000000	0.074700	0.001210	0.081400	0.000000	0.087200

9FTAR

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.076300	0.000000	0.054500	0.000000	0.038700	0.000000	0.041100	0.000000	0.050500	0.000000	0.098100	0.000000
B	0.000000	0.049700	0.000000	0.018200	0.000000	0.040200	0.000000	0.051000	0.000000	0.118000	0.001560	0.120000
C	0.032000	0.000000	0.035200	0.000000	0.118000	0.000000	0.061000	0.000000	0.026000	0.000000	0.067900	0.000000
D	0.000000	0.015300	0.000000	0.078200	0.000000	0.052400	0.000000	0.065900	0.000000	0.056400	0.000000	0.048000
E	0.052000	0.000000	0.051800	0.000000	0.040500	0.000000	0.027900	0.000000	0.062900	0.000000	0.026300	0.000000
F	0.000000	0.058600	0.000000	0.033700	0.000000	0.056600	0.000000	0.085700	0.000000	0.096500	0.000000	0.104000
G	0.076300	0.000000	Removed	0.000000	0.081300	0.000000	0.072600	0.000000	0.006630	0.000000	0.114000	0.000000
H	0.000000	0.081000	0.000000	0.114000	0.000000	0.030500	0.000000	0.122000	0.000000	0.092400	0.000000	0.037500

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	9Plex											
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0358	0.0000	0.001440	0.000000	0.012800	0.000000	0.016500	0.000000	0.009840	0.000000	0.005540	0.000000
B	0.0184	0.0000	0.032800	0.000000	0.037700	0.000000	0.013700	0.000000	0.034600	0.000000	0.026500	0.000000
C	0.0212	0.0000	0.000000	0.000000	0.012100	0.000000	0.008350	0.000000	0.021700	0.000000	0.074200	0.000000
D	0.0324	0.0000	0.025900	0.000000	0.016900	0.000000	0.003010	0.000000	0.024800	0.000000	0.046000	0.000000
E	0.0175	0.0000	0.020000	0.000000	0.026700	0.000000	0.010600	0.000000	0.034700	0.000000	0.033300	0.000000
F	0.0282	0.0000	0.025400	0.000000	0.028700	0.000000	0.039800	0.000000	0.025700	0.000000	0.047100	0.000000
G	0.0144	0.0000	0.017800	0.000000	0.019100	0.000000	0.036200	0.000000	0.069900	0.000000	0.029200	0.000000
H	0.0348	0.0000	0.002230	0.000000	0.009310	0.000000	0.006010	0.000000	0.020200	0.000000	0.031600	0.000000
	9FTAR											
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0534	0.0000	0.015700	0.000000	0.067500	0.000000	0.109000	0.000000	0.093500	0.000000	0.051000	0.009600
B	0.0444	0.0000	0.037600	0.000000	0.047300	0.000000	0.055500	0.000000	0.076100	0.000000	0.076800	0.000000
C	0.0426	0.0000	0.054800	0.000000	0.043100	0.000000	0.001140	0.000000	0.066200	0.000000	0.079300	0.000000
D	0.0386	0.0000	0.046600	0.000000	0.079200	0.000000	0.014700	0.000000	0.093700	0.000000	0.087900	0.000000
E	0.0525	0.0000	0.054500	0.000000	0.043200	0.000000	0.042000	0.000000	0.113000	0.000000	0.050500	0.000000
F	0.0273	0.0000	0.048100	0.000000	0.086800	0.000000	0.022500	0.000000	0.102000	0.000000	0.044300	0.000000
G	0.0408	0.0000	0.070800	0.000000	0.068900	0.000000	0.006870	0.000000	0.075700	0.000000	0.047300	0.000000
H	0.0566	0.0000	0.031100	0.000000	0.079300	0.000000	0.045600	0.000000	0.066900	0.000000	0.050300	0.000000

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6.3 **Verification of the Automated Extraction Protocol**

7. Summary and Recommendations

We recommend the following:

- Use of MPII for automated extraction of reference samples
- Use of MPII for automated extraction of casework samples
- Ongoing development of the automated extraction program to increase the efficiency of the extraction

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Project 13. Report on the Verification of an Automated DNA IQ™ Protocol using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform

Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C., Ientile, V.
Automation/LIMS Implementation Project, DNA Analysis FSS (August 2008)

1. Abstract

A manual method for extracting DNA from forensic samples using the DNA IQ™ system (Promega Corp., Madison, WI, USA) was validated for routine use in DNA Analysis (FSS). We have verified an automated DNA IQ™ protocol in 96-well format for use on the MultiPROBE® II PLUS HT EX Forensic Workstation platforms (PerkinElmer, Downers Grove, IL, USA). Data indicate that results from the automated procedure are comparable to those from the manual procedure. Contamination checks were performed using samples prepared in checkerboard and zebra-stripe format, and results were as expected. We recommend the use of the MultiPROBE® II PLUS HT EX platforms to perform automated DNA extraction using the DNA IQ™ system.

2. Introduction

The MultiPROBE® II PLUS HT EX FORENSIC WORKSTATION platforms (PerkinElmer, Downers Grove, IL, USA) are equipped to perform automated DNA extractions, as they include a DPC shaker and individual heat controllers to enable on-board lysis and incubation steps. Currently in DNA Analysis, the MultiPROBE® platforms allow walk-away operation of PCR setup protocols for DNA quantitation and amplification.

The DNA IQ™ protocol has been verified or validated by various laboratories for use on the MultiPROBE® II PLUS platform. The laboratories that perform an automated DNA IQ™ protocol include PathWest (Western Australia), Forensic Science South Australia (South Australia) and Centre of Forensic Sciences in Toronto (Ontario). The MultiPROBE® II PLUS instrument comes pre-loaded with an automated DNA IQ™ protocol. Unlike the other laboratories, however, we did not validate the included protocol, but instead validated a manual DNA IQ™ protocol which was based on the CFS automated protocol (PerkinElmer, 2004), followed by verification of an automated protocol based on the validated manual method.

The verified automated DNA IQ™ protocol is identical to the validated manual protocol used in-house: there are no differences in reagents or volumes. The adopted DNA IQ™ protocol differs slightly, however, from the manufacturer's protocol, as it includes a lysis step using Extraction Buffer (10mM Tris, 1mM EDTA, 100mM NaCl, 20% SDS) in the presence of Proteinase K, before incubating in the DNA IQ™ Lysis Buffer. Furthermore, the lysis incubation conditions were lowered from 70°C to 37°C in order to accommodate extraction of DNA from heat labile materials such as nylon and polyester. In addition, the automated protocol utilises the SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA) for simultaneous processing of samples in a 96-well format.

3. Aim

To verify an automated DNA IQ™ protocol for use on the MultiPROBE® II PLUS HT EX platforms to allow extraction of DNA from various sample types.

4. Equipment and Materials

- MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (PerkinElmer, Downers Grove, IL, USA)
- Gravimetric Performance Evaluation Option with Mettler SAG285/L balance (Mettler-Toledo, Greifensee, Switzerland)
- DNA IQ™ System (Promega Corp., Madison, WI, USA)
- Extraction Buffer (10mM Tris, 1mM EDTA, 100mM NaCl, 20% SDS)
- SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA)
- Nunc™ Bank-It tubes (Nunc A/S, Roskilde, Denmark)
- 175µL non-conductive sterile filter RoboRack tips (PerkinElmer, Downers Grove, IL, USA)
- 1000µL Conductive sterile filter Robotix tips (Molecular BioProducts, San Diego, CA, USA)
- ABI Prism® 7000 SDS (Applied Biosystems, Foster City, CA, USA)
- Quantifiler™ Human DNA Quantification kits (Applied Biosystems, Foster City, CA, USA)
- AmpFℓSTR® Profiler Plus Amplification kits (Applied Biosystems, Foster City, CA, USA)
- GeneAmp® 9700 thermalcycers (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 96-well optical reaction plates (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA)
- GeneScan™ 500 ROX™ Size Standard (Applied Biosystems, Foster City, CA, USA)
- Hi-Di™ Formamide (Applied Biosystems, Foster City, CA, USA)
- 3100 POP-4™ Polymer (Applied Biosystems, Foster City, CA, USA)
- Cytobrush® Plus Cell Collector (Cooper Surgical, Inc., Trumbull, CT, USA)
- 0.9% saline solution (Baxter Healthcare, Old Toongabbie, NSW, Australia)
- Stem digital tilting head thermometer
- For mock samples:
  - FTA™® Classic Card (Whatman Inc., Florham Park, NJ, USA)
  - Sterile cotton swabs (Medical Wire & Equipment, Corsham, Wiltshire, England)
  - Sterile rayon swabs (Copan Italia SPA, Brescia, Italy)

5. Methods

5.1 Gravimetric Evaluation of Pipetting Accuracy and Precision

Gravimetric analysis was performed by placing the SAG285/L balance on the platform deck and instructing the MP II to repeatedly pipette certain volumes of system liquid onto the balance pan. Readings were taken automatically by the software and compiled into a results table, which was then used to automatically generate an Excel-based results chart containing mean, %CV and %inaccuracy values. The mean values obtained were used to calculate R^2, slope and Y-intercept (offset) values to calibrate the system's pipetting.

Pipetting performance was assessed for various volumes using three different tips in order to calculate appropriate R^2, slope and Y-intercept (offset) values which were then added to the performance file. Values were calculated for both Blowout (single-liquid transfer) and Waste (multidispense) modes for the 1000µL conductive tips, and Blowout mode only for the 175µL non-conductive tips and fixed tips.

For the addition of resin, a specialised performance file was created based on the performance file for 175µL tips in blowout mode, except the "Blowout Volume" column values were set to 0 to allow pipetting performance that is similar to waste mode. Retesting was performed to confirm accurate and precise pipetting with these settings.

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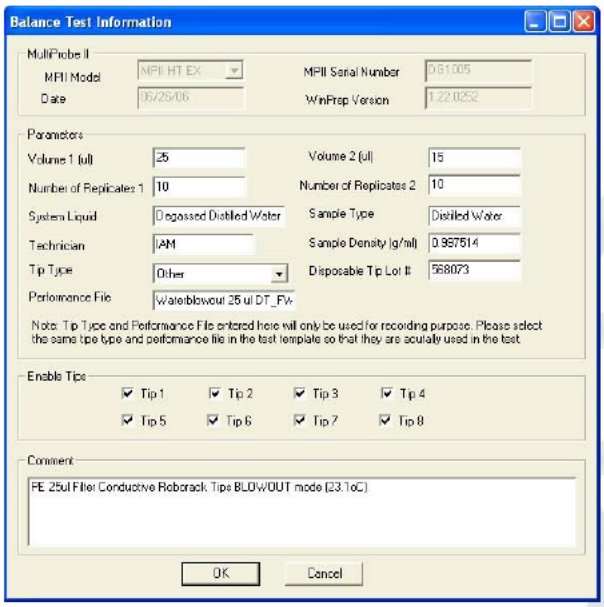

Figure 1. The Balance Test Information Window as present within the Balance Test DT program. All pipetting parameters are entered here and are subsequently transferred to the result output file.

All gravimetric testing was performed using the Balance Test DT test program within WinPrep®. Parameter values that needed to be entered into the Balance Test Information Window (Figure 1) included those as outlined in Table 1.

Table 1. Input values that are required for the various Balance Test Information parameters.

Parameter(s)	Value
Volume 1 and Volume 2	For 175µL tips: 175, 100, 50, 15µL For 1000µL tips: 1000, 700, 400, 100µL For fixed tips: 1000, 700, 400, 100µL
Number of Replicates	10
System Liquid	Degassed Nanopure Water
Sample Type	Nanopure Water
Technician	Initials of the operator performing the test
Sample Density (g/ml)	The density of water at environmental temperature*
Tip Type	Other
Disposable Tip Lot #	The lot number of the particular tips in use
Performance File	The appropriate Performance File for the tip (175µL, 1000µL or fixed tips) and pipetting mode (Blowout or Waste) in use
Enable Tips (checkboxes)	Select the actual tips (1 to 8) to be tested
Comments	Free text box to add additional information (eg. Tip type, mode, current environmental room temperature, etc).

* Water density values were obtained from http://www.simetric.co.uk/si_water.htm

Pipetting accuracy and precision were examined at four different volumes for each tip size: 175, 100, 50, 15µL for 175µL tips and 1000, 700, 400, 100µL for the 1000µL and fixed tips. In order to calculate unbiased values for each set of volumes, the slope and offset values in the relevant Performance File were changed to the default 1 and 0 respectively prior to

testing. The mean volumes that were pipetted by each tip (10 replicates per tip) at the four designated volumes were used to generate a standard curve. The slope and offset calculated from this curve was used to calibrate the relevant Performance File. The final Performance File settings were then tested at the highest and lowest volumes (as per Table 1) to confirm accurate and precise pipetting.

5.2 Blood Collection

Blood samples were collected from 2 staff donors (DJC/VKI) by a phlebotomist as per normal in three 4mL EDTA vials. Blood samples were stored at 4ºC.

5.3 Cell Collection

Buccal cells were collected using a modified Cytobrush® protocol (Mulot *et al*., 2005; Satia-Abouta *et al*., 2002). The donor was instructed to brush the inside of one cheek for one minute using a Cytobrush®. Then, with another Cytobrush®, the other cheek was also sampled. Once each cheek was swabbed, the cells on the brush were suspended in 2mL of 0.9% saline solution. Buccal cell samples were stored at 4ºC.

5.4 FTA cell Collection

Cells were collected from two staff donors (VKI/CJA) by using a "lolly-pop" swab to sample the inside of the donor's cheek for 15 seconds before pressing the swab onto the FTA™ paper to transfer the DNA. FTA™ cards were stored at room temperature.

5.5 Heater tile temperature verification

Heat tiles supplied with the MultiPROBE® II PLUS HT EX platforms were modified to accept the SlicPrep™ 96 Device. For testing, 1mL of nanopure water (at room temperature) was added to each well. The plate was then placed on a heater tile (controlled by the MP II heater controller) and allowed to reach temperature. The temperatures tested were 37ºC and 65ºC. Temperature readings for specific outer and inner wells (i.e. A1, A6, A12, D1, D6, D12, H1, H6, H12) were taken at regular intervals up to and including 45 minutes, using calibrated stem digital tilted head thermometer probes. The data were collated and means calculated to determine the distribution of heat over the tile.

5.6 Verification of automated DNA IQ™ Protocol

The automated DNA IQ™ protocol, based on the validated manual method (refer to Project 11), was programmed in WinPrep™ software. The final, optimised protocol was named "DNA IQ Extraction_Ver1.1.mpt". A screenshot of the Test Outline window for this protocol is depicted below in Figure 2. The deck layout is illustrated in Figure 3.

The automated DNA IQ™ protocol was designed to mimic the validated manual method, with minor modifications. Briefly, the changes include:

- Increasing the volume of Extraction Buffer to 500µL;
- A SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA) was used for sample lysis;
- Incubation steps and any shaking steps were performed on the integrated DPC shaker;
- CRS toroid magnet (P/N 5083175) was used for isolating the DNA IQ™ resin.
- Instead of a single elution of 100µL, a double elution method (2 x 50µL) is used.

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Reagents used in the automated protocol were as per the manual method.

Figure 2. The Test Outline window displaying individual nodes within the DNA IQ Extraction_Ver1.1.mpt program test file.

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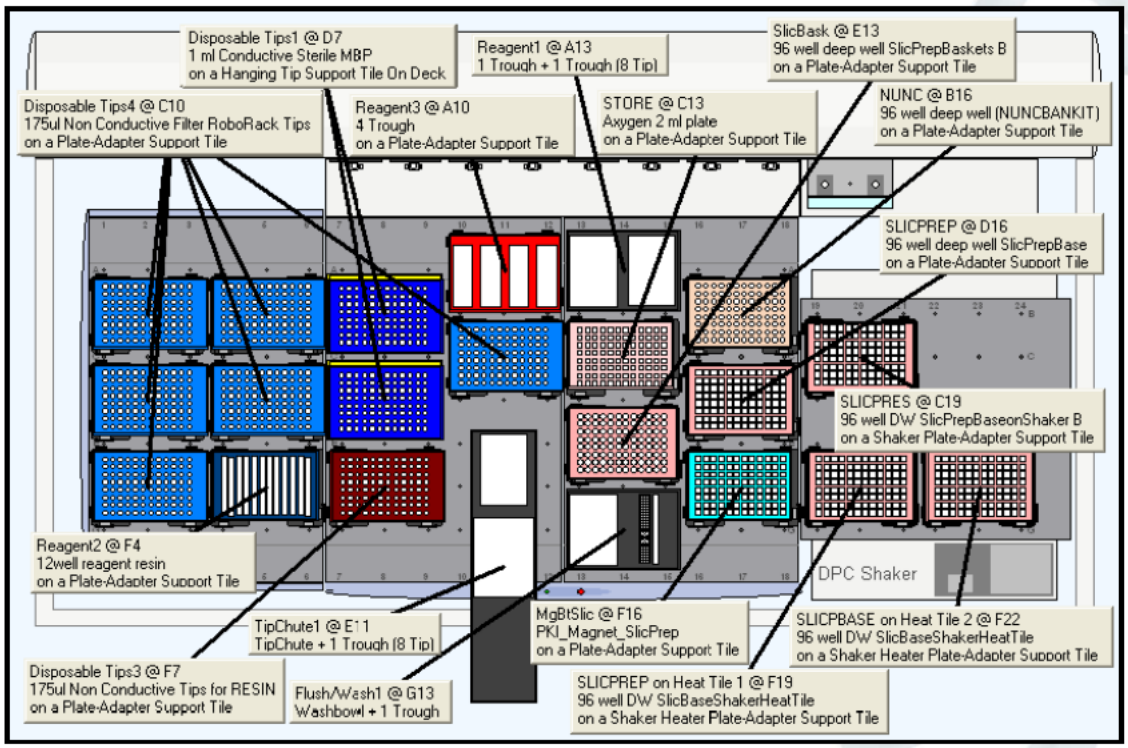

Figure 3. The deck layout for DNA IQ Extraction_Ver1.1.mpt, displaying the required labware on the platform deck.

The automated DNA IQ™ protocol was used to perform the following tests.

5.6.1. Contamination Check via Checkerboard and Zebra-stripe Patterns

Samples consisting of two 3.2mm FTA® discs (containing blood, buccal cells, or blank cards) were arranged in a checkboard and zebra-stripe pattern (Figure 4) in SlicPrep™ plates using the BSD Duet 600 instrument (BSD Robotics, Brisbane, QLD, Australia) and extracted on the MultiPROBE® II PLUS HT EX platforms using the automated DNA IQ™ protocol. One checkerboard and one zebra-stripe plate was processed on each platform.

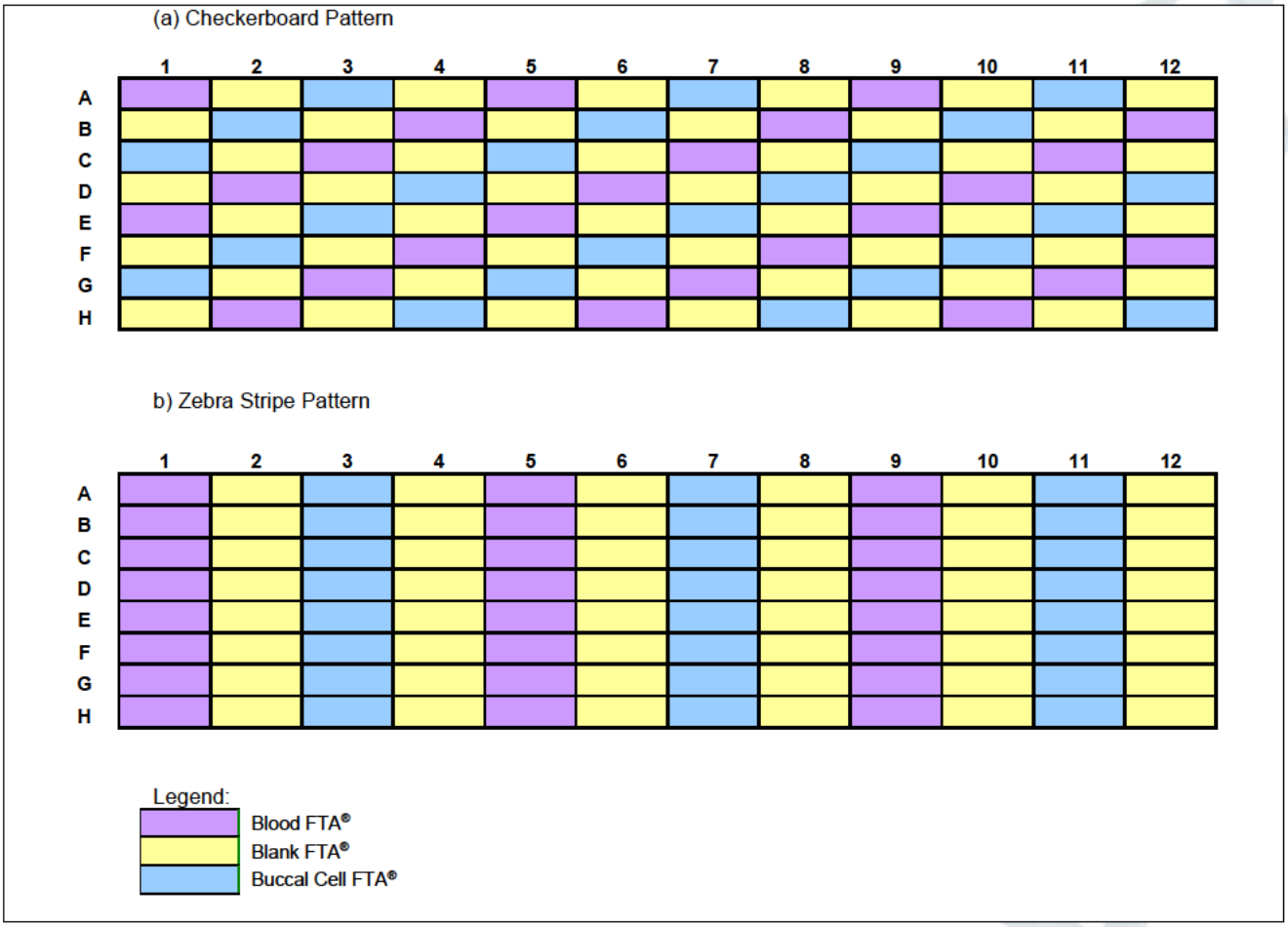

Figure 4. Checkerboard and zebra-stripe patterns utilised in the contamination check.

5.6.2. Comparisons with the manual DNA IQ™ method

Comparisons were made between results generated by the automated and manual methods to verify the performance of the automated DNA IQ™ protocol.

Verification samples consisted of different dilutions of blood and cells spotted in 30µL aliquots onto quartered cotton and rayon swabs. Four blood dilutions of neat, 1/10, 1/100 and 1/1000 and four cell dilutions of neat, 1/5.2, 1/52.2 and 1/522 were used to test the sensitivity of both the manual and automated methods. Dilutions were created using 0.9% saline solution for both sample types. Four replicates of each dilution were made up for each substrate and sample type.

The blood was collected using the same method as in 5.2. Four separate extractions were performed for the manual set based on the combination of sample type and swab type: Blood Rayon, Blood Cotton, Cell Rayon and Cell Cotton. For the automated verification, all

sample types were extracted together after being transferred to a SlicPrep™ 96 Device to allow automated processing.

5.6.3. Resin volume

The performance of the automated DNA IQ™ protocol was assessed when either 7µL or 14µL of DNA IQ™ resin was used in the protocol.

5.6.4. Modifying extraction volumes

The performance of the automated DNA IQ™ protocol was assessed for varying volumes of extraction buffer at 300, 350, 400, 450 and 500µL. In each case, the volume of DNA IQ™ Lysis Buffer was kept at 2x the volume of extraction buffer. Samples extracted were blood swabs, prepared as per ???.

5.6.5. Sensitivity of the automated DNA IQ™ protocol

The sensitivity of the automated DNA IQ™ protocol was assessed using dilutions of whole blood at neat, 1:10, 1:50, 1:100 and 1:1000.

6. Results and Discussion

6.1 Gravimetric Evaluation of Pipetting Accuracy and Precision

EXTN A:
WaterBlowoutFixedTips_08112007.prf
WaterBlowout 175 ul DT_FW 13112007RESIN.prf
WaterWasteFT_1 ml_27082007.prf
WaterBlowout 1ml DT _QHSS09112007.prf
WaterBlowout 175 ul DT_FW QHSS 13112007.prf
WaterWaste 1 ml DT_FW QHSS12112007.prf

EXTN B:
WaterBlowout 1ml DT_QHSS23102007.prf
WaterBlowoutFixed Tips_1ml_26102007.prf
WaterWaste_1ml_27082007.prf
WaterBlowout 175ul DT_FW 25102007.prf
WaterWaste 1ml DT_FW 24102007.prf
WaterBlowout 175 ul DT_FW 25102007RESIN.prf
WaterBlowout 25ul DT_FW_QHSS_060907.prf

6.2 Heater tile temperature verification

Two heater tiles on each MP II platform was verified to reach either 37ºC or 65ºC, the optimum incubation temperatures for sample lysis and DNA elution respectively (using the DNA IQ™ kit). Each tile, upon completion of the verification process, could only be used for a specific temperature, and as such was labelled appropriately to ensure use of the correct tile for specific incubation steps (Table 2).

Table 2. Verified heater tiles for use in the automated DNA IQ™ protocol.

Extraction platform	Tile number	Heater Controller Setting	Average ºC reached	Verified temperature	Incubation Step
EXTN A	3 (45W)	50ºC	37ºC	37ºC	Sample Lysis
EXTN A	1 (45W)	85ºC		65ºC	DNA Elution
EXTN B	1 (45W)	50ºC		37ºC	Sample Lysis
EXTN B	2 (45W)	85ºC	65ºC	65ºC	DNA Elution

A slight variation in the incubation temperature to achieve sample lysis is acceptable, because Proteinase K exhibits stable activity and broad specificity over a wide range of temperatures between 20-60ºC, at which the serine protease still retains greater than 80% of its activity (Sweeney & Walker, 1993).

The efficiency of the elution step is dependent on heating the sample to 65ºC in the presence of DNA IQ™ Elution Buffer (Huston, 2002). If the sample is not sufficiently heated, the extraction yield may be lower than expected. Two heater tiles were able to be verified for this crucial incubation step with minimal variation.

6.3 Contamination Check via Checkerboard and Zebra-stripe Patterns

Table 2 below lists the Extraction Batch ID's of the contamination checks.

Table 2. Extraction Batch ID's for the various contamination check plates that were processed on the MP II platforms using the automated DNA IQ™ protocol.

Type of plate	Extraction batch Id	Extraction Platform	Check passed
Checkerboard 1	VALB20070817_02	Extraction A	Invalidated
Checkerboard 2	VALB20070803_02	Extraction B	Yes
Zebra-Stripe 1	VALB20070803_03	Extraction A	Yes
Zebra-Stripe 2	VALB20070817_03	Extraction B	Yes
Checkerboard/Zebra	VALB20071022_01	Extraction A	Yes

Checkerboard 1

Position E3 (Sample Cells 6) was known to have been contaminated prior to the start of the extraction, during the STORstar process (???). The result showed a mixed DNA profile, with contributing alleles originating from the expected wells. See figure X. In addition to this contamination event, eight of the designated blank samples (positions D3, A10, F1, H5, C4, E4, B7 and E6), two of the cell samples (A1 and B10) and two of the blood samples (F4 and G7) all exhibited a partial DNA profile that was previously unknown (Table 3). This profile did not match any of the positive control samples present on the batch. The DNA profile was searched against the Staff Database and no matches were found. The source of this contaminating DNA profile could not be identified.

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None of the other blank samples yielded any DNA profile. The rest of the cell and blood samples yielded the correct DNA profile. Although there is no evidence of well-to-well contamination, the unknown DNA profile obtained has invalidated this plate. A further checkerboard/Zebra-Stripe combination plate was performed to ensure…

Table 3. The DNA profile of the unknown contaminant that was observed in Checkboard-1.

Sample description	D3	vWA	FGA	Amel	D8	D21	D18	D5	D13	D7
Blk23-E6	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	11,13
Blk25-B7	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	
Blk15-E4	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	11,13
Blk14-C4	14,17									
Blk20-H5										
Blk3-F1										
Blk10-D3										
Blk37-A10										
Cells19-B10	14,17	14,17	20,21,22,24	X,Y	11,13,16	29,30,	14,15,16	11,15	11,12	11,11
Cells13-A1										
Blood14-G7										
Blood8-F4										

Checkerboard 2

None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile. Figure 5 illustrates the DNA quantitation results from this plate. DNA was not detected in any of the blank samples.

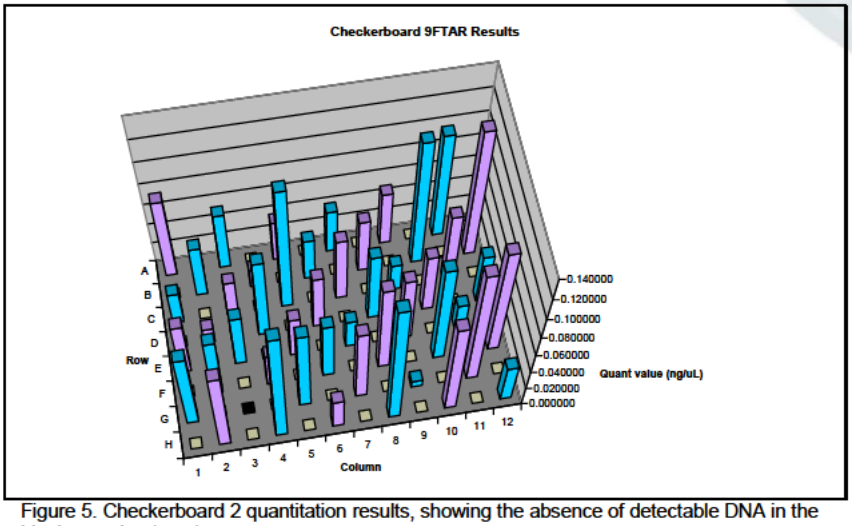

Figure 5. Checkerboard 2 quantitation results, showing the absence of detectable DNA in the blank samples (grey).

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Zebra-Stripe 1
None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile. Figure 6 illustrates the absence of detectable DNA in the blank samples.

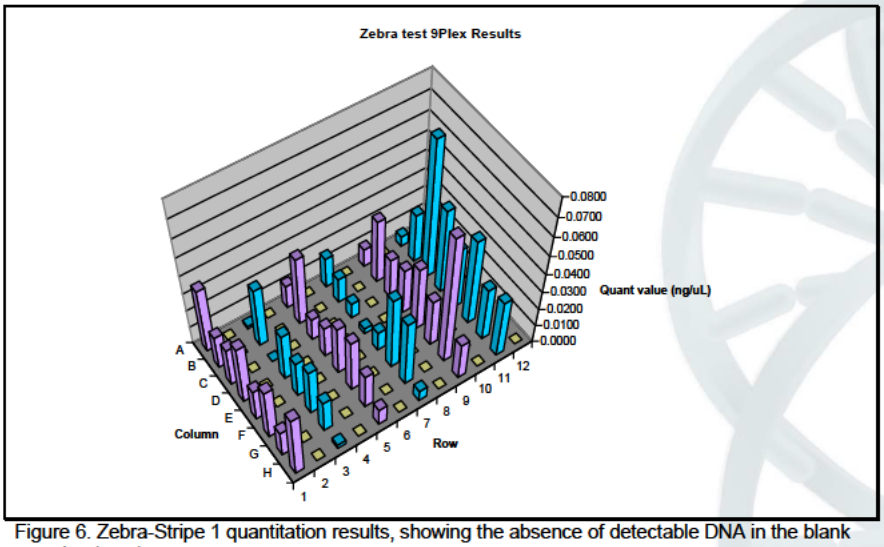

Figure 6. Zebra-Stripe 1 quantitation results, showing the absence of detectable DNA in the blank samples (grey).

Zebra-Stripe 2
None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile. Figure 7 shows the absence of detectable DNA in the blank samples.

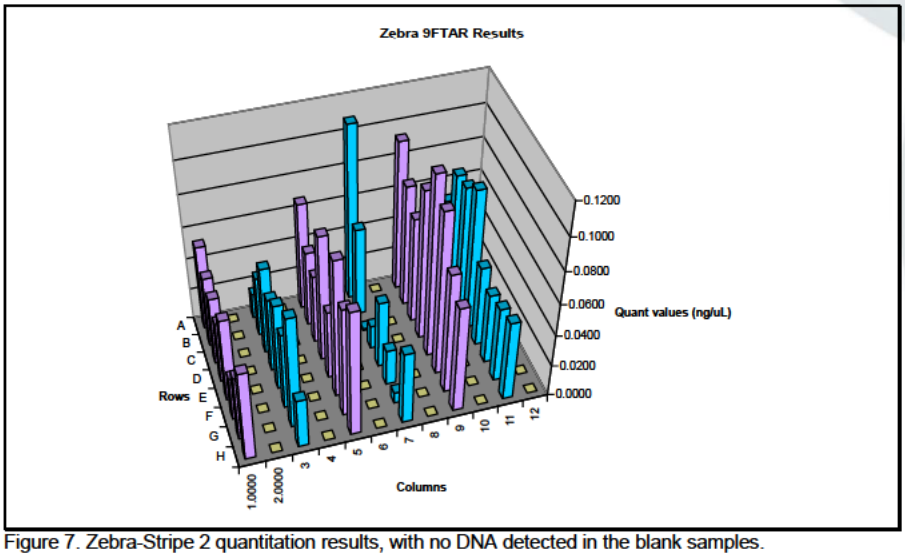

Figure 7. Zebra-Stripe 2 quantitation results, with no DNA detected in the blank samples.

Checkerboard/Zebra
None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile. DNA was undetected in the blank samples (Figure 8).

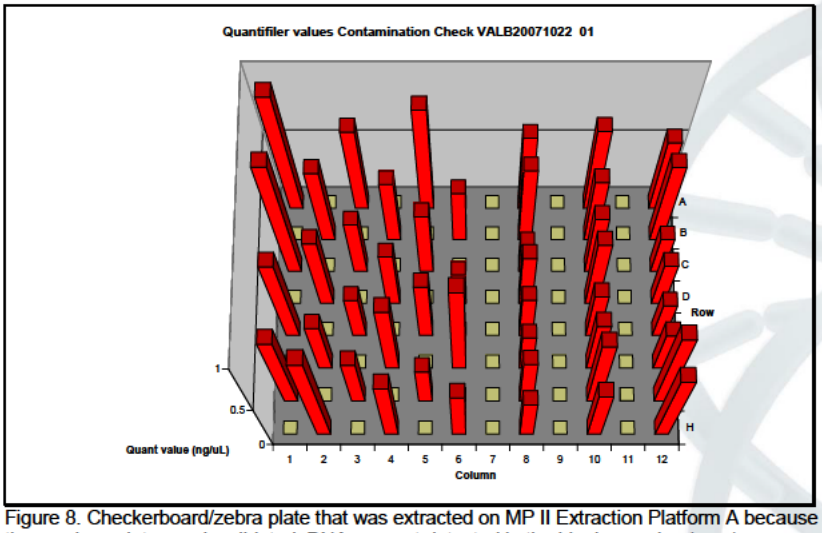

Figure 8. Checkerboard/zebra plate that was extracted on MP II Extraction Platform A because the previous plate was invalidated. DNA was not detected in the blank samples (grey).

6.4 Comparisons with the manual DNA IQ™ method

When dilutions of either blood or cells were applied on to either rayon or cotton swabs, followed by extraction using the DNA IQ™ method, the results of the automated method were always lower in yield compared to the manual method. For blood samples on rayon swabs, the automated method generated yields that were on average around 8% (SD 8.45%) of the automated method. For blood on cotton swabs, the yield from the automated method was also around 8% (SD 3.62%). The yields for cell samples were more comparable at around 33% (SD 16.29%) and 25% (10.32%) for cells on rayon and cotton swabs respectively.

The manual method was found to be more sensitive than the automated method. Out of five replicates at the 1/100 and 1/1000 dilutions for blood on rayon swabs that were processed using the manual method, five and three replicates respectively were detected (and none from the automated method) (see Figure X). The trend is repeated for blood on cotton swabs (Figure XX). For cell samples on either rayon or cotton swabs, the automated method was found to be more sensitive as evidenced by detection of DNA at the 1/522 dilutions (Figure XXX and XXXX).

Cell clumping may have occurred with the cell dilutions, therefore causing inaccurate dilutions as can be observed in the ratios between each dilution.

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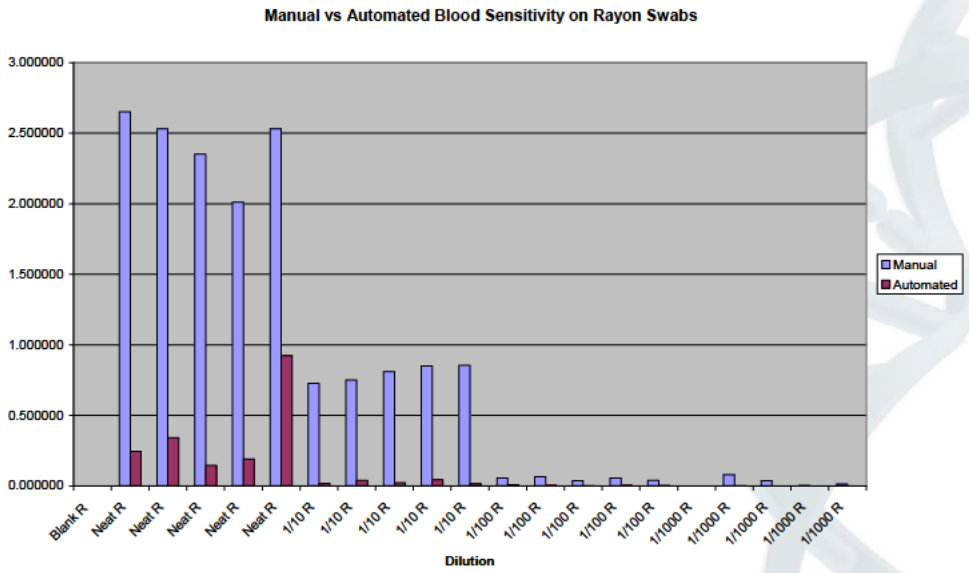

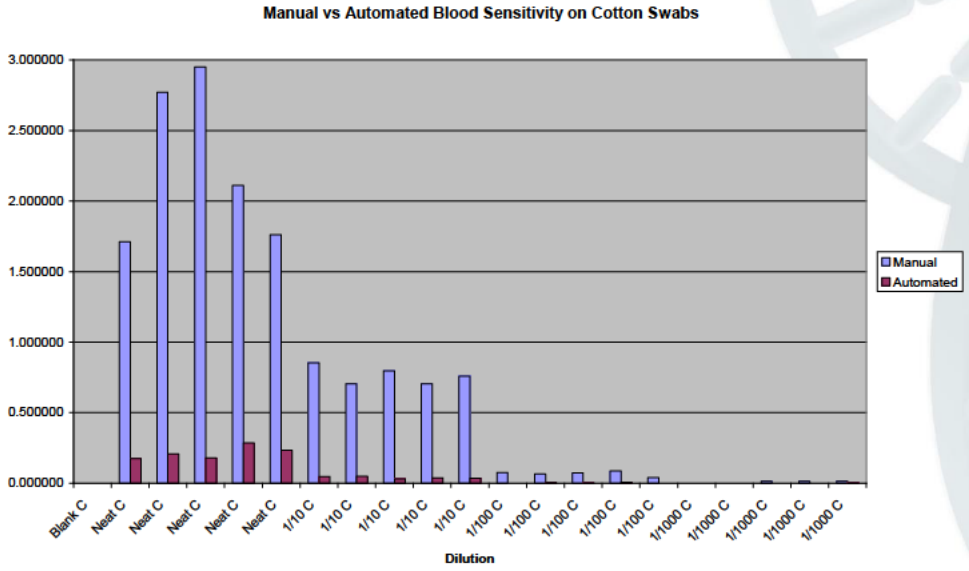

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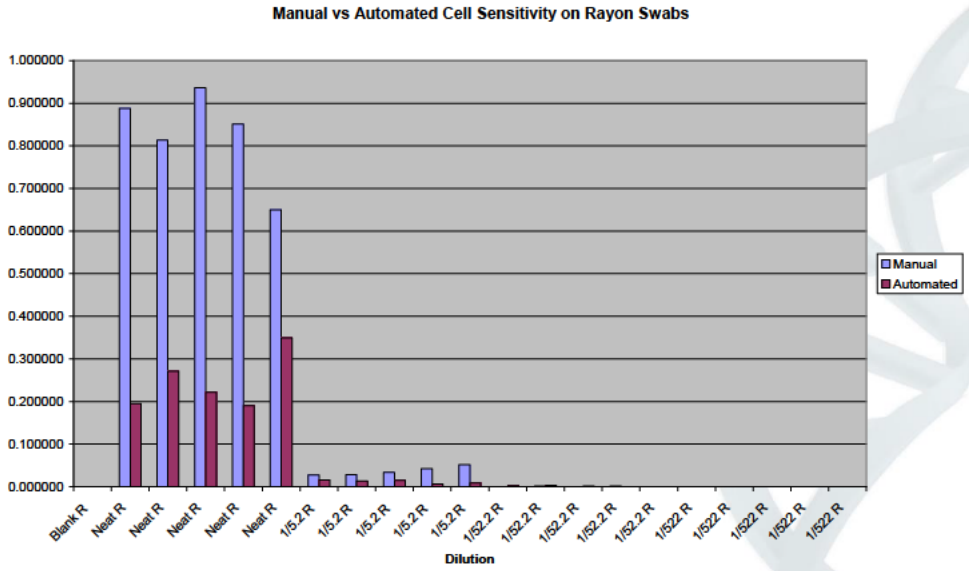

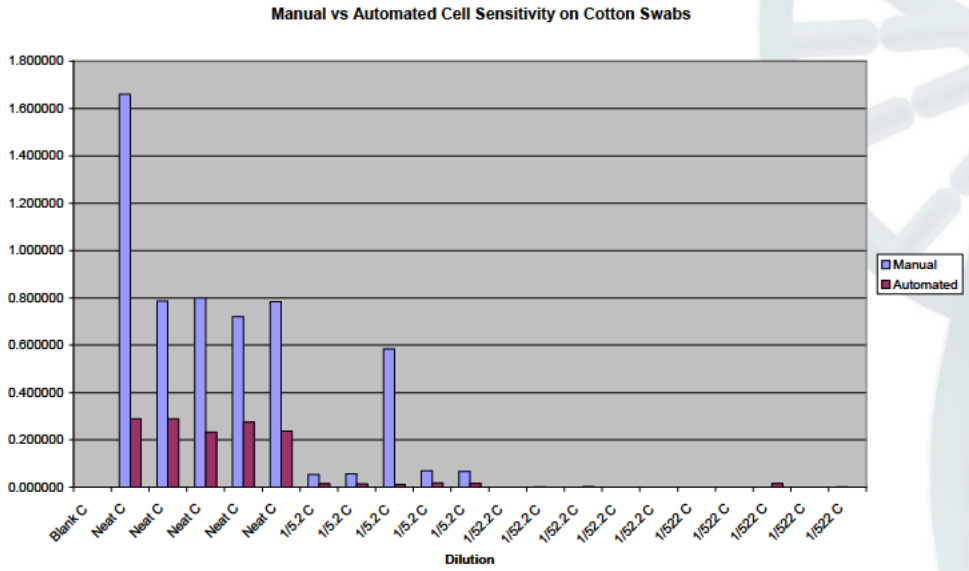

6.5 Protocol

7. Summary and Recommendations

We recommend the following:

- Use of MPII for automated extraction of reference samples

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- Use of MPII for automated extraction of casework samples
- Ongoing development of the automated extraction program to increase the efficiency of the extraction

Sweeney, P.J. and Walker, J.M., Burrell, M.M., Enzymes of molecular biology. *Methods Mol. Biol.* Towanam NJ , (1993) **16**, 306

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Project 13. Report on the Verification of an Automated DNA IQ™ Protocol using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform

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Automation/LIMS Implementation Project, DNA Analysis FSS (August 2008)

1. Abstract

A manual method for extracting DNA from forensic samples using the DNA IQ™ system (Promega Corp., Madison, WI, USA) was validated for routine use in DNA Analysis (FSS). We have verified an automated DNA IQ™ protocol in 96-well format for use on the MultiPROBE® II PLUS HT EX Forensic Workstation platforms (PerkinElmer, Downers Grove, IL, USA). Data indicate that results from the automated procedure are comparable to those from the manual procedure. Contamination checks were performed using samples prepared in checkerboard and zebra-stripe format, and results were as expected. We recommend the use of the MultiPROBE® II PLUS HT EX platforms to perform automated DNA extraction using the DNA IQ™ system.

2. Introduction

The MultiPROBE® II PLUS HT EX FORENSIC WORKSTATION platforms (PerkinElmer, Downers Grove, IL, USA) are equipped to perform automated DNA extractions, as they include a DPC shaker and individual heat controllers to enable on-board lysis and incubation steps. Currently in DNA Analysis, the MultiPROBE® platforms allow walk-away operation of PCR setup protocols for DNA quantitation and amplification.

The DNA IQ™ protocol has been verified or validated by various laboratories for use on the MultiPROBE® II PLUS platform. The laboratories that perform an automated DNA IQ™ protocol include PathWest (Western Australia), Forensic Science South Australia (South Australia) and Centre of Forensic Sciences in Toronto (Ontario). The MultiPROBE® II PLUS instrument comes pre-loaded with an automated DNA IQ™ protocol. Unlike the other laboratories, however, we did not validate the included protocol, but instead validated a manual DNA IQ™ protocol which was based on the CFS automated protocol (PerkinElmer, 2004), followed by verification of an automated protocol based on the validated manual method.

The verified automated DNA IQ™ protocol is identical to the validated manual protocol used in-house: there are no differences in reagents or volumes. The adopted DNA IQ™ protocol differs slightly, however, from the manufacturer's protocol, as it includes a lysis step using Extraction Buffer (10mM Tris, 1mM EDTA, 100mM NaCl, 20% SDS) in the presence of Proteinase K, before incubating in the DNA IQ™ Lysis Buffer. Furthermore, the lysis incubation conditions were lowered from 70°C to 37°C in order to accommodate extraction of DNA from heat labile materials such as nylon and polyester. In addition, the automated protocol utilises the SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA) for simultaneous processing of samples in a 96-well format.

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3. Aim

To verify an automated DNA IQ™ protocol for use on the MultiPROBE® II PLUS HT EX platforms to allow extraction of DNA from various sample types.

4. Equipment and Materials

- MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (PerkinElmer, Downers Grove, IL, USA)
- Gravimetric Performance Evaluation Option with Mettler SAG285/L balance (Mettler-Toledo, Greifensee, Switzerland)
- DNA IQ™ System (Promega Corp., Madison, WI, USA)
- Extraction Buffer (10mM Tris, 1mM EDTA, 100mM NaCl, 20% SDS)
- SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA)
- Nunc™ Bank-It tubes (Nunc A/S, Roskilde, Denmark)
- 175µL non-conductive sterile filter RoboRack tips (PerkinElmer, Downers Grove, IL, USA)
- 1000µL Conductive sterile filter Robotix tips (Molecular BioProducts, San Diego, CA, USA)
- ABI Prism® 7000 SDS (Applied Biosystems, Foster City, CA, USA)
- Quantifiler™ Human DNA Quantification kits (Applied Biosystems, Foster City, CA, USA)
- AmpFℓSTR® Profiler Plus Amplification kits (Applied Biosystems, Foster City, CA, USA)
- GeneAmp® 9700 thermalcycers (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 96-well optical reaction plates (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA)
- GeneScan™ 500 ROX™ Size Standard (Applied Biosystems, Foster City, CA, USA)
- Hi-Di™ Formamide (Applied Biosystems, Foster City, CA, USA)
- 3100 POP-4™ Polymer (Applied Biosystems, Foster City, CA, USA)
- Cytobrush® Plus Cell Collector (Cooper Surgical, Inc., Trumbull, CT, USA)
- 0.9% saline solution (Baxter Healthcare, Old Toongabbie, NSW, Australia)
- Stem digital tilting head thermometer
- For mock samples:
  - FTA™® Classic Card (Whatman Inc., Florham Park, NJ, USA)
  - Sterile cotton swabs (Medical Wire & Equipment, Corsham, Wiltshire, England)
  - Sterile rayon swabs (Copan Italia SPA, Brescia, Italy)

5. Methods

5.1 Gravimetric Evaluation of Pipetting Accuracy and Precision

Gravimetric analysis was performed by placing the SAG285/L balance on the platform deck and instructing the MP II to repeatedly pipette certain volumes of system liquid onto the balance pan. Readings were taken automatically by the software and compiled into a results table, which was then used to automatically generate an Excel-based results chart containing mean, %CV and %inaccuracy values. The mean values obtained were used to calculate R^2, slope and Y-intercept (offset) values to calibrate the system's pipetting.

Pipetting performance was assessed for various volumes using three different tips in order to calculate appropriate R^2, slope and Y-intercept (offset) values which were then added to the performance file. Values were calculated for both Blowout (single-liquid transfer) and Waste (multidispense) modes for the 1000µL conductive tips, and Blowout mode only for the 175µL non-conductive tips and fixed tips.

For the addition of resin, a specialised performance file was created based on the performance file for 175µL tips in blowout mode, except the "Blowout Volume" column values were set to 0 to allow pipetting performance that is similar to waste mode. Retesting was performed to confirm accurate and precise pipetting with these settings.

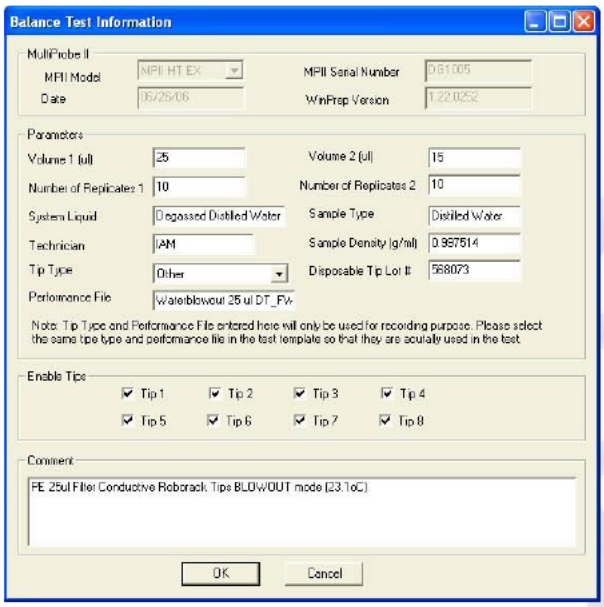

Figure 1. The Balance Test Information Window as present within the Balance Test DT program. All pipetting parameters are entered here and are subsequently transferred to the result output file.

All gravimetric testing was performed using the Balance Test DT test program within WinPrep®. Parameter values that needed to be entered into the Balance Test Information Window (Figure 1) included those as outlined in Table 1.

Table 1. Input values that are required for the various Balance Test Information parameters.

Parameter(s)	Value
Volume 1 and Volume 2	For 175µL tips: 175, 100, 50, 15µL For 1000µL tips: 1000, 700, 400, 100µL For fixed tips: 1000, 700, 400, 100µL
Number of Replicates	10
System Liquid	Degassed Nanopure Water
Sample Type	Nanopure Water
Technician	Initials of the operator performing the test
Sample Density (g/ml)	The density of water at environmental temperature*
Tip Type	Other
Disposable Tip Lot #	The lot number of the particular tips in use
Performance File	The appropriate Performance File for the tip (175µL, 1000µL or fixed tips) and pipetting mode (Blowout or Waste) in use
Enable Tips (checkboxes)	Select the actual tips (1 to 8) to be tested
Comments	Free text box to add additional information (eg. Tip type, mode, current environmental room temperature, etc).

* Water density values were obtained from http://www.simetric.co.uk/si_water.htm

Pipetting accuracy and precision were examined at four different volumes for each tip size: 175, 100, 50, 15µL for 175µL tips and 1000, 700, 400, 100µL for the 1000µL and fixed tips. In order to calculate unbiased values for each set of volumes, the slope and offset values in the relevant Performance File were changed to the default 1 and 0 respectively prior to

testing. The mean volumes that were pipetted by each tip (10 replicates per tip) at the four designated volumes were used to generate a standard curve. The slope and offset calculated from this curve was used to calibrate the relevant Performance File. The final Performance File settings were then tested at the highest and lowest volumes (as per Table 1) to confirm accurate and precise pipetting.

5.2 Blood Collection

Blood samples were collected from 2 staff donors (DJC/VKI) by a phlebotomist as per normal in three 4mL EDTA vials. Blood samples were stored at 4ºC.

5.3 Cell Collection

Buccal cells were collected using a modified Cytobrush® protocol (Mulot *et al*., 2005; Satia-Abouta *et al*., 2002). The donor was instructed to brush the inside of one cheek for one minute using a Cytobrush®. Then, with another Cytobrush®, the other cheek was also sampled. Once each cheek was swabbed, the cells on the brush were suspended in 2mL of 0.9% saline solution. Buccal cell samples were stored at 4ºC.

5.4 FTA cell Collection

Cells were collected from two staff donors (VKI/CJA) by using a "lolly-pop" swab to sample the inside of the donor's cheek for 15 seconds before pressing the swab onto the FTA™ paper to transfer the DNA. FTA™ cards were stored at room temperature.

5.5 Heater tile temperature verification

Heat tiles supplied with the MultiPROBE® II PLUS HT EX platforms were modified to accept the SlicPrep™ 96 Device. For testing, 1mL of nanopure water (at room temperature) was added to each well. The plate was then placed on a heater tile (controlled by the MP II heater controller) and allowed to reach temperature. The temperatures tested were 37ºC and 65ºC. Temperature readings for specific outer and inner wells (i.e. A1, A6, A12, D1, D6, D12, H1, H6, H12) were taken at regular intervals up to and including 45 minutes, using calibrated stem digital tilted head thermometer probes. The data were collated and means calculated to determine the distribution of heat over the tile.

5.6 Verification of automated DNA IQ™ Protocol

The automated DNA IQ™ protocol, based on the validated manual method (refer to Project 11), was programmed in WinPrep™ software. The final, optimised protocol was named "DNA IQ Extraction_Ver1.1.mpt". A screenshot of the Test Outline window for this protocol is depicted below in Figure 2. The deck layout is illustrated in Figure 3.

The automated DNA IQ™ protocol was designed to mimic the validated manual method, with minor modifications. Briefly, the changes include:

- Increasing the volume of Extraction Buffer to 500µL;
- A SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA) was used for sample lysis;
- Incubation steps and any shaking steps were performed on the integrated DPC shaker;
- CRS toroid magnet (P/N 5083175) was used for isolating the DNA IQ™ resin.
- Instead of a single elution of 100µL, a double elution method (2 x 50µL) is used.

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Reagents used in the automated protocol were as per the manual method.

Figure 2. The Test Outline window displaying individual nodes within the DNA IQ Extraction_Ver1.1.mpt program test file.

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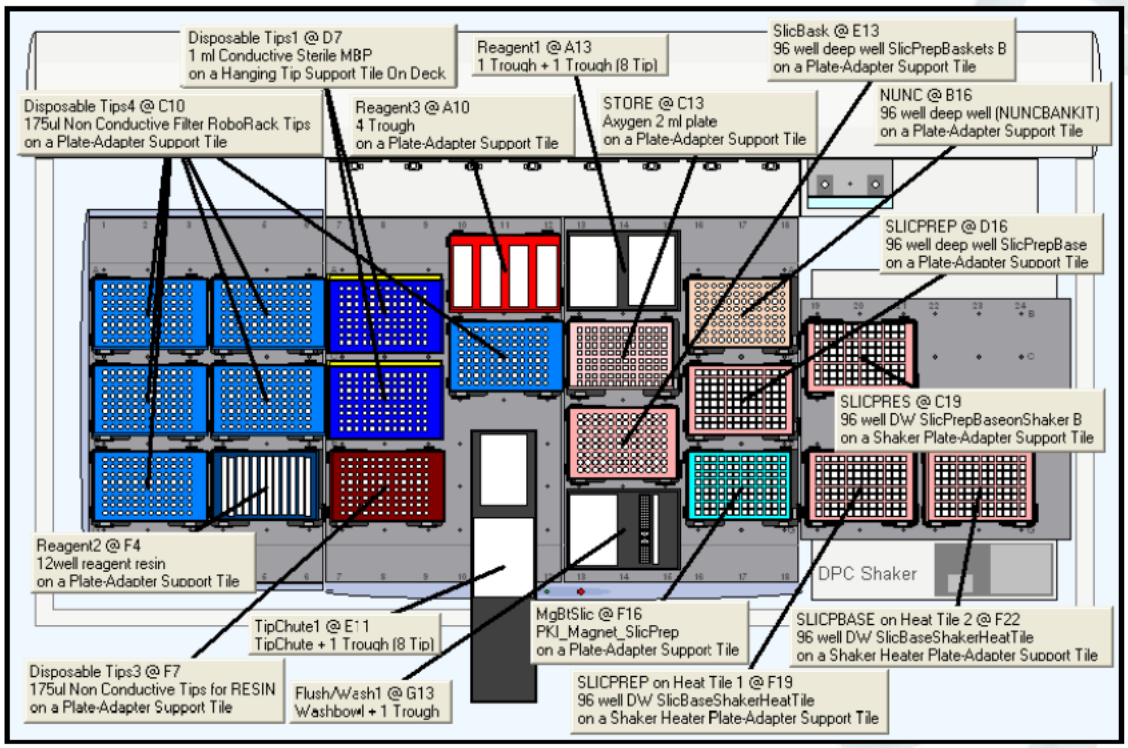

Figure 3. The deck layout for DNA IQ Extraction_Ver1.1.mpt, displaying the required labware on the platform deck.

The automated DNA IQ™ protocol was used to perform the following tests.

5.6.1. Contamination Check via Checkerboard and Zebra-stripe Patterns

Samples consisting of two 3.2mm FTA® discs (containing blood, buccal cells, or blank cards) were arranged in a checkerboard and zebra-stripe pattern (Figure 4) in SlicPrep™ plates using the BSD Duet 600 instrument (BSD Robotics, Brisbane, QLD, Australia) and extracted on the MultiPROBE® II PLUS HT EX platforms using the automated DNA IQ™ protocol. One checkerboard and one zebra-stripe plate was processed on each platform.

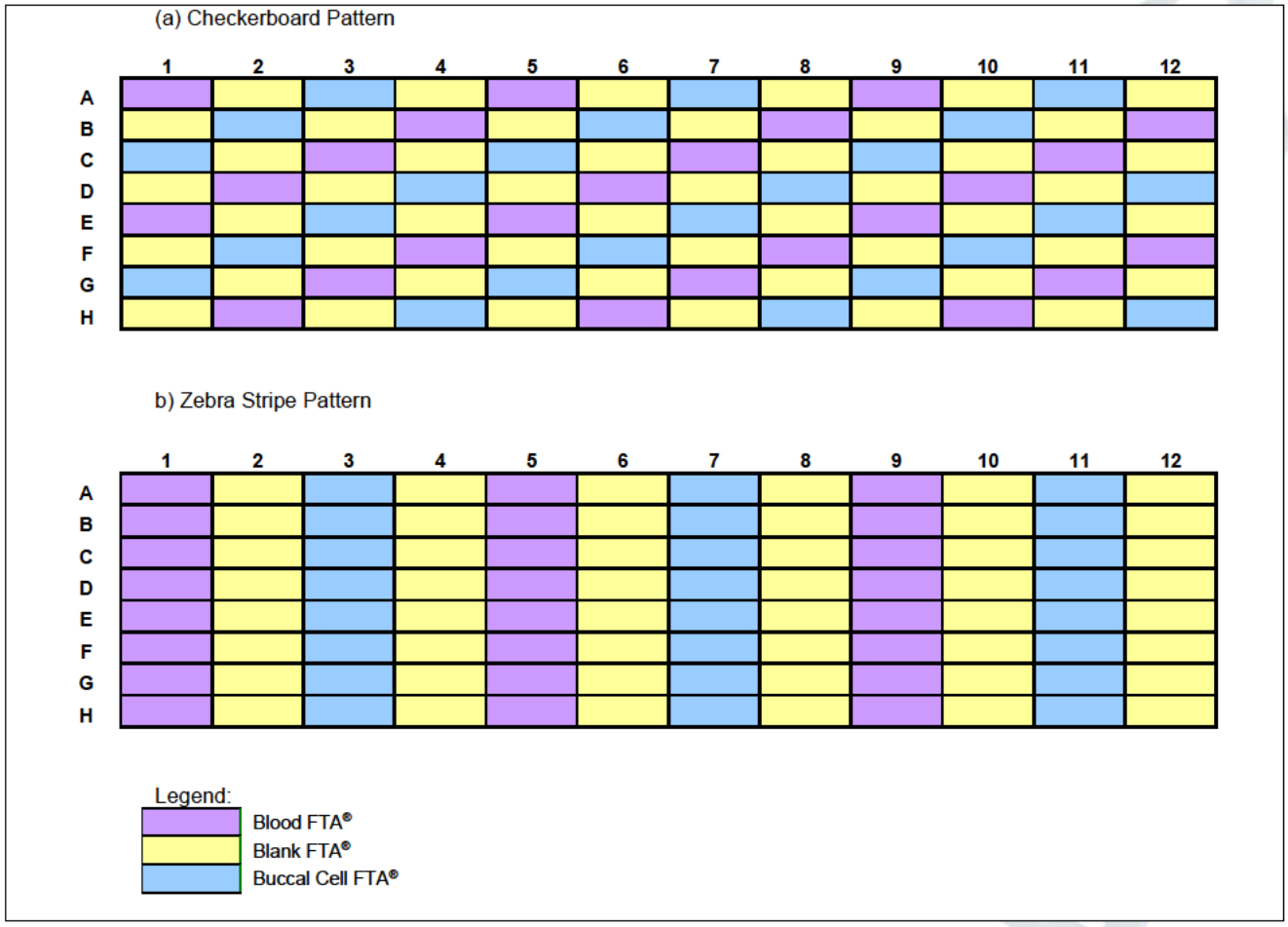

Figure 4. Checkerboard and zebra-stripe patterns utilised in the contamination check.

5.6.2. Comparisons with the manual DNA IQ™ method

Comparisons were made between results generated by the automated and manual methods to verify the performance of the automated DNA IQ™ protocol.

Verification samples consisted of different dilutions of blood and cells spotted in 30µL aliquots onto quartered cotton and rayon swabs. Four blood dilutions of neat, 1/10, 1/100 and 1/1000 and four cell dilutions of neat, 1/5.2, 1/52.2 and 1/522 were used to test the sensitivity of both the manual and automated methods. Dilutions were created using 0.9% saline solution for both sample types. Four replicates of each dilution were made up for each substrate and sample type.

The blood was collected using the same method as in 5.2. Four separate extractions were performed for the manual set based on the combination of sample type and swab type: Blood Rayon, Blood Cotton, Cell Rayon and Cell Cotton. For the automated verification, all

sample types were extracted together after being transferred to a SlicPrep™ 96 Device to allow automated processing.

5.6.3. Resin volume

The performance of the automated DNA IQ™ protocol was assessed when either 7µL or 14µL of DNA IQ™ resin was used in the protocol to extract blood samples.

5.6.4. Modifying extraction volumes

The performance of the automated DNA IQ™ protocol was assessed for varying volumes of extraction buffer at 300, 350, 400, 450 and 500µL. In each case, the volume of DNA IQ™ Lysis Buffer was kept at 2x the volume of extraction buffer. Samples extracted were blood swabs, prepared as per ???.

5.6.5. Sensitivity of the automated DNA IQ™ protocol

The sensitivity of the automated DNA IQ™ protocol was assessed using dilutions of whole blood at neat, 1:10, 1:50, 1:100 and 1:1000.

6. Results and Discussion

6.1 Gravimetric Evaluation of Pipetting Accuracy and Precision

EXTN A:
WaterBlowoutFixedTips_08112007.prf
WaterBlowout 175 ul DT_FW 13112007RESIN.prf
WaterWasteFT_1 ml_27082007.prf
WaterBlowout 1ml DT _QHSS09112007.prf
WaterBlowout 175 ul DT_FW QHSS 13112007.prf
WaterWaste 1 ml DT_FW QHSS12112007.prf

EXTN B:
WaterBlowout 1ml DT_QHSS23102007.prf
WaterBlowoutFixed Tips_1ml_26102007.prf
WaterWaste_1ml_27082007.prf
WaterBlowout 175ul DT_FW 25102007.prf
WaterWaste 1ml DT_FW 24102007.prf
WaterBlowout 175 ul DT_FW 25102007RESIN.prf
WaterBlowout 25ul DT_FW_QHSS_060907.prf

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6.2 Heater tile temperature verification

Two heater tiles on each MP II platform was verified to reach either 37ºC or 65ºC, the optimum incubation temperatures for sample lysis and DNA elution respectively (using the DNA IQ™ kit). Each tile, upon completion of the verification process, could only be used for a specific temperature, and as such was labelled appropriately to ensure use of the correct tile for specific incubation steps (Table 2).

Table 2. Verified heater tiles for use in the automated DNA IQ™ protocol.

Extraction platform	Tile number	Heater Controller Setting	Average ºC reached	Verified temperature	Incubation Step
EXTN A	3 (45W)	50ºC	37ºC	37ºC	Sample Lysis
EXTN A	1 (45W)	85ºC		65ºC	DNA Elution
EXTN B	1 (45W)	50ºC		37ºC	Sample Lysis
EXTN B	2 (45W)	85ºC	65ºC	65ºC	DNA Elution

A slight variation in the incubation temperature to achieve sample lysis is acceptable, because Proteinase K exhibits stable activity and broad specificity over a wide range of temperatures between 20-60ºC, at which the serine protease still retains greater than 80% of its activity (Sweeney & Walker, 1993).

The efficiency of the elution step is dependent on heating the sample to 65ºC in the presence of DNA IQ™ Elution Buffer (Huston, 2002). If the sample is not sufficiently heated, the extraction yield may be lower than expected. Two heater tiles were able to be verified for this crucial incubation step, with both tiles exhibiting minimal variation.

6.3 Contamination Check via Checkerboard and Zebra-stripe Patterns

Table 2 below lists the Extraction Batch ID's of the contamination checks.

Table 2. Extraction Batch ID's for the various contamination check plates that were processed on the MP II platforms using the automated DNA IQ™ protocol.

Type of plate	Extraction batch Id	Extraction Platform	Check passed
Checkerboard 1	VALB20070817_02	Extraction A	Invalidated
Checkerboard 2	VALB20070803_02	Extraction B	Yes
Zebra-Stripe 1	VALB20070803_03	Extraction A	Yes
Zebra-Stripe 2	VALB20070817_03	Extraction B	Yes
Checkerboard/Zebra	VALB20071022_01	Extraction A	Yes

Checkerboard 1

Position E3 (Sample Cells 6) was known to have been contaminated prior to the start of the extraction, during the STORstar process (???). The result showed a mixed DNA profile, with contributing alleles originating from the expected wells. See figure X. In addition to this contamination event, eight of the designated blank samples (positions D3, A10, F1, H5, C4, E4, B7 and E6), two of the cell samples (A1 and B10) and two of the blood samples (F4 and G7) all exhibited a partial DNA profile that was previously unknown (Table 3). This profile did not match any of the positive control samples present on the batch. The DNA profile was searched against the Staff Database and no matches were found. The source of this contaminating DNA profile could not be identified.

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None of the other blank samples yielded any DNA profile. The rest of the cell and blood samples yielded the correct DNA profile. Although there is no evidence of well-to-well contamination, the unknown DNA profile obtained has invalidated this plate. A further checkerboard/Zebra-Stripe combination plate was performed to ensure…

Table 3. The DNA profile of the unknown contaminant that was observed in Checkboard-1.

Sample description	D3	vWA	FGA	Amel	D8	D21	D18	D5	D13	D7
Blk23-E6	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	11,13
Blk25-B7	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	
Blk15-E4	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	11,13
Blk14-C4	14,17									
Blk20-H5										
Blk3-F1										
Blk10-D3										
Blk37-A10										
Cells19-B10	14,17	14,17	20,21,22,24	X,Y	11,13,16	29,30,	14,15,16	11,15	11,12	11,11
Cells13-A1										
Blood14-G7										
Blood8-F4										

Checkerboard 2

None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile. Figure 5 illustrates the DNA quantitation results from this plate. DNA was not detected in any of the blank samples.

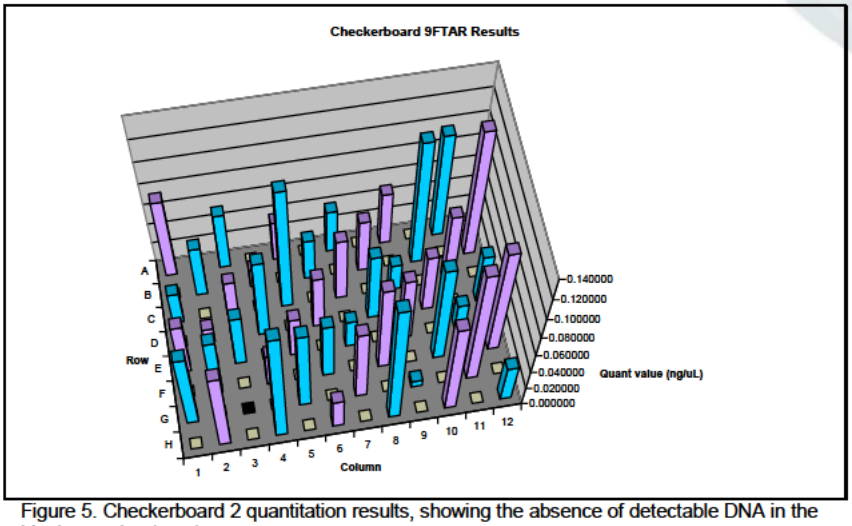

Figure 5. Checkerboard 2 quantitation results, showing the absence of detectable DNA in the blank samples (grey).

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Zebra-Stripe 1
None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile. Figure 6 illustrates the absence of detectable DNA in the blank samples.

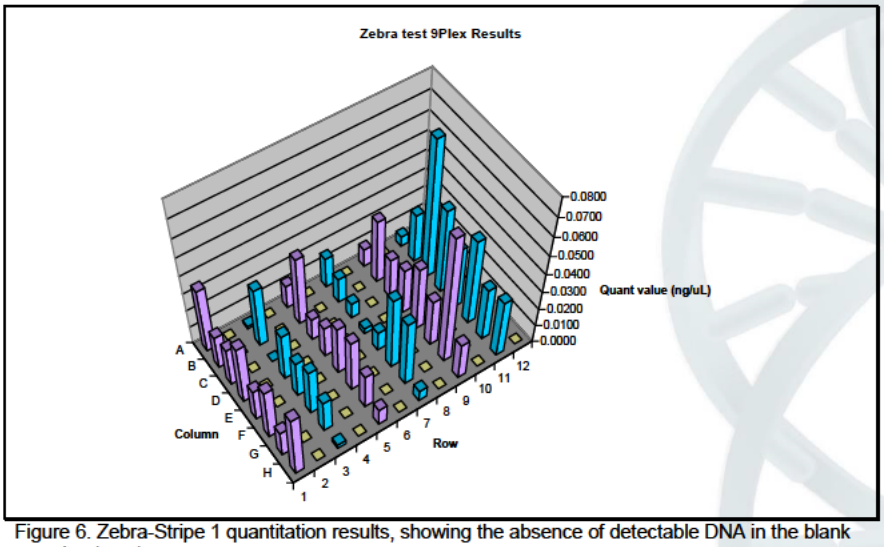

Figure 6. Zebra-Stripe 1 quantitation results, showing the absence of detectable DNA in the blank samples (grey).

Zebra-Stripe 2
None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile. Figure 7 shows the absence of detectable DNA in the blank samples.

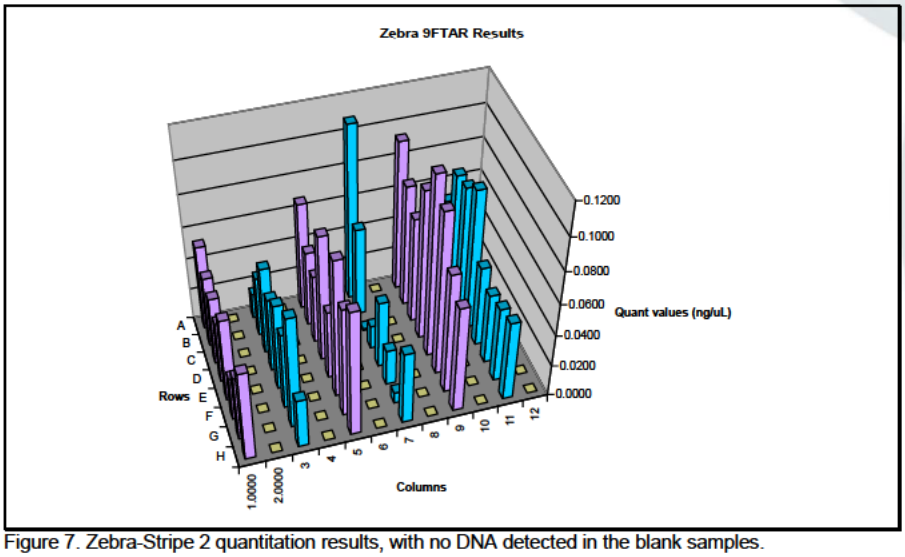

Figure 7. Zebra-Stripe 2 quantitation results, with no DNA detected in the blank samples.

Checkerboard/Zebra
None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile. DNA was undetected in the blank samples (Figure 8).

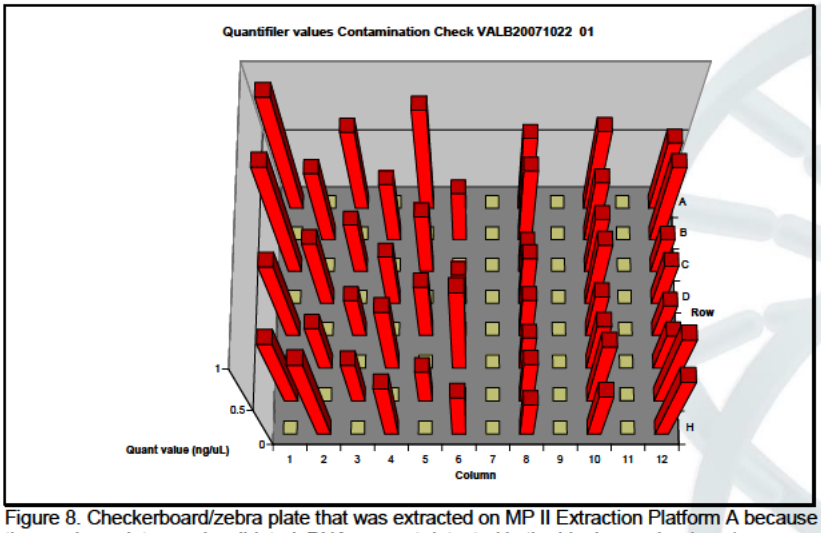

Figure 8. Checkerboard/zebra plate that was extracted on MP II Extraction Platform A because the previous plate was invalidated. DNA was not detected in the blank samples (grey).

6.4 Comparisons with the manual DNA IQ™ method

When dilutions of either blood or cells were applied on to either rayon or cotton swabs, followed by extraction using the DNA IQ™ method, the results of the automated method were always lower in yield compared to the manual method. For blood samples on rayon swabs, the automated method generated yields that were on average around 8% (SD 8.45%) of the automated method. For blood on cotton swabs, the yield from the automated method was also around 8% (SD 3.62%). The yields for cell samples were higher at around 33% (SD 16.29%) and 25% (10.32%) for cells on rayon and cotton swabs respectively.

The manual method was found to be more sensitive than the automated method. Out of five replicates at the 1/100 and 1/1000 dilutions for blood on rayon swabs that were processed using the manual method, five and three replicates respectively were detected (and none from the automated method) (see Figure 9). The trend is repeated for blood on cotton swabs (Figure 10). For cell samples on either rayon or cotton swabs, the automated method was found to be more sensitive as evidenced by detection of DNA at the 1/522 dilutions (Figure 11 and 12).

Cell clumping may have occurred with the cell dilutions, therefore causing inaccurate dilutions as can be observed in the ratios between each dilution.

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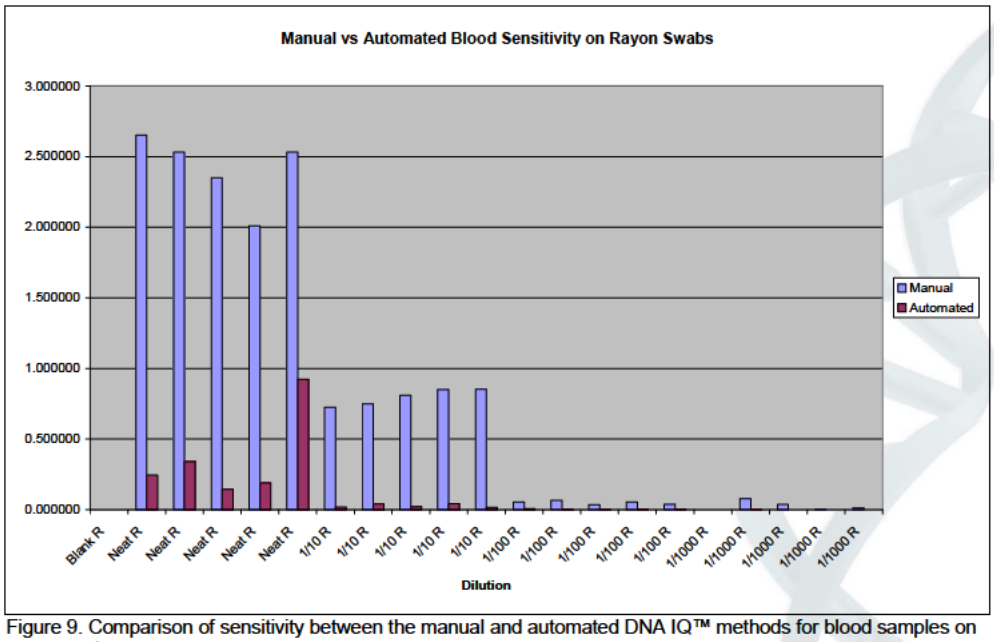

Figure 9. Comparison of sensitivity between the manual and automated DNA IQ™ methods for blood samples on rayon swabs.

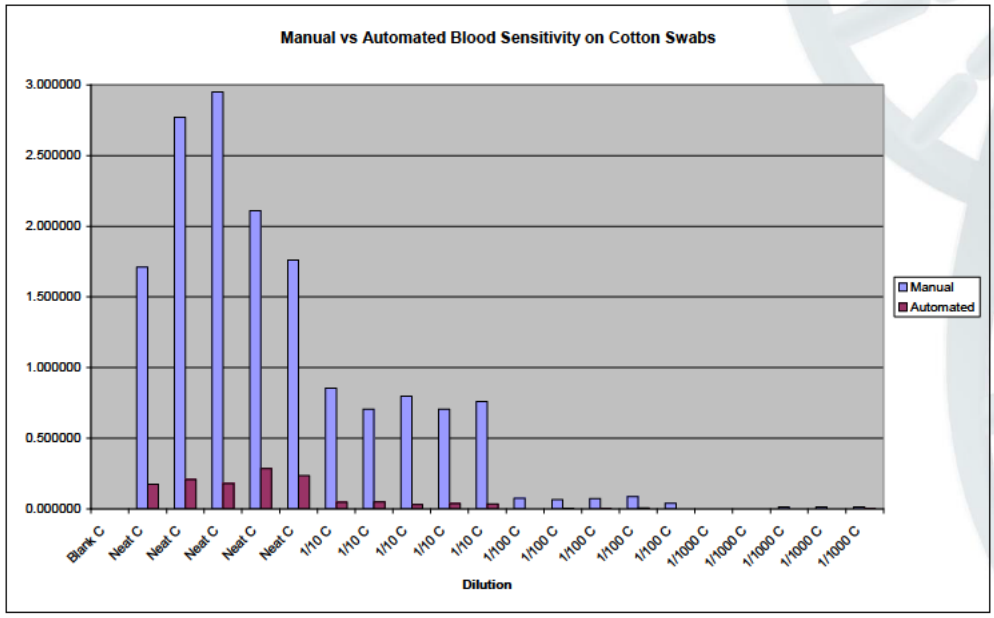

Figure 10. Comparison of sensitivity between the manual and automated DNA IQ™ methods for blood samples on cotton swabs.

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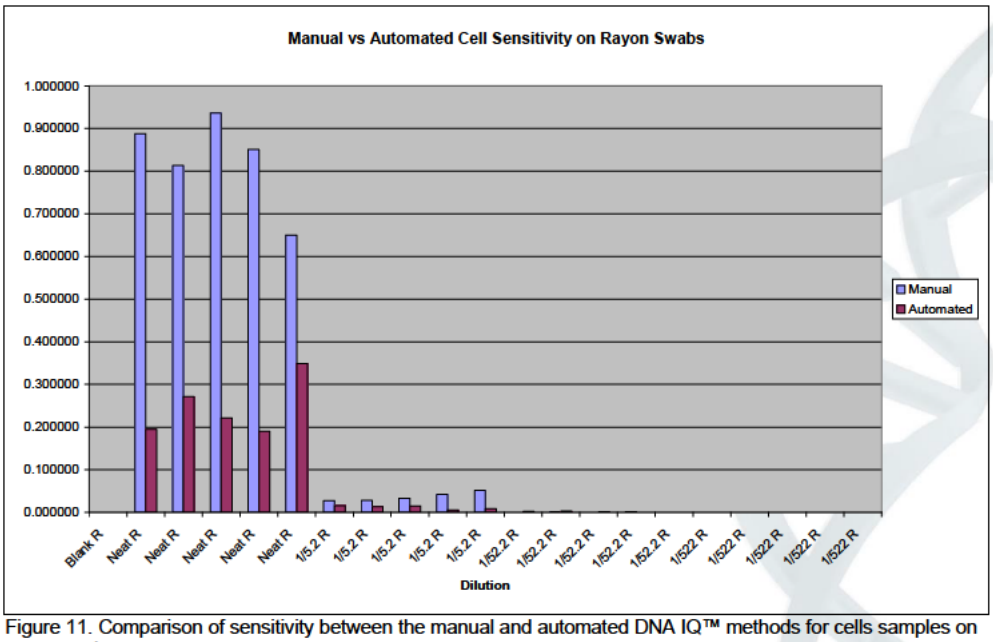

Figure 11. Comparison of sensitivity between the manual and automated DNA IQ™ methods for cells samples on rayon swabs.

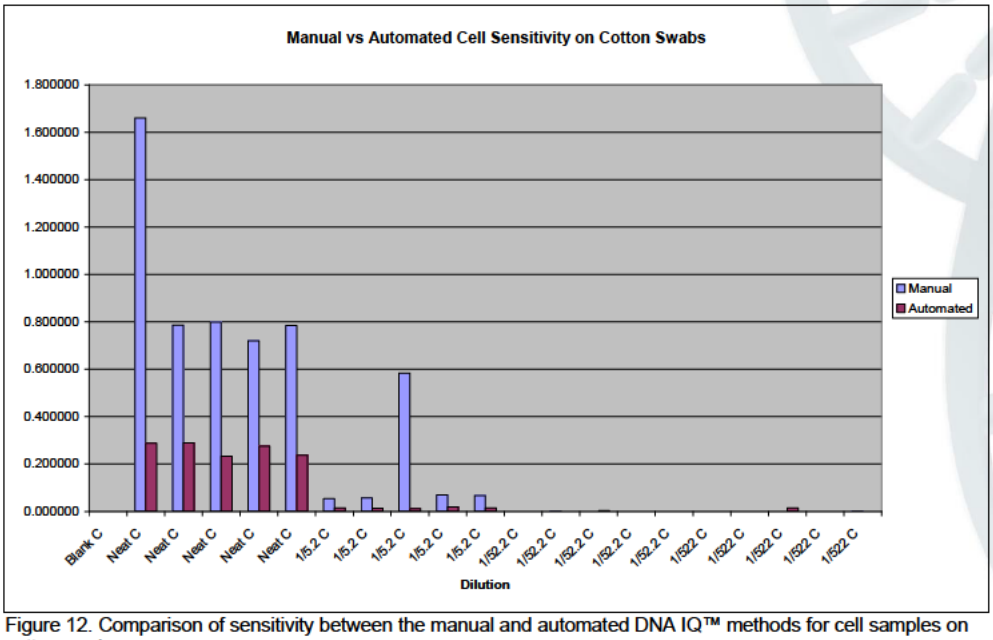

Figure 12. Comparison of sensitivity between the manual and automated DNA IQ™ methods for cell samples on cotton swabs.

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6.5 Investigating resin volume

Promega recommends the use of 7µL of DNA IQ™ resin with their protocol. We investigated the performance of the protocol with double the amount of resin (14µL) in order to assess any benefits that may be gained in terms of the resulting yield and quality of the STR profile.

It was observed that doubling the resin resulted in a proportional doubling of the yield. On average, doubling the resin increased the yield by an additional 77.28% (*n*=4). The average yield from an extraction using 7µL of resin was 64.725ng (SD 32.21ng, *n*=4), whereas 14µL resin generated 114.75ng (SD 10.72ng, *n*=4) (Table CRAP). At the higher resin concentration, the amount of DNA isolated appears to be capped at around 100ng, indicating no change in the ability of the reaction to isolate more DNA due to saturation of resin.

Table CRAP. Comparison of the effects of doubling the amount of recommended DNA IQ™ resin.

Sample ID	Resin volume	[DNA] ng/µL	Reportable alleles
33383-4216	7µL	0.701	18/18
33383-4225		1.070	18/18
33383-4239		0.319	18/18
33383-4248		0.499	18/18
33383-4252	14µL	1.140	18/18
33383-4261		1.270	18/18
33383-4270		1.010	18/18
33383-4284		1.170	18/18

Samples extracted using either amount of resin generated concordant full DNA profiles (18/18 alleles). Samples processed using the 14µL method produced peaks that were slightly higher. The difference in peak heights between alleles within the same loci ranged from 59-86%, with a mean of 71%, indicating minimal difference between the two methods.

Doubling the amount of resin did not appear to provide any additional benefits compared to the original recommended protocol. Therefore, the costs associated with increasing the amount of resin cannot be justified at this stage.

6.6 Modifying extraction volumes

An investigation into optimising extraction volumes ranging from 300 to 500µL was performed in order to ensure that buffer coverage over the samples was sufficient to enable optimal lysis and release of DNA. In addition, the use of an optimum volume of extraction reagents increases efficiency and economy, therefore allowing lower costs.

Spotting

6.7 Protocol

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7. Summary and Recommendations

We recommend the following:

- Use of MPII for automated extraction of reference samples
- Use of MPII for automated extraction of casework samples
- Ongoing development of the automated extraction program to increase the efficiency of the extraction

Sweeney, P.J. and Walker, J.M., Burrell, M.M., Enzymes of molecular biology. *Methods Mol. Biol.* Towanam NJ , (1993) **16**, 306

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Project 1: Report on the Verification of automated Quantifiler™ Human DNA Quantification Setup using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform

2006

Automation and LIMS Implementation Project Team,

DNA Analysis

Forensic And Scientific Services

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Project 1: Report on the Verification of automated Quantifiler™ Human DNA Quantification Setup using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform

Iman Muharam, Allan McNevin, Thomas Nurthen, Cathie Allen, Vanessa Ientile
Automation Project, DNA Analysis QHSS (August 2006)

1. Abstract

The quantification of forensic DNA samples within DNA Analysis QHSS is performed using the Quantifiler™ Human DNA Quantification Kit (Applied Biosystems, Foster City, CA, USA), in combination with the ABI Prism® 7000 SDS instrument (Applied Biosystems, Foster City, CA, USA). The quantification procedure is currently performed manually in 96-well format. We have verified the use of the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform to automatically setup a Quantifiler reaction in 96-well plate format. Data indicate that results from the automated procedure are comparable to those from the manual procedure. Contamination checks were performed using samples prepared in checkerboard and zebra-stripe format, and results were as expected.

2. Introduction

The Queensland Government has allocated 11 million dollars to be spent over 3 years as part of the February 2004 “Law and Order” election commitment to clear the DNA backlog at QHSS. DNA Analysis QHSS has formulated a number of initiatives to address the backlog, including the recruitment of new scientific staff members, the refurbishment of existing laboratory space to enhance workflow efficiency, and the purchasing of additional instrumentation and equipment to improve efficiency and turn around times for DNA profiling.

In October 2005, DNA Analysis decided to purchase four MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms from PerkinElmer after an extensive tender evaluation process. The four platforms and their intended applications are tabulated below (Table 1). The PCR platforms were installed in DNA Analysis on 7 December 2005, while the extraction platforms were installed soon after on 3 January 2006. Installation was performed by Fraser Smith (PerkinElmer engineer) and training was delivered by Desley Pitcher (PerkinElmer Automated Liquid Handling Product Specialist). DNA Analysis’s purchase of the four platforms represents the largest automation project undertaken in a DNA Analysis facility within Australia. Whereas other Australian forensic laboratories have only purchased automated platforms with the view of automating specific steps within their DNA processing workflows, DNA Analysis QHSS aims to automate all DNA analysis steps from DNA extraction up to post-PCR setup and the analysis of data by expert systems.

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Table 1. The proposed functions of the four MultiPROBE® II platforms in DNA Analysis.

Platform	Function
Extraction (Reference)	To extract and purify DNA from reference or non-casework samples in the form of FTA cards, blood stains or cellular materials.
Extraction (Casework)	To extract and purify DNA from casework samples, originating from a variety of matrices and sample types.
Pre-PCR	i. To perform set-up of DNA quantitation using Quantifiler real-time quantitative PCR (Applied Biosystems, Foster City, CA, USA), involving the addition of reaction mastermix, DNA standards and controls, and extracted DNA samples, into a 96-well reaction plate. ii. To perform set-up of STR genotyping reactions using Profiler Plus® and COfiler® AmpFℓSTR® PCR (Applied Biosystems, Foster City, CA, USA), involving the addition of reaction mastermix, DNA controls, and extracted DNA samples, into a 96-well reaction plate.
Post-PCR	To perform the set-up of 96-well plates containing a sub-sample of amplified DNA (PCR products) for capillary electrophoresis using the ABI Prism 3100 Genetic Analyzer (ABI Foster City, CA, USA).

The MultiPROBE® II PLUS HT EX platform is a computer-controlled Cartesian X-Y-Z liquid handling system designed for the efficient automation of sample preparation. For the DNA Analysis platforms, liquid transfer is performed by an 8-tip system with VersaTip® and Varispan™ options, allowing the use of fixed and/or disposable tips, with variable probe spacing to access a wide variety of labware including microplates, tubes, vials and reagent containers. Each sample probe is capable of independent motion in the Z direction due to independent Z-drives. Accusense™ technology enables multichannel liquid level sensing using a capacitive mode of detection that also allows detection of low or non-ionic polar solutions and solvents. To perform pipetting, the platforms transfer liquid using a positive displacement of system liquid (nanopure H_2O) driven by 8 individually-controlled syringes. The Gripper™ Integration platform (installed on all systems except the post-PCR instrument) allows relocation of labware across the deck and automated identification of labware via the scanning of barcodes. All platforms include the EXpanded Left Extension deck to provide increased microplate tile capacity.

Control of the platforms is performed via WinPrep® application software using pull-down menus and simple user prompts. The application interface includes a virtual representation of the platform deck and functions via drag-and-drop operations. Standard tests (programs) are created by performing three basic steps: placing labware on deck, creation of procedures, and linking labware to procedure operation steps. A library of standard labware definitions is available which allows labware to be placed anywhere on the deck without the need to define additional labware parameters. Additional labware can be added to the labware library to meet user-specific application requirements. New labware only needs to be defined once for unlimited future use. Data logs or audit reports are generated by the software for every test run. An Enhanced Security Option upgrade to the existing WinPrep® application software was purchased to enable individual log-in options and account restrictions.

3. Aim

To verify the performance of the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (“Pre-PCR MP II”) to prepare Quantifiler™ Human DNA Quantification assay (“Quantifiler plates”) for the quantitation of forensic DNA samples on the ABI Prism 7000 SDS instrument (“ABI 7000”). This includes a verification of the Pre-PCR MP II’s pipetting accuracy and precision, as determined colorimetrically and gravimetrically, and an assessment or comparison of the output data to the manual setup method.

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4. Equipment and Materials

- MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (PerkinElmer Life Sciences, Downers Grove, IL, USA)
- Gravimetric Performance Evaluation Option with Mettler SAG285/L balance (Mettler-Toledo, Greifensee, Switzerland), PN 7601823
- Tecan Spectra Reader (Tecan GmbH, Salzburg, Austria)
- ABI Prism® 7000 SDS (Applied Biosystems, Foster City, CA, USA)
- Quantifiler™ Human DNA Quantification kits (Applied Biosystems, Foster City, CA, USA)
- Tartrazine yellow (for colorimetric analysis)
- 25µL Conductive MP II sterile filter RoboRack tips (PerkinElmer)
- 175µL Conductive MP II sterile filter RoboRack tips (PerkinElmer)
- Isoplate™ microtitre plates (Wallac Oy, Turku, Finland)
- ABI Prism® 96-well optical reaction plates (Applied Biosystems, Foster City, CA, USA)
- Microcon® Centrifugal Filter Devices (YM-100) (Millipore Corp., Bedford, MA, USA)
- For mock samples:
  - FTA® Classic Card (Whatman Inc., Florham Park, NJ, USA)
  - Sterile cotton s abs (Medical Wire & Equipment, Corsham, Wiltshire, England)
- Other general consumables were supplied by Analytical Section, DNA Analysis QHSS

5. Methods

5.1 Gravimetric Evaluation of Pipetting Accuracy and Precision

The Gravimetric Performance Evaluation Option with Mettler SAG285/L balance (Mettler-Toledo, Greifensee, Switzerland), hereby referred to as the “gravimetric kit”, was purchased from PerkinElmer (PN 7601823) as an optional accessory upon recommendations made by Forensic Science South Australia staff members. The gravimetric kit consists of a SAG285/L balance and various accessories including a humidified chamber and software for integration with the WinPrep® software. All components were installed by a qualified PerkinElmer service engineer, and subsequently calibrated by a Mettler-Toledo engineer for NATA certification. A NATA certificate for the kit (Report Number Q0737-001-1) was produced on 13 March 2006.

Gravimetric analysis is performed by placing the balance on the platform deck and instructing the MP II to repeatedly pipette certain volumes of system liquid onto the balance pan. Readings are taken automatically by the software and compiled into a results table, which is then used to automatically generate an Excel-based results chart containing mean, %CV and %inaccuracy values. The mean values obtained are used to calculate R^2, slope and Y-intercept (offset) values to calibrate the system’s pipetting.

The pipette tips required for use on the Pre-PCR MP II only include 25µL and 175µL conductive MP II sterile filter RoboRack tips, for the purposes of DNA sample delivery and mastermix dispensing respectively. Pipetting performance was assessed for various volumes using different tip sizes in order to calculate appropriate R^2, slope and Y-intercept (offset) values which were then added to the performance file. Values were calculated for both Blowout (single-liquid transfer) and Waste (multidispense) modes for the 175µL tips, and Blowout mode only for 25µL tips. Retesting was performed to confirm accurate and precise pipetting with these settings.

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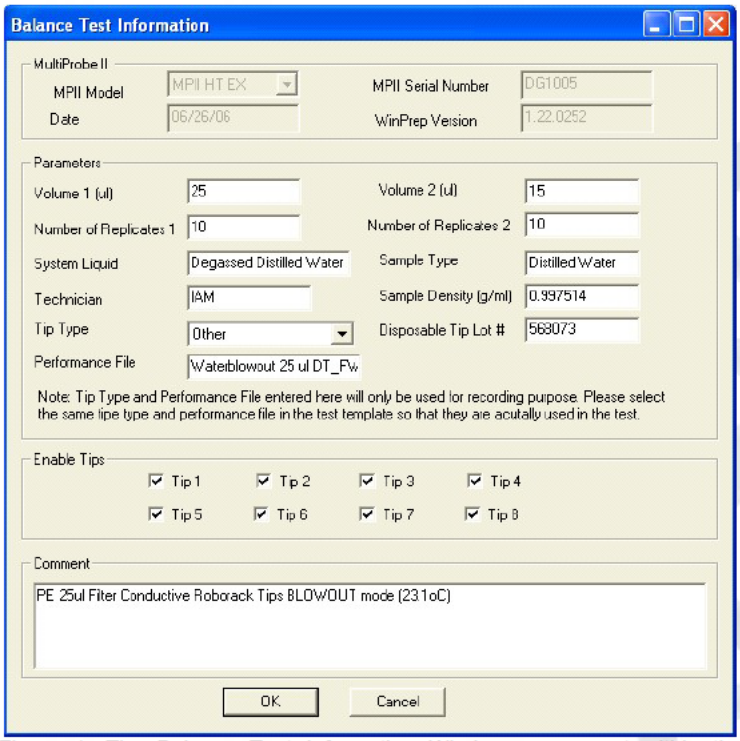

Figure 1. The Balance Test Information Window as present within the Balance Test DT program. All pipetting parameters are entered here and are subsequently transferred to the result output file.

All gravimetric testing was performed using the Balance Test DT test program within WinPrep®. Parameter values that needed to be entered into the Balance Test Information Window (Figure 1) included those as outlined in Table 2.

Table 2. Input values that are required for the various Balance Test Information parameters.

Parameter(s)	Value
Volume 1 and Volume 2	For 25µL tips: 25, 15, 10, 5µL For 175µL tips: 175, 100, 50, 15µL
Number of Replicates	10
System Liquid	Degassed Distilled Water
Sample Type	Distilled Water
Technician	Initials of the operator performing the test
Sample Density (g/ml)	The density of water at environmental temperature*
Tip Type	Other
Disposable Tip Lot #	The lot number of the particular tips in use
Performance File	The appropriate Performance File for the tip (25µL or 175µL) and pipetting mode (Blowout or Waste) in use
Enable Tips (checkboxes)	Select the actual tips (1 to 8) to be tested
Comments	Free text box to add additional information (eg. Tip type, mode, current environmental room temperature, etc).

* Water density values were obtained from http://www.simetric.co.uk/si_water.htm

Pipetting accuracy and precision were examined at four different volumes for each tip size: 25, 15, 10, 5µL for 25µL tips and 175, 100, 50, 15µL for 175µL tips. In order to calculate unbiased values for each set of volumes, the slope and offset values in the relevant

Performance File were changed to the default 1 and 0 respectively prior to testing. The mean volumes that were pipetted by each tip (10 replicates per tip) at the four designated volumes were used to generate a standard curve. The slope and offset calculated from this curve was used to calibrate the relevant Performance File. The final Performance File settings were then tested at the highest and lowest volumes to confirm accurate and precise pipetting.

5.2 Colorimetric Evaluation of Pipetting

Tartrazine yellow (Colour 102) was obtained from Food Chemistry (QHSS), and consisted of tartrazine at 80.3% purity (λ = 429nm). A saturated solution of tartrazine was made using nanopure water and used to perform all colorimetric assays. Briefly, using the Colorimetric Dilutions test program, the Pre-PCR MP II was used to add tartrazine solution (either neat or diluted 1:2) to the first column in a 96-well Isoplate™ (Wallac Oy, Turku, Finland). A serial dilution across the plate was then performed automatically by the Pre-PCR MP II (refer to Table 3). Nanopure water was dispensed into the 12th column to serve as a background control. Due to technical limitations, only 175µL conductive MP II sterile filter RoboRack tips were used for all pipetting procedures. Results were analysed using a Tecan Spectra Reader (Tecan GmbH, Salzburg, Austria), located in Virology, Public Health Unit, QHSS. Measurement wavelength was set at 450nm and reference wavelength was set at 620nm.

Table 3. The arrangement of pipetting modes and serial dilution sets that were analysed via colorimetric assays.

Plate ID	Pipetting Mode	Tartrazine concentration	Serial Dilution	Total Volume per well (µL)
607	Blowout	Neat	1:2	60
608	Blowout	Neat	1:2	60
609	Waste	Neat	1:2	60
622	Blowout	1:2	1:2 → 1:5 → 1:5 → 1:5 set in tandem	80
623	Waste	1:2	1:2 → 1:5 → 1:5 → 1:5 set in tandem	80

5.3 Contamination Check via Checkboard and Zebra-stripe Patterns using Quantifiler Assays

Promega Human Genomic DNA: Male (Promega Corp., Madison, WI, USA; Catalogue Number G1471, lot number 20479703) of a known concentration of 190µg/mL was diluted to 25ng/µL and aliquoted into 40 tubes. Water blanks were prepared using in-house autoclaved nanopure water or Sigma Water (Sigma-Aldrich Corp., St. Louis, MO, USA; Catalogue Number W4502, lot number 83K2357). Quantifiler reactions in checkerboard and zebra-stripe patterns were prepared using these samples in alternating DNA-water-DNA arrangements. A representation of the plate layouts are shown below in Figure 2. The Quantifiler reagents used were as per the in-house validated method (QIS 21963). Reaction controls included “High” and “Low” dilutions of Promega Human Genomic DNA: Female (Promega Corp., Madison, WI, USA; Catalogue Number G1521, lot number 20683601) at 1ng/µL and 0.1ng/µL respectively. A reagent blank “RB” was included in every run and consisted of mastermix only (no DNA or water) to monitor the concentration of background contamination present in the Quantifiler kit reagents (Applied Biosystems, 2005 [letter]). Wells A11 and A12 were omitted from analysis due to the inconsistency of results from these positions, as reported previously (Hlinka et al., 2006).

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(a) Checkerboard Pattern

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD1	STD1									Omitted	Omitted
B	STD2	STD2										
C	STD3	STD3										
D	STD4	STD4										
E	STD5	STD5										
F	STD6	STD6										
G	STD7	STD7										
H	STD8	STD8								High	Low	RB

(b) Zebra-stripe Pattern

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD1	STD1									Omitted	Omitted
B	STD2	STD2										
C	STD3	STD3										
D	STD4	STD4										
E	STD5	STD5										
F	STD6	STD6										
G	STD7	STD7										
H	STD8	STD8								High	Low	RB

Legend:

- DNA Standards / Controls
- Water blanks
- Promega 25ng/µL gDNA

Figure 2. Checkerboard and zebra-stripe patterns utilised in the Quantifiler contamination check.

The automated Quantifiler Setup MP II test program was compiled to mimic the manual setup of Quantifiler assays as performed by scientists in the Analytical Section, DNA Analysis QHSS (refer to QIS 21963). The final Test Outline tree structure is presented below as Figure 3. Refer to Figure 4 for the deck layout.

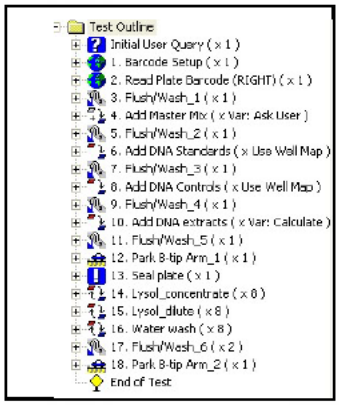

Figure 3. Test Outline for the automated setup of Quantifiler assays using the Pre-PCR MP II.

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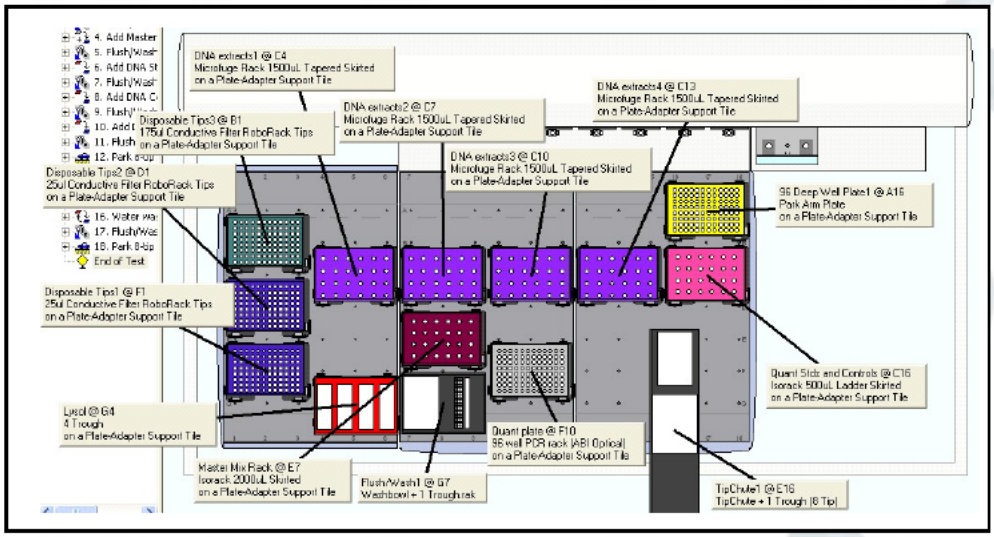

Figure 4. The WinPrep® virtual deck view displaying the necessary labware required to perform automated Quantifiler setup.

5.4 Verification of the Automated Quantifiler Protocol

Comparisons were made between results generated by the automated and manual methods to verify the performance of the automated Quantifiler setup protocol.

Samples that were quantified included mock forensic samples of buccal FTA, blood FTA and whole blood on cotton swab. Samples were obtained from 2 female donors (VKI, MMH) and 2 male donors (PAC, DJC). Each sample type from each donor was prepared within a sterilised Biohazard Class II cabinet on different days. DNA extractions (QIS 17171) for each sample type were also performed on different days. These measures were implemented to prevent the occurrence of cross-contamination between sample types and the different donors prior to handling on the automated platform.

6. Results and Discussion

6.1 Gravimetric Evaluation of Pipetting Accuracy and Precision

Pipetting performance of the various tip types and pipetting modes at the highest and lowest volumes tested are outlined in Table 4. The individual Performance Files required for each combination of pipette size and pipetting mode are presented in Table 5.

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Table 4. Pipetting performance for 25µL and 175µL Conductive MP II sterile filter RoboRack tips under Blowout and Waste modes.

Tip Type	Volume	Blowout Mode		Waste Mode	
25µL Conductive MP II sterile filter RoboRack	25µL	Mean (µL)	24.96	Not tested	
		CV (%)	0.72		
		Inaccuracy (%)	0.15		
	2µL	Mean (µL)	2.06		
		CV (%)	6.53		
		Inaccuracy (%)	2.91		
175µL Conductive MP II sterile filter RoboRack	175µL	Mean (µL)	174.90	Mean (µL)	175.13
		CV (%)	0.35	CV (%)	1.57
		Inaccuracy (%)	0.06	Inaccuracy (%)	0.07
	15µL	Mean (µL)	15.19	Mean (µL)	15.31
		CV (%)	0.90	CV (%)	5.84
		Inaccuracy (%)	1.25	Inaccuracy (%)	2.08

Table 5. Calibrated slope and offset values for individual pipette types under different pipetting modes.

Tip Size	Pipetting Mode	Performance File	Slope	Offset (Y-intercept)	R^2	Pipetting Range (µL)
25µL Conductive MPII sterile filter RoboRack	Blowout	WaterBlowout 25 ul DT_FW_QHSS_11042006.prf	0.962803	-0.390539	0.999915	1-25
175µL Conductive MPII sterile filter RoboRack	Blowout	WaterBlowout 175 ul DT_FW_QHSS_21032006.prf	0.978905	-1.082636	0.999991	15-175
175µL Conductive MPII sterile filter RoboRack	Waste	WaterWaste 175 ul DT_FW_QHSS_27032006.prf	0.974194	-1.246471	0.999971	15-175

Screenshots of the final Performance Files with pipetting performance sets and calibrated slope and offset values are presented in Figures 5, 6, and 7.

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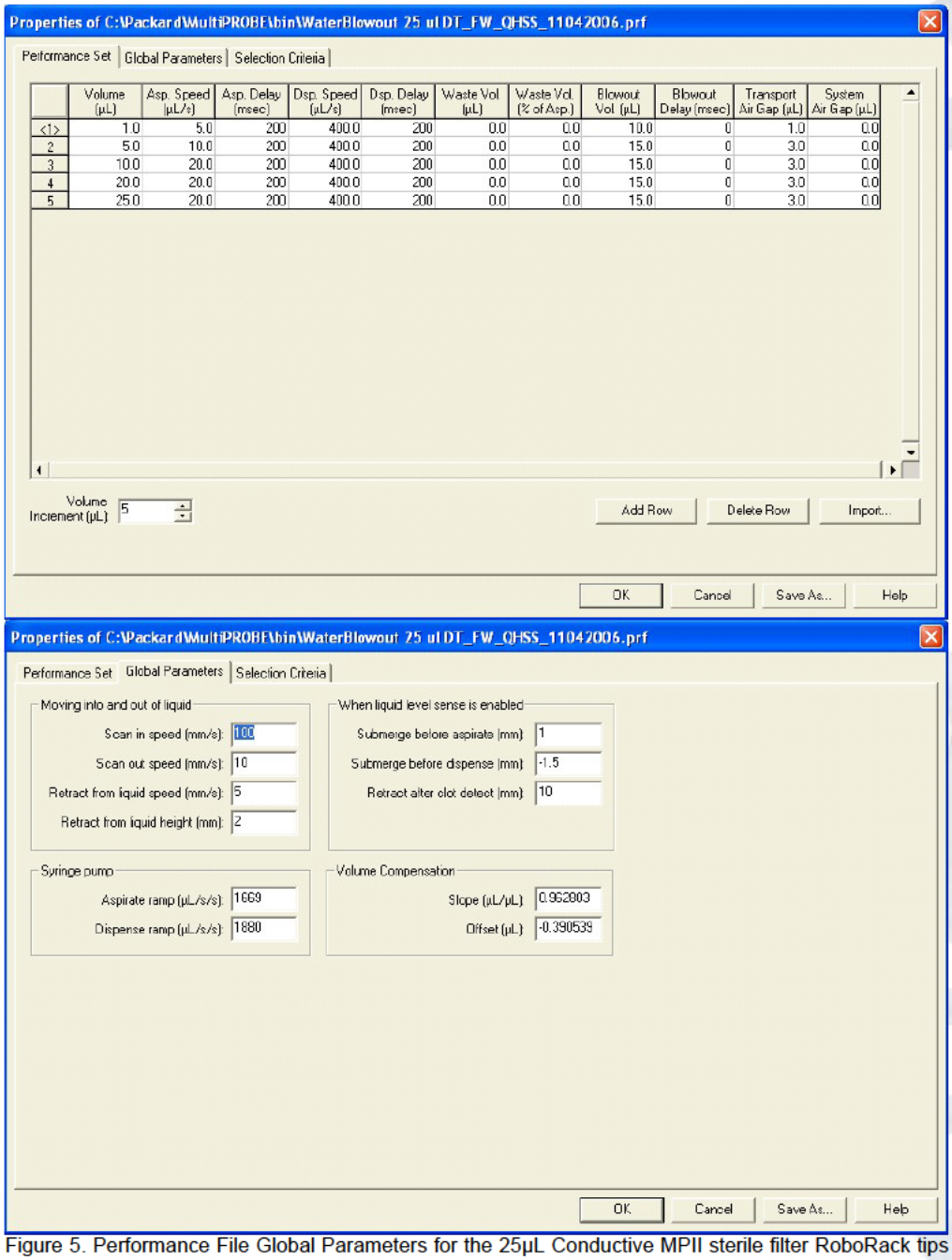

Figure 5. Performance File Global Parameters for the 25µL Conductive MPII sterile filter RoboRack tips under Blowout Mode.

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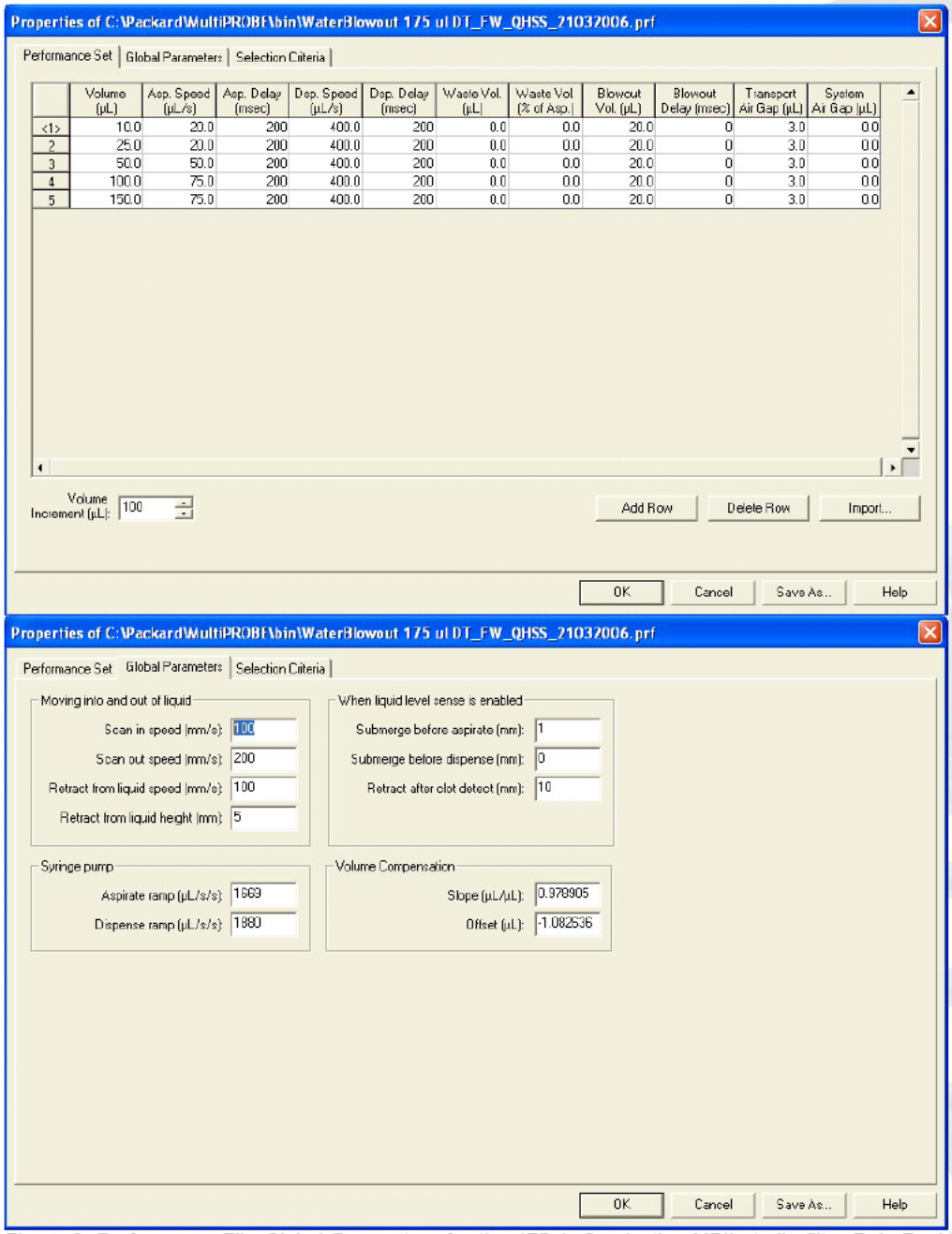

Figure 6. Performance File Global Parameters for the 175µL Conductive MPII sterile filter RoboRack tips under Blowout Mode.

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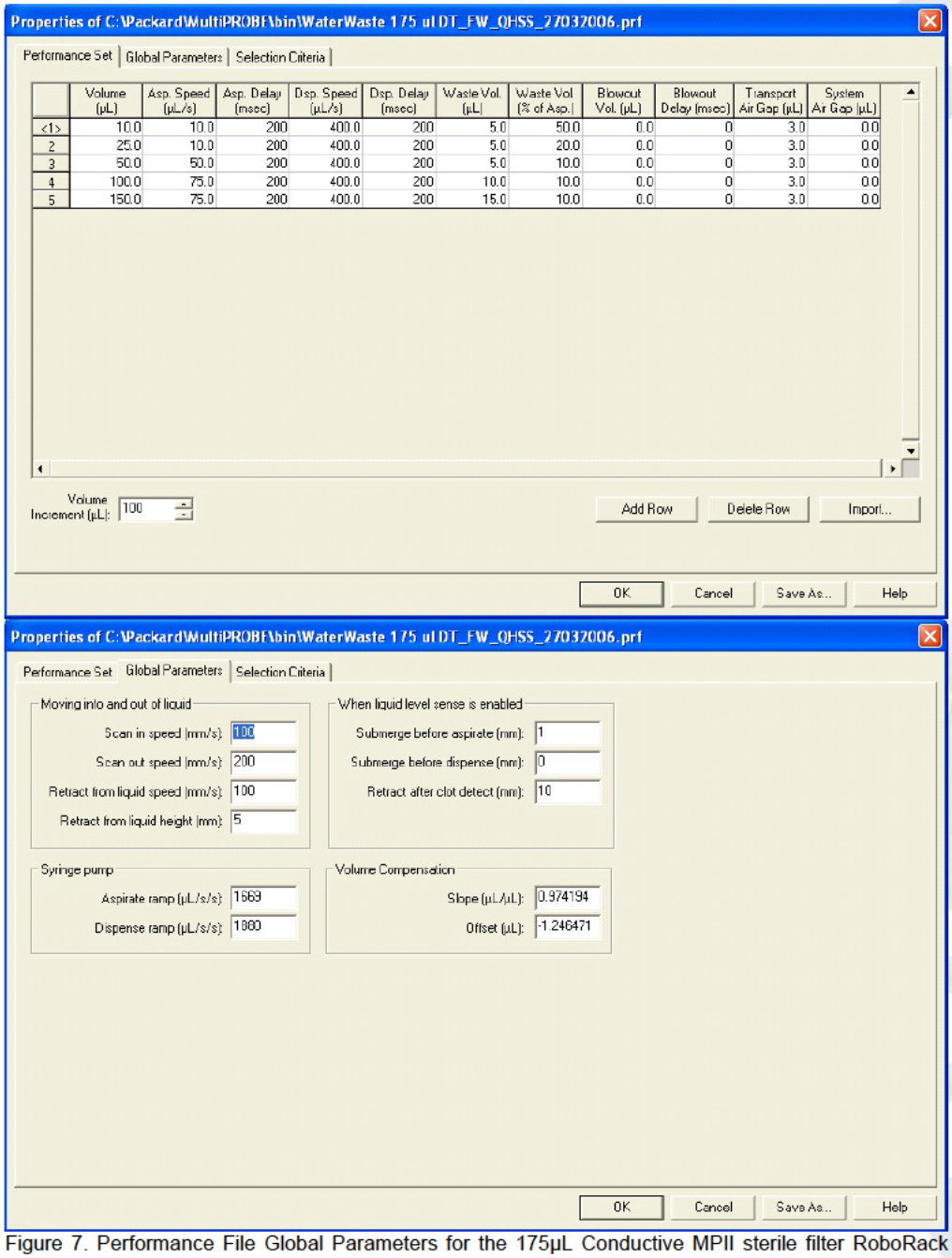

Figure 7. Performance File Global Parameters for the 175µL Conductive MPII sterile filter RoboRack tips under Waste Mode.

Initial pipetting performance at low volumes (1 and 2µL) using the 25µL tips was considered substandard due to the observation of sporadic "zero" volumes, where liquid did not appear to be pipetted and this error was not detected by the Pre-PCR MP II. This observation occurred at a frequency of at least once per 96-well plate. Discussions were made with colleagues at Forensic Science South Australia (Mark Webster and Chris Hefford). It was put forward that the dispense height is a critical factor for low volume pipetting, and dispensing approximately 1.5mm above the liquid surface allows the expelled liquid to form a drop at the end of the tip which touches the liquid surface, whereby surface tension is then able to pull the drop off the tip. Based on these findings, the "Submerge before dispense (mm)" parameter was changed from 0 to -1.5 (Figure 5). The double negative value results in a dispense height 1.5mm above the liquid surface. This adjustment significantly enhanced pipetting performance at low volumes. To further ensure total delivery of all pipette tip contents, the Performance Set for each Performance File was also adjusted and tested to maximise pipetting accuracy/precision while maintaining minimal drip formation and cross-contamination risks.

6.2 Colorimetric Evaluation of Pipetting

Results for pipetting performance over a range of serial dilutions as determined via colorimetric detection are outlined below in Table 6.

Table 6. Results of colorimetric assays to assess pipetting performance.

Plate ID	Pipetting mode	Absolute		Relative	
607*	Blowout	%CV	13.65	%CV	6.79
		%Inacc	12.12	%Inacc	2.12
		R^2	0.99890		
608*	Blowout	%CV	18.80	%CV	11.73
		%Inacc	2.92	%Inacc	0.27
		R^2	0.99852		
609*	Waste	%CV	10.63	%CV	9.23
		%Inacc	13.18	%Inacc	4.22
		R^2	0.99966		
622†	Blowout	%CV	3.14	%CV	1.63
		%Inacc	4.22	%Inacc	1.92
		R^2	1.00000		
623†	Waste	%CV	5.88	%CV	2.25
		%Inacc	7.51	%Inacc	3.13
		R^2	0.99993		

* Plate consists of ten serial dilutions at 1:2.
† Plate consists of four serial dilutions at 1:2 → 1:5 → 1:5 → 1:5 in tandem.

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6.3 Contamination Check via Checkboard and Zebra-stripe Patterns using Quantifiler Assays

The same sets of High and Low female control gDNA, Promega 25ng/µL gDNA, water blanks and DNA standards were used for both plates. The Promega 25ng/µL gDNA was purposely diluted to this concentration as splashback or carryover of 1µL from a well containing Promega 25ng/µL DNA into a well containing water blank would result in detectable contamination at the level of 0.04ng/µL. Carryover of 0.117µL would result in contamination at the lower limit of detection (0.00467ng/µL) as previously established by in-house validation of the Quantifiler system (Hlinka et al., 2006). This value is similar to the concentration of detectable background readings that are known to occur in Quantifiler reagents (Applied Biosystems, 2005 [letter]).

All wells in the checkerboard pattern exhibited results as expected. Quantitation of Promega 25ng/µL gDNA resulted in concentration values equal to or greater than 25ng/µL (mean 30.65ng/µL, SD 3.65ng/µL). All of the reaction controls showed expected values and amplification was not detected in any of the water blank reactions.

The zebra-stripe layout displayed expected results for all controls and Promega 25ng/µL gDNA. The majority of water blank reactions did not result in amplification, however a small number of reactions yielded low concentration values as presented in Table 7 below.

Table 7. Water blank reactions that displayed amplification in the zebra-stripe pattern plate 1 (run ID: QUACWZebra).

Sample ID	Well	Quantifiler value (ng/µL)
Water_20	D7	0.000233
Water_5	E3	0.000124
Water_29	E9	0.00724
Water_14	F5	0.00192

The whole test was repeated and only the following reaction provided a DNA concentration value:

Table 8. Water blank reactions that displayed amplification in the zebra-stripe pattern plate 2 (run ID: QUACWZebra2).

Sample ID	Well	Quantifiler value (ng/µL)
Water_29	E9	0.00590

Water_29 provided similar concentration values across two different Quantifiler runs. It was suspected that this tube may contain a DNA contaminant. The Water_29 sample (620µL of water) was given a DNA # ID (94355) and concentrated using a Microcon-100 device as per the in-house protocol (QIS 19544). Briefly, 500µL of the sample was transferred into a fresh tube and centrifuged for 6mins at 500g. The remaining 120µL was further transferred to this tube and centrifuged for 6mins at 500g. The Microcon-100 column containing concentrated DNA was inverted into a new tube and centrifuged for 3mins at 1000g. The retentate (approx 260µL) was concentrated in a fresh Microcon-100 filter for 12mins at 500g. The filter was inverted and centrifuged for 3mins at 1000g. The final concentrate (45µL) was quantified in duplicate using the Quantifiler kit (run ID QF#748, setup manually) and resulted in undetermined concentration values (0ng/µL). The concentrated sample was amplified using

the AmpFℓSTR® Profiler Plus® kit at 20µL in a 50µL reaction (run ID CW#1184), which resulted in an NSD profile. It appears that the presumed contaminant present in the concentrated Water_29 sample was either too low in concentration and could not result in a detectable Quantifiler result or DNA profile, or the initial detected concentration values were the result of detectable background inherently present in the Quantifiler reagents (Applied Biosystems, 2005 [letter]).

The contamination check in zebra-stripe pattern was repeated using sterile molecular grade Sigma Water (Sigma-Aldrich Corp., St. Louis, MO, USA; Catalogue Number W4502, lot number 83K2357). All controls and Promega 25ng/µL DNA showed expected results. The majority of water blank reactions did not result in amplification, but four samples yielded low concentration values as per Table 9 below.

Table 9. Water blank reactions that displayed amplification in the zebra-stripe pattern plate 3 (run ID: QUACWZebra3).

Sample ID	Well	Quantifiler value (ng/µL)
Sigma Water_25	A9	0.0027
Sigma Water_29	E9	0.000179
Sigma Water_38	G11	0.00211
Sigma Water_8	H3	0.000291

All water samples that previously presented with DNA concentration values were re-quantified in 5 replicates using the Quantifiler kit, which resulted in undetermined concentration values (0.0ng/µL) for all reactions. These results suggest that there was no contamination of the original water samples by the MP II but rather is the result of the inherent detectable background within the Quantifiler reagents.

6.4 Verification of the Automated Quantifiler Protocol

Refer to Table 10 and Figure 8 for results from the comparison of automated versus manual Quantifiler setup.

Table 10. Differences in run results between automated and manual Quantifiler setup methods for 132 DNA samples.

Average CT difference	-0.1293939
Average quant diff (%)	-1.265
Average quant diff (ng/µL)	-0.003
Maximum quant diff (%)	67.391
Maximum quant diff (ng/µL)	0.460
Minimum quant diff (%)	-41.232
minimum quant diff (ng/µL)	-0.420

Quant difference = automated result - manual result
Quant difference % = (quant difference/manual result * 100)

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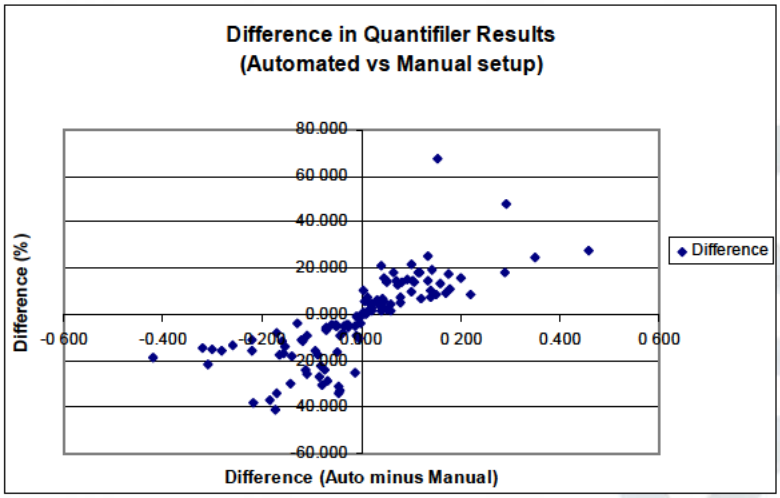

Figure 8. Plot of difference between automated and manual methods for Quantifiler setup. Most samples cluster close to 0, indicating minimal difference between sample results.

The maximum quant value was for sample ID 228675605 (PAC mouth swab), at 3.67ng/µL (auto) and 3.61ng/µL (manual). For both results, this equates to a 3:10 dilution of the original extract before adding 1µL of sample volume in order to give a total of 1ng DNA in the subsequent Profiler Plus® or COfiler® amplification. The lowest quant values obtained were 0.0374ng/µL (auto) and 0.0455ng/µL (manual), for sample ID 228675715 (DJC mouth swab) and 228675706 (DJC mouth swab) respectively. The corresponding manual or auto results for these samples were 0.0499ng/µL and 0.0409ng/µL respectively, accounting for a difference of 0.0129ng/µL (~25.0%) and 0.0046ng/µL (~10.1%). The low DNA concentration of these samples contribute to stochastic effects during the real-time QPCR DNA quantitation process, reflected in the differences in results between the automated and manual quant setup procedures.

Overall, the maximum quant difference observed was 67.4% for sample ID 129466814 (MMH blood swab), with a maximum quant value of 0.385ng/µL (auto) and a minimum of 0.230ng/µL (manual). The manual result was checked by repeating the quantitation assay manually (DNA # ID 102305, run ID QF#873), to yield a concentration of 0.397ng/µL, yielding a revised difference value of 1.2%. The lower quantitation result obtained previously using the manual Quantifiler setup protocol most likely reflects the occurrence of pipetting error. The minimum quant difference observed was -41.2% for sample ID 228675056 (PAC blood swab), with a maximum quant value of 0.422ng/µL (manual) and a minimum of 0.248ng/µL (auto).

A comparison of standard curve parameter values that are observed after manual or automated setup of Quantifiler assays is outlined in Table 11 below. In both runs, the automated plate displayed Y-intercepts that were closer to the mean of 28.011482. The Y-intercept differences from the mean for the automated plates were 0.13081 and 0.035412 for the first and second runs respectively, compared to the manual values of 0.220831 and 0.362122. The R^2 values for the automated plates were closer to 1 compared to the manual plate results. Although these results suggest that the standard curve parameters from the automated plates appear to out-perform those from the manual plates, a calculation of statistical significance cannot be performed due to limited data.

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Table 11. Comparison of standard curve parameter values between manual and automated Quantifiler plates.

Run ID:		QUACW20060419_01		QUACW20060419_02	
Parameter	**Threshold**	**Manual Result**	**Auto Result**	**Manual Result**	**Auto Result**
Slope	-2.9 to -3.3	-3.037914	-3.176350	-3.190838	-3.168825
Y-Intercept*	27.655034 - 28.367930	28.232313	28.142292	28.373604	28.046894
R^2	≥0.98	0.995921	0.998229	0.992763	0.996018

*Y-intercept range at 2SD.

No amplified product was detected in the reagent blank reactions. Samples that did not contain sufficient DNA were undetermined on both the automated and manual plates, indicating reproducibility and consistency in results. This also indicates that the automated procedure does not introduce contamination into the DNA samples.

7. Summary

Results indicate that the automated setup of the Quantifiler assay for the purposes of quantifying forensic DNA samples is comparable in performance and reproducibility to the current manual setup.

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Queensland Government
Queensland Health

Automation Team – Weekly meetings (Chief Scientist & Project Manager)

VKI / TEN 9.10.07

Agenda Item	Discussion/Issues	Actions	Due date	Action Status
Implementation	lower yield - auto to manual. manual working well. Post-PCR - ready to go - awaits live ~ 3:30	VH to contact Promega.		Impact on going live.
Staff issues/concerns	looking @ alternative colourmetric system for tip change - min d'time	use gravimetric as annual check.		TEN to chase details.
Validation	cont plates. - looks like it cannot confirm source.			
AUSLAB	Need wshops for func spec^	TEN to arrange meet) ~ mark team.		
Communication				
Training status report (sign off, timeframes, issues)				
Own workload, concerns, issues or suggestions				

Automation Team – Weekly meetings (Chief Scientist & Project Manager)

VKI/TEN 16.10.07

Agenda Item	Discussion/Issues	Actions	Due date	Action Status
Implementation	yield expts 50% lower on auto process than manual ruled out reagents.	Need to discover where source - comparing methods focus on wash + elution	- need to address contam issue + yield but need to consider	implementation then optimisation
Staff issues/concerns	forecasting meeting reqd.			
Validation	Setting implementation date. Validation reports being prepared (Post-PCR included)			
AUSLAB	2 batches id where completed in automation trail but didn't appear on next batch type.	→ might need physical check step	TEN to log job	
Communication	TEN to m/c meeting to discuss size charts.			
Training status report (sign off, timeframes, issues)	SOP/training modules in QIS.	Ready for approval Speak to AM re staff to be trained.		
Own workload, concerns, issues or suggestions				

TN-31

Notice number: 2022/ 00178

COMMISSION OF INQUIRY INTO FORENSIC DNA TESTING IN QUEENSLAND

Section 5(1)(d) of the *Commissions of Inquiry Act 1950*

REQUIREMENT TO GIVE INFORMATION IN A WRITTEN STATEMENT

To: Thomas Nurthen

Of: Queensland Health

I, Walter Sofronoff QC, Commissioner, appointed pursuant to Commissions of Inquiry Order (No. 3) 2022 to inquire into certain matters pertaining to forensic DNA testing in Queensland require you to attend to give a written statement to the Commission pursuant to section 5(1)(d) of the *Commissions of Inquiry Act 1950* in regard to your knowledge of the matters set out in the Schedule annexed hereto.

YOU MUST COMPLY WITH THIS REQUIREMENT BY:

Giving a written statement prepared either in affidavit form or verified as a statutory declaration under the *Oaths Act 1867* to the Commission of Inquiry on or before **COB Tuesday 20 September 2022** by delivering it to Level 21, 111 George Street, Brisbane.

A copy of the written statement must also be provided electronically by email at admin@dnainquiry.qld.gov.au, with the subject line "Requirement for Written Statement".

If you believe that you have a reasonable excuse for not complying with this notice, you will need to satisfy me of this prior to the above date.

DATED this 14th day of September 2022

[redacted]

Walter Sofronoff QC
Commissioner
Commission of Inquiry into Forensic DNA Testing in Queensland

Notice 2022/ 00178

Schedule of topics for statement

Thomas Nurthen

1. State your full name, current position/title and where you work.
2. List your tertiary qualifications, the year you obtained them, and the institute from which the qualifications were obtained.
3. Identify the duties/responsibilities of your current position.
4. Identify what positions you held at Queensland Health Forensic and Scientific Services from 2008 to 2014, and the duties/responsibilities of those positions.

DNAIQ

5. Explain what DNAIQ is. Explain the way or ways in which the DNA laboratory used DNAIQ, and/or DNAIQ system(s), as at the start of 2008.
6. Explain what problems with DNAIQ were experienced in approximately 2008. Explain, to the best of your knowledge, how these problems were first detected.
7. Identify each OQI and adverse event that relates to DNAIQ problems at around this time, or has since been linked to DNAIQ problems from around this time.
8. What actions did the management committee and/or staff at the DNA laboratory take in response to the discovery of the problem? Provide a clear timeline which covers the problems identified, the decisions taken in response and by whom, and how those decisions were implemented.
9. Was the cause of the issues or problems relating to DNAIQ identified? If yes, what was it?
10. What immediate action was taken after the cause of the issues or problems was identified?
11. Outline your role in responding to issues with DNAIQ, and any audits completed in relation to any OQI concerning DNAIQ, including audit 9175. Provide an explanation of the findings of audit 9175 and actions taken in response to that audit. When were the follow-up actions finalised?
12. Identify each staff member involved in detecting and responding to the problems with DNAIQ, and the nature of each person's involvement.

13. Identify whether any issue or problem with respect to DNAIQ was audited by an external agency? If yes, when did that occur and in respect of what particular issue or issues. Who decided that should occur, provide:

    a. instructions;

    b. list of material;

    c. the report, including any draft report.

14. How were the results of the audit communicated to the DNA laboratory?

15. What permanent changes, or amendments to SOPs, were made as a result of identifying the problems related to DNAIQ?

16. Explain what communications were made to external agencies, including the Queensland Police Service, the Office of the Director of Public Prosecutions, and the Queensland Courts, about the problems with DNAIQ and when the communications were made. Attach copies of any emails or letters sent to the external agencies.

17. Did the problems with DNAIQ lead to the retraction or amendment of results in these cases? If yes, how many cases were affected? By what means were the amendment and retraction of results communicated?

18. Has the DNA laboratory since returned to using DNAIQ processes, systems and/or products? Have there been any further problems with DNAIQ systems or products? Explain all future problems in detail, including what has been done in response to them. Attach any OQI's, Adverse Entry Log's or record of the problem being identified and investigated.

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Re-implementing the automated DNA IQ™ extraction protocol on the MultiPROBE® II PLUS HT EX Forensic Workstation platforms, and associated processes

Interim Report – Extraction Platform B

Chiron Weber, Generosa Lundie, Iman Muharam, Thomas Nurthen, Cathie Allen
Automation/LIMS Implementation Project, DNA Analysis FSS (April 2009)

Keywords: DNA IQ™, MultiPROBE® II PLUS HT EX, adverse event, OQI, verification.

1. Abstract

Some adverse events that were identified in the laboratory were hypothesised to be caused by the automated DNA IQ™ process (including off-deck lysis and STORstar), as evidenced by several OQI's that were noted in the period between February and July 2008 (see report "Investigation into adverse events in the automated DNA IQ™ extraction process" by Nurthen, 2008). The adverse events were notable occurrences whereby possible well-to-well cross contamination may have taken place. Improvements to the extraction procedure were made and tested. The new protocol is fit for routine use within the DNA Analysis Unit for automated DNA IQ™ extractions.

2. Definitions and Abbreviations

DNA	Deoxyribonucleic acid
DNA IQ™	A commercial DNA extraction kit based on paramagnetic bead technology. This is the DNA extraction method performed on the MPII platforms.
MPII	MultiPROBE® II PLUS HT EX
EP-A	Extraction Platform A
EP-B	Extraction Platform B
OQI	Opportunity for Quality Improvement
Sample	A substrate potentially containing DNA material
Lysate	A sample that has undergone off-deck lysis (but not DNA IQ™ extraction)
Extract	A sample that has undergone extraction using DNA IQ™
LOQ	Limit of Quantitation
LOD	Limit of Detection
LOR	Limit of Reporting
RFU	Relative Fluorescence Unit
DWP	Deep Well Plate

3. Background

The MultiPROBE® II PLUS HT EX FORENSIC WORKSTATION platforms (PerkinElmer, Downers Grove, IL, USA) (MPII) are equipped to perform automated DNA extractions, they include a DPC shaker and individual heat controllers to enable on-board lysis and incubation steps. The DNA IQ™ protocol has been verified or validated by various forensic laboratories for use on the MultiPROBE® II PLUS HT EX platform. The laboratories that perform an automated DNA IQ™ protocol include PathWest (Western Australia), Forensic Science South Australia (South Australia) and Centre of Forensic Sciences in Toronto (Ontario), amongst others. In DNA Analysis, the MPII platform also allows walk-away operation of

PCR setup protocols for DNA quantitation and amplification (Muharam *et al* 2006 (Report 1) (Report 2)).

Validation of the manual DNA IQ™ method commenced in April 2007, evaluation of various commercial DNA extraction kits showed DNA IQ™ to be superior over other kits in several aspects (Gallagher *et al*., 2007). Verification of an automated DNA IQ™ method on the MultiPROBE® II PLUS HT EX FORENSIC WORKSTATION platform followed soon after. In October 2007, routine automated DNA IQ™ extractions commenced in the laboratory.

In April 2008, two automated DNA extraction batches that were processed in February 2008 were observed to exhibit possible instances of cross contamination. With the introduction of the off-deck lysis method in March 2008, several other instances were identified. On 14 July 2008, troubleshooting procedures to identify potential issues were commenced, and internal audit 8227 on the automated DNA IQ™ process was performed over the period 15 to 28 July 2008. The auditors identified several trends that needed to be addressed immediately, resulting in the creation of 3 OQI's. On 28 July 2009, the laboratory ceased using the automated DNA IQ™ protocol, and returned to previous manual methods, using a Chelex® 100 resin (BioRad, Hercules, CA, USA) protocol QIS 17171.

At this point in time, a series of actions were undertaken in order to hold off the reporting of results from the automated DNA IQ™ process and also review the results on a batch-per-batch basis. A review of the DNA extraction process commenced.

The automated DNA IQ™ protocol was reviewed internally and also externally by the PerkinElmer National Liquid Handling Specialist, and the necessary changes made. Some of the changes included modifications to dispense heights; optimisation of scan, aspirate, dispense and retract speeds; insertion of post-dispense transport air gaps to remove bubbles; and the removal of flush protocols. A report of the observations was made available to DNA Analysis (Pitcher, 3 October 2008).

Further enhancements and changes to the protocol were made to increase efficiency and further lower the risk of well-to-well cross contamination events. Another review was performed by the PerkinElmer National Liquid Handling Specialist, and a report was again made available (Pitcher, 4 November 2008).

At the final review, the enhancements that were made to the automated DNA IQ™ protocol included:

1. Syringes on the MultiPROBE® II PLUS HT EX FORENSIC WORKSTATION platforms for performing automated DNA IQ™ extractions were changed from 500µL to 1000µL in order to minimise the number of syringe draws and therefore prolong syringe lifetime. The added benefit includes a reduction in the time spent performing pipetting checks/calibrations due to a decrease in the number of syringe replacements.
2. Off-deck lysis volumes were reduced from 500µL to 300µL to minimise the risk of well-to-well splashing that may result in cross contamination. A separate study showed that this change in volume creates a minimal difference in yield.
3. Off-deck lysate transferred into individual Nunc Bank-It™ tubes rather than 2.-ml screw cap tubes. This removes the requirement for performing liquid STORstar. The Bank-It™ tubes containing lysates are arranged using STORstar and placed on the MPII deck for automated lysate transfer automatically to a 96-deep well plate for DNA IQ™ processing.
4. The deck layout for performing automated DNA IQ™ extractions was changed to minimise 8-tip arm movement in the right-left or left-right directions when sample is

Commented [W1]: References

contained within tips. The 8-tip arm now moves back-front or front-back, within the same column (Figure 1).

5. Resin is added automatically by the MPII, whereas before the resin was added manually by the operator.
6. Post-dispense resin mixing is not performed by the MPII. Instead, the 96-deep well plate is sealed with a septa mat and shaken on a MixMate unit, then centrifuged. The septa is removed and the plate is returned to the MPII by the operator.
7. The magnet has been changed from a PKI magnet to an ABI magnet. The ABI magnet does not have corner holders and therefore the 96-deep well plate fits easily onto the magnet (Figure 2).
8. System and transport air gaps have been optimised to reduce the chances of bubbles/drips forming on the end of disposable tips.
9. The electronic platemap that provides sample identification information and pipetting volumes for the MPII has been changed, to accommodate the changes.
10. Risks were considered with regards to droplets on the side of disposable tips.

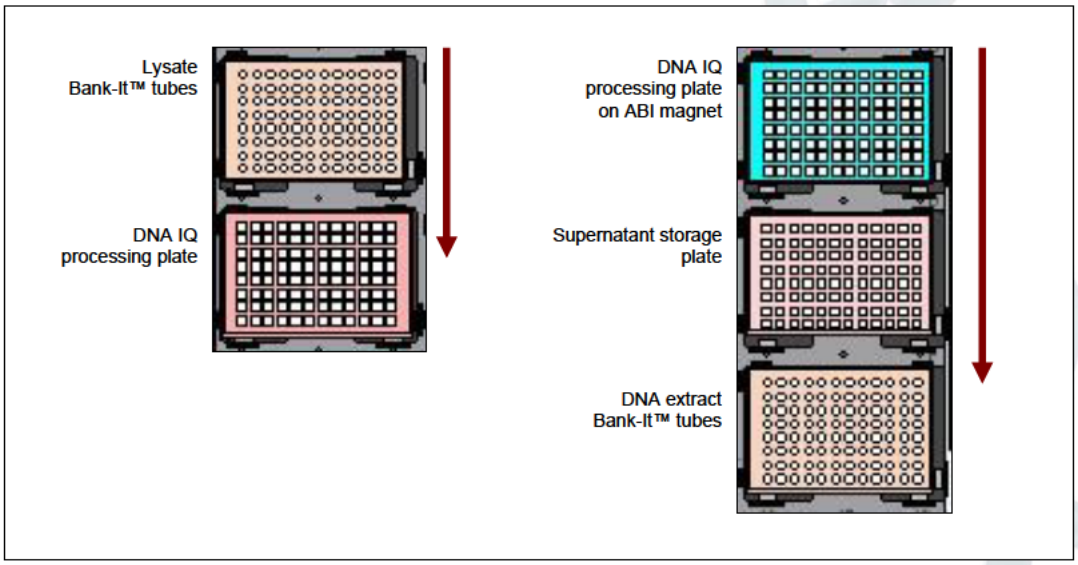

Figure 1 Movement of samples between different racks and plates occur from the back to the front of the instrument, therefore only passing over one column at a time. No diagonal movements occur when samples are present in tips, therefore minimising the risk of contamination across the plate.

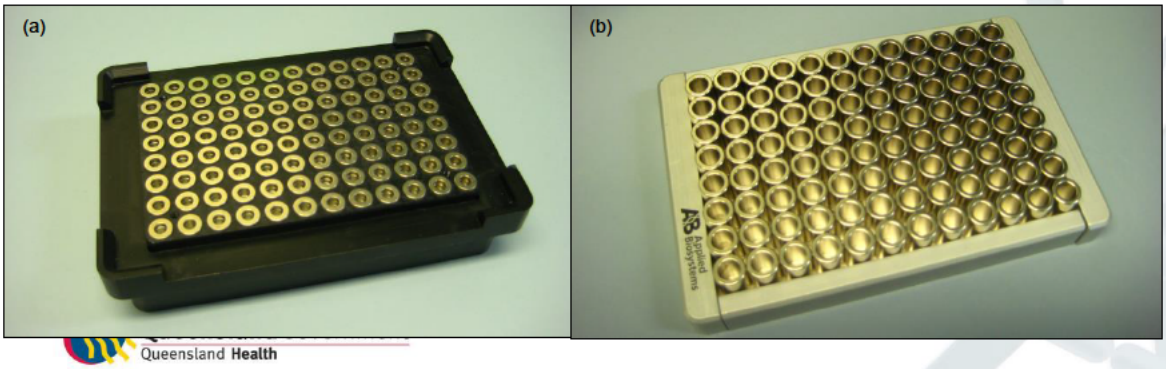

Figure 2 The PKI magnet (a) has corners that often required manual intervention to allow full contact between the plate surface and the magnet. The ABI magnet (b) has no corner holders and the magnets are slightly raised, therefore allowing full contact with the plate.

These changes were tested in order to determine sensitivity and efficiency/recovery of the new protocol. To determine the risk of well-to-well cross contamination, several anti-contamination plates were processed in various layouts, including soccerball, checkerboard and zebra stripe. In addition to these plates, batches containing 96 FTA® reference samples with different DNA profiles were extracted in order to further assess the risk of cross contamination. The combination of all these plates provided crucial information regarding contamination risks that could be attributed to the automated DNA IQ™ process. The procedure was further optimised based on the results obtained from these plates.

The further change made to the process was the use of a pierceable aluminium seal in place of the septa mat for off-deck mixing using an Eppendorf MixMate. This change was made due to the inability of the septa mat to appropriately seal the 2.2ml Deep Well Plate (DWP) during mixing. The aluminium seal is pierced, this is a preferable option due to the risks associated with peeling or removing a plate seal. The updated procedure is referred to as V6.3 within this report. The aluminium adhesive seal was then superseded by an aluminium heat seal. The heat sealer has been incorporated into the automated procedure V6.4 and V6.5.

This report presents the results on the verification of changes made to the automated DNA IQ™ extraction process using extraction platform B. The same verification plates and program will be used to evaluate extraction platform A. During the validation process parts of the automated extraction procedure changed, this is reflected in the report and the results are separated pre and post the change of plate sealing and seal piercing. A re-implementation strategy is also proposed in this report, along with the expectation that a formal review process (audit) will be performed 2 and 8 months post-implementation to ensure that the changes are effective and viable for long-term use.

4. Aim

- To verify changes made to the automated DNA IQ™ extraction process on the MultiPROBE® II PLUS HT EX FORENSIC WORKSTATION platforms and determine the risk of well-to-well cross contamination events.
- To re-implement an improved automated DNA IQ™ extraction process (with the verified changes) into the laboratory for routine processing of casework samples.

5. Equipment and Materials

- MultiPROBE® II PLUS HT EX FORENSIC WORKSTATION (PerkinElmer, Downers Grove, IL, USA)
- 1000µL syringes for MultiPROBE® II PLUS (Tecan Systems, Inc., San Jose, CA, USA)
- Gravimetric Performance Evaluation Option with Mettler SAG285/L balance (Mettler-Toledo, Greifensee, Switzerland)
- DNA IQ™ System (Promega Corp., Madison, WI, USA)
- MixMate (Eppendorf AG, Hamburg, Germany)
- *automate.it* STORstar (Process Analysis & Automation, Hampshire, UK)
- 96-well magnetic ring stand (Applied Biosystems, Foster City, CA, USA)
- 2.2mL 96-deep well plate (ABgene, Epsom, Surrey, UK)

- Aluminium sealing film (Axygen,)
- 96 square cap sealing mat (ABgene, Epsom, Surrey, UK)
- TNE Buffer (10mM Tris, 1mM EDTA, 100mM NaCl, pH 8.0)
- TE^{-4} Buffer (10mM Tris HCl, 0.1mM EDTA, pH 8.0)
- 20mg/mL Proteinase K
- 40% Sarcosyl (N-lauroylsarcosine sodium)
- 5% TriGene
- 70% ethanol
- 1% Amphyl
- 0.2% Amphyl
- Nanopure water
- Isopropyl alcohol
- Molecular Biology Grade Water (Sigma-Aldrich Corp., St Louis, MO, USA)
- Human Genomic DNA: Male (Promega Corp., Madison, WI, USA)
- Human Genomic DNA: Female (Promega Corp., Madison, WI, USA)
- 1.0mL Nunc Bank-It™ tubes (Nunc A/S, Roskilde, Denmark)
- 175µL non-conductive sterile filter RoboRack tips (PerkinElmer, Downers Grove, IL, USA)
- 1000µL Conductive sterile filter Robotix tips (Molecular BioProducts, San Diego, CA, USA)
- BSD Duet 600 (BSD Robotics, Brisbane, QLD, Australia)
- RECAP-96M™ (LifeTool™, UK)
- ABI Prism® 7000 SDS (Applied Biosystems, Foster City, CA, USA)
- Quantifiler™ Human DNA Quantification kits (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 96-well optical reaction plates (Applied Biosystems, Foster City, CA, USA)
- AmpFℓSTR® Profiler Plus® Amplification kits (Applied Biosystems, Foster City, CA, USA)
- 96-well half skirt clear PCR microplate (Axygen, Union City, CA, USA)
- GeneAmp® 9700 thermalcycers (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 3130*x*/ Genetic Analyzer (Applied Biosystems, Foster City, CA, USA)
- GeneScan™ 500 ROX™ Size Standard (Applied Biosystems, Foster City, CA, USA)
- Hi-Di™ Formamide (Applied Biosystems, Foster City, CA, USA)
- 3130*x*/ POP-4™ Polymer (Applied Biosystems, Foster City, CA, USA)
- 4titude 4Seal Variable Temperature Sealer (4TITUDE, Ockley, Surrey, UK)
- Pierce seal (4titude Ockley, Surrey, UK)

6. Methods

6.1 Gravimetric evaluation of pipetting accuracy and precision of the 1000µL syringes

Gravimetric analysis was performed (according to QIS 24732) by placing a SAG285/L balance on the platform deck and instructing the MPII to repeatedly pipette set volumes of system liquid into a receptacle on the balance pan. Readings were taken automatically by the software and compiled into a results table, which was then used to automatically generate an Excel-based results chart containing mean, %CV and %inaccuracy values. The mean values obtained were used to calculate R^2, slope and Y-intercept (offset) values to calibrate the system's pipetting.

Pipetting performance was assessed for various volumes using three different tip types (1ml conductive, fixed and 175ul non-conductive) in order to calculate appropriate R^2, slope and Y-intercept (offset) values which were then added to the performance file for each tip type. Values were calculated for both Blowout (single-liquid transfer) and Waste (multidispense) modes for the 1000µL conductive tips, and Blowout mode only for the 175µL non-conductive tips and fixed tips.

All gravimetric testing was performed using the Balance Test DT test program within WinPrep®. Parameter values that needed to be entered into the Balance Test Information Window (Figure 3) included those as outlined in Table 1.

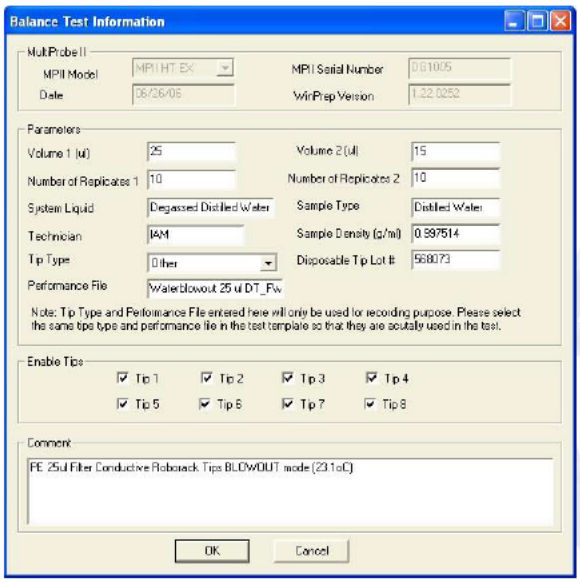

Figure 3 The Balance Test Information Window as present within the Balance Test DT program. All pipetting parameters are entered here and are subsequently transferred to the result output file.

Pipetting accuracy and precision were examined at four different volumes for each tip size: 175, 100, 50, 15µL for 175µL tips and 1000, 700, 400, 100µL for the 1000µL and fixed tips. In order to calculate unbiased values for each set of volumes, the slope and offset values in the relevant Performance File were changed to the default 1 and 0 respectively prior to testing. The mean volumes that were pipetted by each tip (10 replicates per tip) at the four designated volumes were used to generate a standard curve. The slope and offset calculated from this curve was used to calibrate the relevant Performance File. The final Performance File settings were then tested at the highest and lowest volumes (as per Table 1) to confirm accurate and precise pipetting.

Table 1. Input values that are required for the various Balance Test Information parameters.

Parameter(s)	Value
Volume 1 and Volume 2	For 175µL tips: 175, 100, 50, 15µL For 1000µL tips: 1000, 700, 400, 100µL For fixed tips: 1000, 700, 400, 100µL
Number of Replicates	10
System Liquid	Degassed Nanopure Water
Sample Type	Nanopure Water
Technician	Initials of the operator performing the test
Sample Density (g/ml)	The density of water at environmental temperature*
Tip Type	Other
Disposable Tip Lot #	The lot number of the particular tips in use
Performance File	The appropriate Performance File for the tip (175µL, 1000µL or fixed tips) and pipetting mode (Blowout or Waste) in use
Enable Tips (checkboxes)	Select the actual tips (1 to 8) to be tested
Comments	Free text box to add additional information (eg. Tip type, mode, current environmental room temperature, etc).

* Water density values were obtained from http://www.simetric.co.uk/si_water.htm

6.2 Sensitivity and efficiency/recovery

Male Human Genomic DNA (Promega Corp., Madison, WI, USA) was diluted using molecular grade water (Sigma-Aldrich Corp., St Louis, MO, USA) and added to Nunc Bank-It™ tubes in duplicate at a total amount of 0, 2, 5, 10, 25, 50 and 100ng with a total volume of 300µL. Samples were stored at 4°C until processing using the automated DNA IQ™ method (version 6.1).

6.3 Anti-contamination checks with water

Male Human Genomic DNA and Female Human Genomic DNA (Promega Corp., Madison, WI, USA) were added separately to individual Nunc Bank-It™ tubes at a total amount of 1125ng, in a total volume of 300µL water (Sigma-Aldrich Corp., St Louis, MO, USA). Along with Nunc Bank-It™ tubes that only contained 300µL water (no DNA template), the tubes were arranged using STORstar (Process Analysis & Automation, Hampshire, UK) (according to QIS 24256) in soccerball, checkerboard or zebra stripe format, referred to in section 6.10. Samples were stored at 4°C until processing using the automated DNA IQ™ method (version 6.1 – 6.4).

6.4 Anti-contamination checks with DNA IQ™ extraction buffer

Male Human Genomic DNA and Female Human Genomic DNA (Promega Corp., Madison, WI, USA) were added separately to individual Nunc Bank-It™ tubes at a total amount of 1125ng, made up to a total volume of 300µL using extraction buffer (Promega Corp., Madison, WI, USA). Along with Nunc Bank-It™ tubes that only contained 300µL of extraction buffer (no DNA template), the tubes were arranged using STORstar (Process Analysis & Automation, Hampshire, UK) in soccerball, checkerboard or zebra stripe format. Samples were stored at 4°C until processing using the automated DNA IQ™ method (version 6.5).

6.5 Punching of reference buccal cell samples on FTA® Classic Card (QIS 24823) – Method 1

A BSD Duet 600 (BSD Robotics, Brisbane, QLD, Australia) instrument was used to punch 4 x 3.2mm disks from FTA® Classic Cards containing reference buccal cell samples into individual 1.5mL tubes. The samples were processed in batches of 22 (plus 2 controls) using the modified off-deck lysis procedure, prior to processing using the automated DNA IQ™ method (version 6.1).

6.6 Punching of reference buccal cell samples on FTA® Classic Card – Method 2

A BSD Duet 600 (BSD Robotics, Brisbane, QLD, Australia) instrument was used to punch 4 x 3.2mm disks from FTA® Classic Cards containing reference buccal cell samples into individual 1.5mL tubes. The samples were processed in batches of 23 (plus 1 negative control) using the modified off-deck lysis procedure, prior to processing using the automated DNA IQ™ method (version 6.4/6.5).

6.7 Modified off-deck lysis procedure (300µL, no retained supernatant) for lysis of reference samples on FTA® Classic Card

300µL Extraction Buffer was added to tubes containing reference FTA® samples, fully submerging the 4 x 3.2mm disks. The tubes were incubated at 37°C for 45 minutes in a hot block (Thermomixer comfort), vortexed briefly, then incubated at 65°C for 10 minutes. The tubes were centrifuged on a tabletop microcentrifuge at maximum speed (14,000rpm) for 1 minute. The lysate was then transferred to Nunc Bank-It™ tubes labelled with the

corresponding barcodes. The samples were arranged in sequence using STORstar prior to processing using the automated DNA IQ™ method (version 6.x).

6.8 DNA extraction using the automated DNA IQ™ protocol (version 6.1-6.3)

DNA extraction using the DNA IQ™ system (Promega Corp., Madison, WI, USA) was performed on the MultiPROBE® II PLUS HT EX FORENSIC WORKSTATION platform (PerkinElmer, Downers Grove, IL, USA) using the modified automated DNA IQ™ protocol (version 6.1-6.3) as per QIS 24897R4.

- Nunc Bank-It™ tubes containing off-deck lysate were placed on the MP II platform for automated transfer of 300µL lysate to a 2.2mL ABgene 96-deep well plate.
- The MPII automatically dispensed 50µL DNA IQ™ Resin solution and 557µL DNA IQ™ Lysis Buffer.
- The operator sealed the 96-deep well plate with a 96-square cap sealing mat (septa), ensuring that each well was sealed tightly, and placed the 96-deep well plate on a MixMate instrument to mix at 1100rpm for 5 minutes prior to centrifugation on an Eppendorf 5804 at 3000rpm for 2 minutes.
- The septa mat was pealed carefully and the 96-deep well plate was returned to the ABI magnet on the MPII to continue processing.
- Supernatant was transferred to a second 96-deep well storage plate.
- The DNA IQ™ resin was then washed by a single wash routine using DNA IQ™ Lysis Buffer and three wash routines using DNQ IQ™ Wash Buffer.
- A double elution process of 2 x 50µL was then performed, where each elution involved addition of 50µL DNA IQ™ Elution Buffer followed by incubation at 65°C for 6 minutes (shaking on the DPC Shaker for the last 3 minutes).
- Purified DNA extracts were eluted into individual Nunc Bank-It™ tubes and stored at 4°C for short periods or -20°C for extended periods until PCR quantification and amplification. The difference between versions 6.1 – 6.3 is optimisation of pipetting heights.

6.9 DNA extraction using the automated DNA IQ™ protocol (version 6.4-6.5)

This procedure is the same as outlined above for version 6.1 – 6.3 with the minor modification of replacing the septa mat with an adhesive aluminium seal. The adhesive aluminium seal was later superseded by a heat seal. In version 6.5 the user prompt messages have been updated and the dispense height for DNA IQ™ Resin solution has been optimised.

6.10 Anti-contamination checks consisting of purified genomic DNA – Plate layouts

Anti-contamination checks consisting of purified genomic DNA were extracted in four different orientations. These plate layouts were used to assess the various versions of the extraction method.

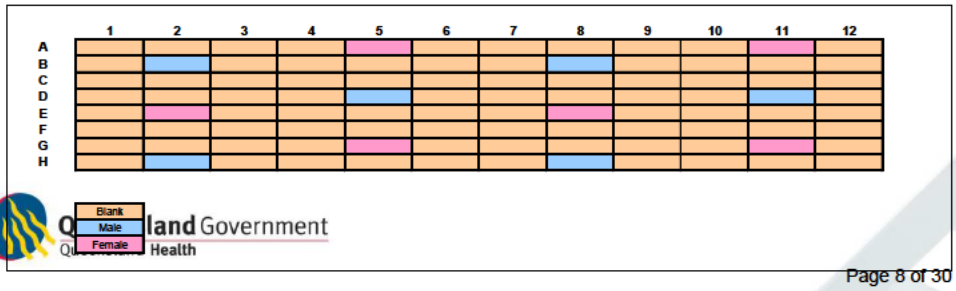

Figure 4 Anti-contamination plate in soccerball format. Each plate contained 6 DNA samples consisting of male DNA and 6 DNA samples consisting of female DNA, and the remainder were negative (blank) samples consisting of water and no template DNA.

For the patterned anti-contamination checks, the Male and Female Human Genomic DNA samples (Promega Corp., Madison, WI, USA) were used as the positive samples, and molecular biology grade water (Sigma-Aldrich Corp., St Louis, MO, USA) was used as the negative (blank) samples. For the soccer ball and zebra stripe plates, all plates were arranged in the same format see Figure 4 and Figure 5 respectively, but the arrangement of samples was re-ordered for the checkerboard plates in order to ensure that each MPII tip is tested for processing and delivery of both DNA samples and negative controls and that no cross contamination between the two occur during DNA IQ™ processing and Figure 6.

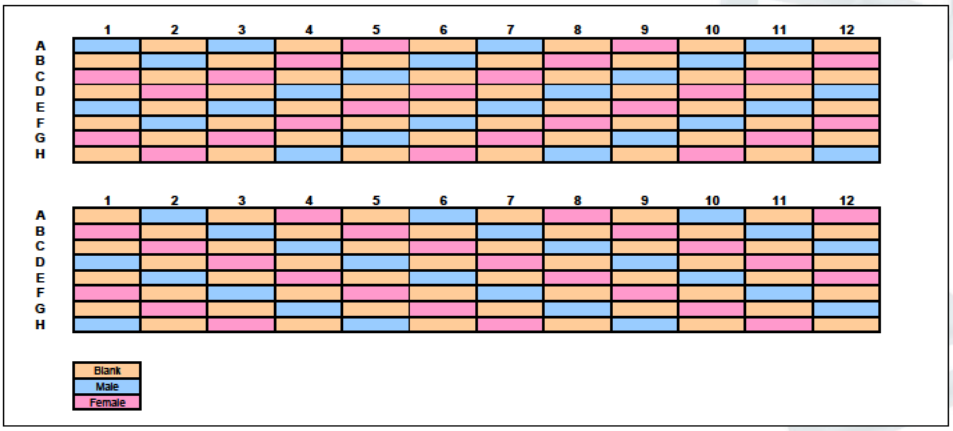

Figure 5 Anti-contamination plates in checkerboard format. Each plate consisted of 48 DNA samples (24 male and 24 female) and 48 negative (blank) samples consisting of water and no template DNA.

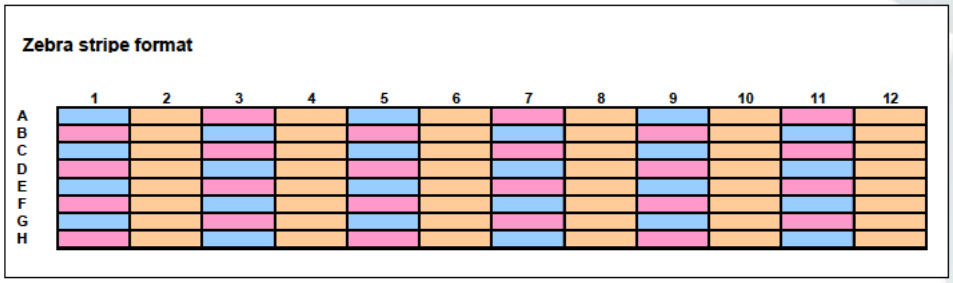

Figure 6 Anti-contamination plates in zebra stripe format. Each plate consisted of 48 DNA samples (24 male and female) and 48 negative (blank) samples consisting of water and no template DNA.

6.11 Assessing the risk of contamination on the septa mat

Various positions on the underside of the septa mat that was used to seal the Soccerball 2 EP-B 96-deep well plate were swabbed, and these swabs, were sampled and placed into individual 1.5mL tubes. In total, 40 swabs were collected. The swabs were processed using the modified off-deck lysis procedure, prior to processing using the automated DNA IQ™ method (version 6.1).

6.12 Sodium Iodide testing of the sealing of a ABgene 96 well DWP using various seals and also the septa mat

Small pieces of paper were cut into squares of about 6x6mm and placed inside each well of an Abgene 96 well DWP. Then 100µL of bleach was dispensed into each well. Into selected wells C4, D7 and F4 800µL of Extraction buffer containing 3M sodium iodide (NaI) was added. The plate was sealed with a septa mat and mixed on an Eppendorf MixMate for 5 minutes at 1100rpm. Following mixing the plate was centrifuged at 3000rpm for 2 minutes. The NaI with bleach reaction was used to determine if NaI had transferred between wells.

The iodide oxidizes to iodine and gives iodine in the presence of bleach. The iodine creates a colour change in solution and especially sensitive in the presence of cardboard or fabric. (http://www.scienceteacherprogram.org/chemistry/Oleary99.html)

6.13 Nunc Bank-It™ de-capping, NaI investigation

The de-capping of Nunc Bank-It™ tubes was investigated using NaI and bleach. Each Nunc Bank-It™ tube had 200µL of extraction buffer and 100µL of 3M NaI added into 16 Nunc Bank-It™ tubes in a Nunc rack. The tubes were then briefly mixed and centrifuged as per the standard operating procedure (QIS 24897). The tubes were de-capped and pressed against cardboard sprayed with bleach. Positive reactions indicate that sample remains adhering to the cap.

The first rack of 16 samples was de-capped immediately at room temperature. The second rack of samples was stored in the fridge for ~24 hours prior to de-capping.

6.14 Sodium Iodide testing of the first part of the Automated DNA IQ extraction

A rack of 96 Nunc Bank-It™ tubes were filled with 300µL DNA IQ™ Extraction Buffer except for positions C4, F4, D7 and G8. These positions were filled with 300µL 6M NaI made up in DNA IQ™ Extraction Buffer. To each well in the storage plate 100µL of bleach and a 6mm x 6mm piece of cardboard was added. This was presented to the MPII EP-B for the first steps of the program. The ABgene 2.2mL DWP was sealed using an Aluminium adhesive seal, mixed on an Eppendorf MixMate, centrifuged and pierced using a sterile PCR plate as per the standard operating procedure. This plate was then presented to the MPII for removal of "lysate" to the Store plate.

6.15 DNA quantification, amplification, capillary electrophoresis and genotyping analysis

Extracted samples were quantified using the Quantifiler™ Human DNA Quantification Kit (Applied Biosystems, Foster City, CA, USA) as per QIS 19977R3, followed by amplification using the AmpF*t*STR® Profiler Plus® kit (Applied Biosystems, Foster City, CA, USA) as per QIS 19976R2. Fragment analysis was performed by capillary electrophoresis using an ABI Prism® 3130*xl* Genetic Analyzer instrument (Applied Biosystems, Foster City, CA, USA) as per QIS 15998R5. Fragment data was analysed using GeneMapper ID-*X*® v1.1 (Applied Biosystems, Foster City, CA, USA), with thresholds for heterozygous and homozygous peaks at 50 and 200 RFU respectively. The allelic imbalance threshold was set at 50%. When needed, some samples were analysed at a lower threshold of 20 RFU or 16 RFU. All results were uploaded to AUSLAB for data storage.

7. Results and Discussion

7.1 Gravimetric evaluation of pipetting accuracy and precision of the 1000µL syringes

Pipetting on both automated platforms was assessed gravimetrically as per laboratory practice. Gravimetric results indicate that pipetting performance for five different pipetting behaviours using 1000µL syringes on the instruments is accurate and precise to within the established threshold of ±5% (Table 2). The maximum CV at the maximum volume was 0.45% (except one performance file due to variations in one VersaTip™, the variation was within acceptable limits for the tip type), whereas the maximum CV at the minimum volume was 0.51%. With 500µL syringes, these values were 0.78% and 1.1% respectively. The maximum CV at the maximum volume dropped by almost half after changing to 1000µL syringes due to the less number of draws required to deliver volumes greater than 500µL. The CV for pipetting at lower volumes is expected to be slightly higher than the CV at higher volumes using 1000µL syringes, because accuracy at small volumes is harder to achieve with larger syringe sizes. Nevertheless, pipetting on the extraction platforms is limited to a minimum of 50µL for the purposes of delivering DNA IQ™ Resin solution, which has shown a very low inaccuracy value of only 0.4%.

Table 2. Gravimetric evaluation results for various performance files used on either MPII EP-A or MPII EP-B with 1000µL syringes installed.

Performance File	Max. Vol. µL	Min. Vol. µL	Max. Vol. µL Mean	Max. Vol. %CV	Max. Vol. %Inac.	Min. Vol. µL Mean	Min. Vol. %CV	Min. Vol. %Inac.
EXTN A								
WaterWaste 175µL DT_FW FSS21102008 prf	175µL	50µL	175.24	0.36	0.1	50.12	0.66	0.2
WaterBlowout 175µL DT_FW FSS18092008 prf	175µL	50µL	176.07	0.26	0.6	49.76	1.40	0.5
WaterWaste 1mL DT_FW FSS15092008.prf	1000µL	100µL	1004.84	0.36	0.5	101.44.	0.52	1.4
WaterBlowout 1mL DT_FSS18092008.prf	1000µL	100µL	1001.35	0.36	0.1	100.41	0.90	0.4
WaterBlowoutFixedTips_FSS19092008.prf	1000µL	100µL	1000.46	0.25	0.0	99.34	0.51	0.7
EXTN B								
WaterWaste_175 DT_FW FSS 20081017.prf	175µL	50µL	175.72	0.51	0.4	49.89	0.86	0.2
Water Blowout_175ul DT_FSS30092008.prf	175µL	50µL	175.93	0.42	0.5	49.64	0.72	0.7
WaterWaste_1mLDT_FSS_02102008.prf	1000µL	100µL	1002.43	0.29	0.2	99.42	0.82	0.6
WaterBlowout_1mL DT_FSS25092008.prf	1000µL	100µL	999.11	0.96	0.1	101.17	0.80	1.2
WaterBlowout Fixed Tips_1mL_FSS_24092008 prf	1000µL	100µL	999.01	0.45	0.1	98.87	1.03	1.1

The results from the gravimetric assessment of the 1000µL syringes indicate that they are comparable in performance to the 500µL syringes. It is envisaged that syringe lifetime will be prolonged, as 1000µL syringes perform a reduced number of draws to deliver larger volumes. Nevertheless, proactive checking of the pipetting performance of the larger syringes should be performed on a regular basis to establish an approximate lifetime for these syringes, and therefore syringe replacements and calibration checks can be scheduled in order to minimise instrument downtime.

7.2 Modifications to the off-deck lysis and automated DNA IQ™ procedures

The modified automated DNA IQ™ procedure, designated version 6.x, is quite different from the previous version that was used routinely in the laboratory (e.g. version 4.1). As described in Section 3 above, several modifications have been made in order to minimise the risk of cross contamination and increase the efficiency of the protocol. Refer to Figure 7 for the virtual deck layouts of both procedures for comparative purposes.

The changes that were made incorporate recommendations from Audit 8227 in addition to suggestions from the PerkinElmer National Liquid Handling Specialist. Changes in various air gap settings and movement speeds were made in order to reduce the risk of drop formation and splashing that may lead to adverse events (see Pitcher 102008 and Pitcher 112008).

The volume of Extraction Buffer added to samples during off-deck lysis was reduced from 500µL to 300µL to minimise the risk of well-to-well splashing that may result in cross contamination. The change in the off-deck lysis procedure, whereby lysates are added to a 96-deep well plate with the aide of STORstar to adding lysates directly into a corresponding Nunc Bank-It™ tube, the automated DNA IQ™ version 6.x procedure performs the automatic transfer of lysate from Bank-It™ tubes to 96-deep well plate therefore a new step in the protocol that was not included in previous versions was required. Version 6.x incorporates automated addition of DNA IQ™ Resin solution; mixing is achieved by placing the plate on a MixMate rather than pipette mixing as utilized in version 4.1. Exclusion of the Resin mixing steps has resulted in a time saving of up to 60 minutes, and the added benefit of increased yield due to high recovery rates, see section 6.2.

The use of the alternative ABI magnet allows the 96-deep well plate to sit securely on the magnet without requiring operator intervention. Furthermore, the restricted back-front movement (in a column) of the 8-tip arm during sample transfers removes the risk of contaminating samples across the entirety of the 96-deep well plate, as the instrument only performs diagonal movements across the plate when moving to the tip chute to dispense tips. This diagonal movement across the plate cannot be removed. The volumes for washing and elution steps have not changed, but the steps have been optimised by changing pipetting speeds and adding post-dispense transport air gaps in order to prevent the formation of air bubbles at the end of the tips (see Pitcher 102008 and Pitcher 112008).

External auditors from the Sir Albert Sakzewski Virus Research Centre were requested by Mr Greg Shaw (Senior Director, Forensic and Scientific Services) to review the automated DNA IQ™ procedure, specifically version 6.1, which included associated procedures such as off-deck lysis and arranging tubes using STORstar. The auditors scrutinised both staff input and instrument operation and found the modified off-deck lysis and automated DNQ IQ™ procedures to be “adequate and specifically designed to prevent cross contamination of test samples” and that previous adverse events that were encountered and recorded in various OQI’s were “most likely related to the use of adhesive film in sealing the deep-well plates used in the off-deck lysis procedure” that is no longer used in the modified off-deck lysis protocol. The reviewers also felt that extensive measures are undertaken in the laboratory to prevent cross contamination of samples these include the application of personal protective equipment and other protective measures (including environmental monitoring) Sloots & Whiley 2008.

Lastly, staff members from the Analytical Team participated actively in the development and testing of the modified protocols by taking ownership of the DNA IQ™ standard operating procedure and performing anti-contamination checks. Staff members also performed pipetting calibration checks of the two MPII instruments dedicated for automated DNA IQ™ extraction. This level of involvement has increased the number of staff members involved in the review of the modified processes, and has made the change process more transparent.

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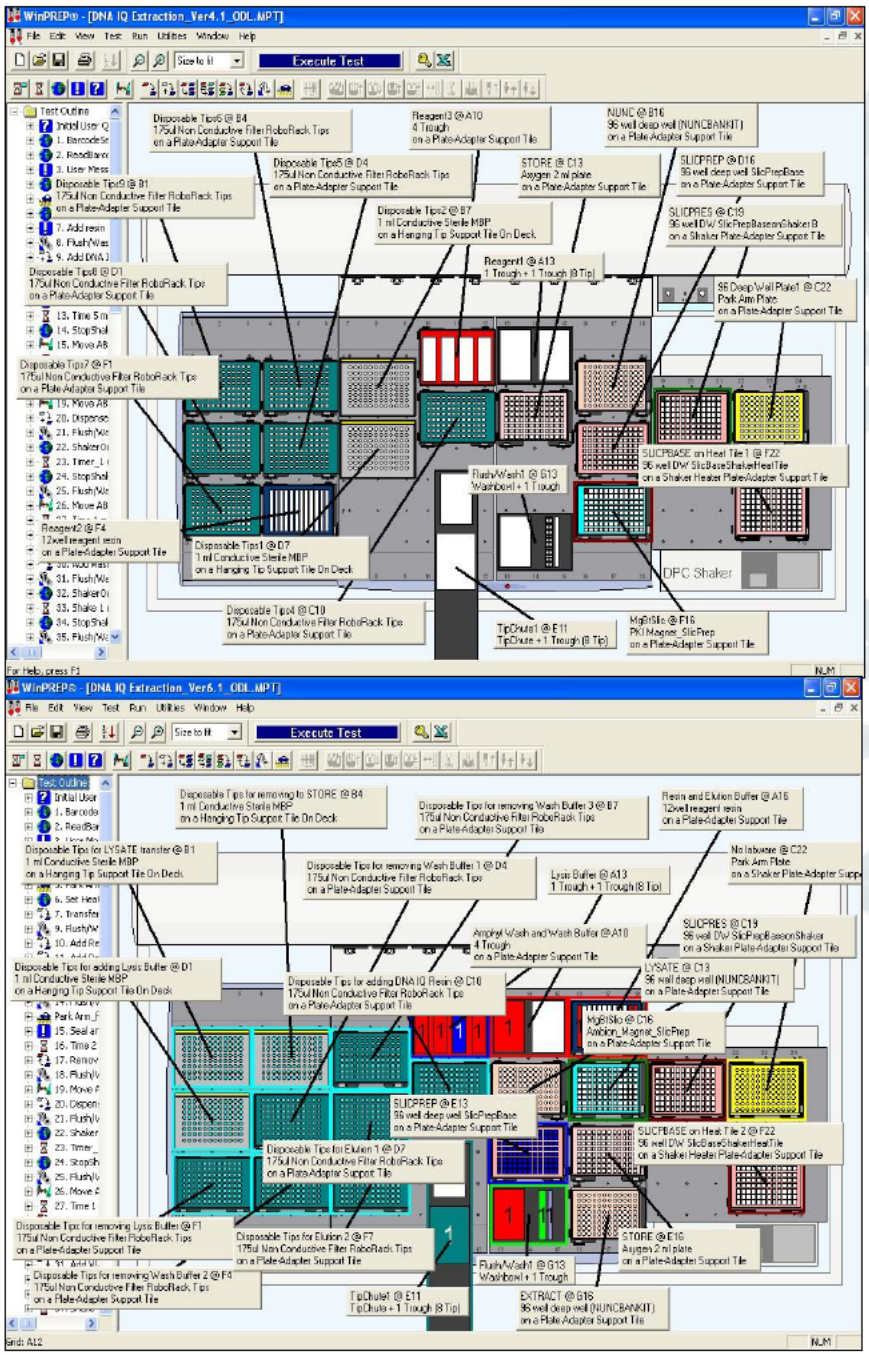

Figure 7 Virtual deck layouts for version 4.1 (top) and version 6.5 (bottom), showing changes in labware and positioning of labware on the MPII deck.

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Based on feedback from numerous reviewers, both external (PerkinElmer and Sir Albert Sakzewski Virus Research Centre) and internal (Analytical Section staff members), the modified off-deck lysis and automated DNA IQ™ procedures are suitable for processing forensic samples, and adequate cross contamination prevention measures are in place.

7.3 Sensitivity and efficiency as assessed by percent recovery

To assess sensitivity and percentage recovery of the modified automated DNA IQ™ procedure, known quantities of purified genomic DNA were processed through the procedure and the resulting output quantity was measured and compared to the original input quantity. Purified Male Human Genomic DNA (Promega Corp., Madison, WI, USA) was selected for use in this study because this product undergoes strict factory QA/QC checks to ensure accurate and reliable DNA quantification. Furthermore, this DNA is currently used in DNA Analysis to create a DNA standard suitable for use in conjunction with the Quantifiler™ system (Applied Biosystems, Foster City, CA, USA), and therefore historical records exist for its reliable performance.

The purpose of this test was to determine the ability of the automated DNA IQ™ procedure (specifically the DNA IQ™ Resin) in binding and releasing the DNA that was present within the sample. Because of this, samples were not subjected to off-deck lysis. Instead, purified DNA was suspended in 300µL molecular biology grade water (Sigma-Aldrich Corp., St Louis, MO, USA) and processed directly using the automated DNA IQ™ procedure.

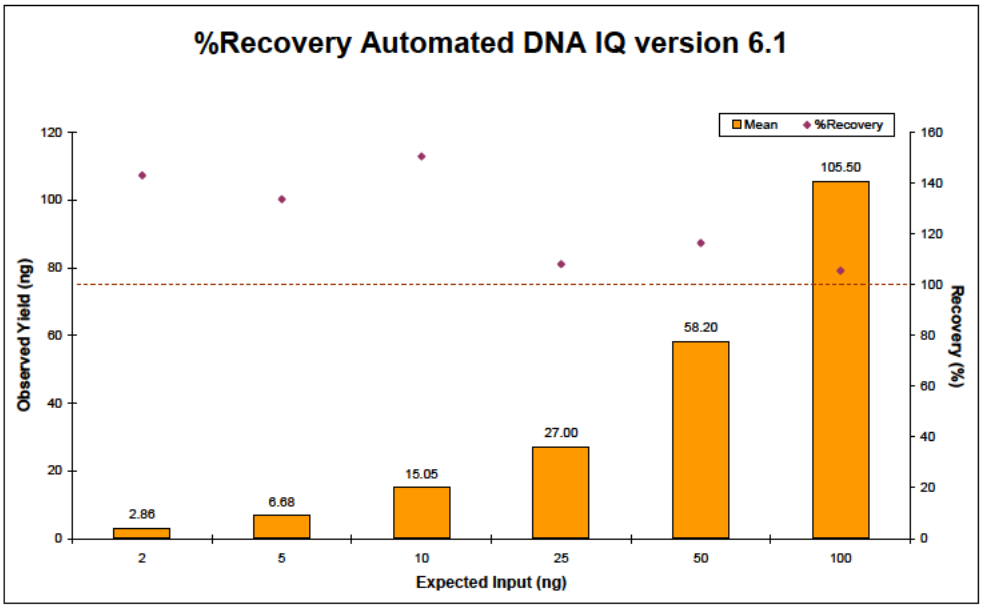

Figure 8 The recovery rate of the modified automated DNA IQ™ procedure (version 6.1).

The 2ng – 100ng samples used are equivalent to concentrations of 0.0067 and 0.3334ng/µL. These concentrations reflect those observed in forensic samples, i.e. approximately 78% of casework samples generate quantitation values of less than 0.1ng/µL (data not shown). DNA IQ™ is optimised for the isolation and recovery of trace

amounts of DNA, and is known to saturate at around 100ng, resulting in decreased recovery rates for samples containing more than 100ng of DNA.

Testing results indicate that the modified automated DNA IQ™ procedure is very sensitive and able to isolate low copy number DNA samples at a very high recovery rate that is close to 100% (Figure 8). The percentage recovery rates in our study were greater than 100% because the observed values were greater than the expected values. Inherent variation in pipetting during dilution of the samples, and inherent variations in the quantification system may have caused additional slight differences in the two values. Nevertheless, it can be postulated that for most samples processed in the laboratory, the modified automated DNA IQ™ procedure will be able to recover most if not all of the DNA that is present in a sample.

7.4 Septa mat swab results

The aim of the experiment was to identify if DNA is present on the septa mat that may cause contamination when the septa mat is peeled back. Prior to removing and swabbing the septa mat, the plate was mixed on a MixMate and centrifuged as per the standard operating procedure for routine automated extractions. In total 40 samples were taken from the septa mat. All samples had an undetermined quantification value and all profiles were NSD. No indications of below threshold peaks were noted. Based on these results it was concluded that the septa mat was fit for purpose. Anti-contamination plates in soccerball, checkerboard and zebra stripe format were extracted using the septa mat for plate sealing. These results are presented in section 7.5.

7.5 Anti-contamination results for Version 6.1-6.3 (Extraction platform B)

Several of the negative (blank) samples that were processed on anti-contamination batches generated quantification values that were below or close to the validated LOD for quantification. The LOD within DNA Analysis is 0.00426ng/µL. The samples ranged from 0.000121 to 0.008730ng/µL; note: some of the higher values resulted from a failed quantification batch that was subsequently repeated and found to satisfy all acceptance criteria). A subset was re-quantified, most of which yielded “undetermined” values indicating that no DNA was detected. A small number of samples yielded quantification values that were below the LOD.

A number of negative controls showed indications of cross contamination. Cross contamination was also observed from male to female samples. The adverse events were not restricted to a certain plate layout.

The occurrence of cross contamination without a clear identifiable cause prompted close scrutiny of each step within the automated extraction procedure. Inadequate sealing of the DWP using the septa mat was identified as a probable cause and was further investigated using a NaI and bleach reaction.

7.6 Septa mat seal testing using NaI

The contamination events observed using the automated extraction process version 6.1-6.3 displayed some contamination occurring to blanks on the right hand side of samples. As such this is not possible with the automated process. The robotic platform does not move from left to right over the plate after contacting sample. Therefore, the contamination events from left to right indicated that contamination was occurring during the manual intervention step(s) within the process. The manual processes are, the plate sealing and mixing or centrifuging using the septa mat.

The sealing capacity of the septa mat of the DWP was assessed using a NaI test. NaI solution when in contact with bleach and cardboard causes the cardboard to turn a purple/black colour.

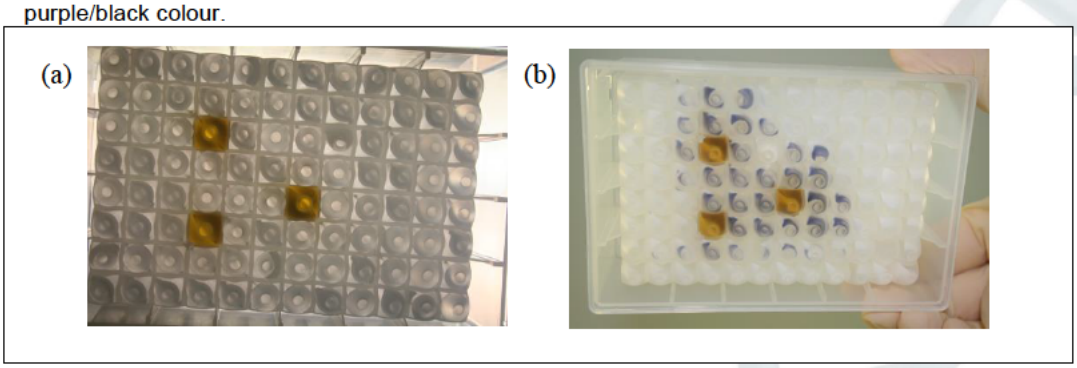

Figure 9 (a) ABgene 2.2ml DWP sealed with septa mat after mixing. Yellow wells contain NaI, surrounding wells contain bleach and cardboard. **(b)** Wells after mixing and centrifuging of 2.2ml DWP. Wells with dark staining contain NaI transferred from the yellow coloured wells.

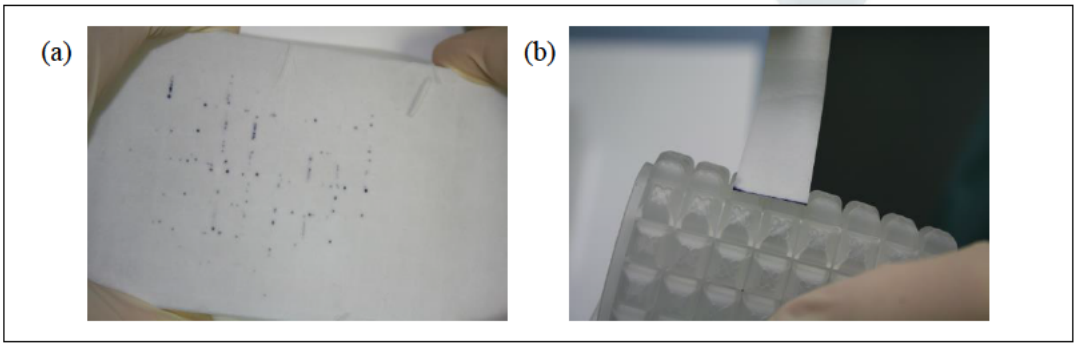

Figure 10 (a) Cardboard swab of DWP after removal of the septa mat. **(b)** Cardboard swab between the grooves of the septa mat.

The NaI test in Figure 9b clearly shows that sample has travelled from a positive control well (yellow in appearance) across a number of other wells within the plate. It is clear that when the septa mat is used for sealing, a sample can travel across the plate up to 3 wells. It is hypothesised that sample travels via capillary action across the top of the wells and when the plate is centrifuged the sample travels into the surrounding wells. This hypothesis is confirmed by the results presented in Figure 10. The presence of sample is clear within the grooves of the septa, Figure 10b, and on top of the wells, Figure 10a.

The inability of the septa mat to seal a 2.2ml DWP for mixing and centrifugation is therefore the most likely cause for the cross-contamination events observed using the automated extraction procedure version 6.1-6.3. The results from this experiment prompted a change of sealing method for the mixing and centrifugation of the 2.2ml DWP. The off-deck mixing is a required step as this is responsible for increasing the extraction efficiency of the automated method. Earlier investigations of adhesive seals showed condensation on the underside of the adhesive seal that does not centrifuge down, and therefore is a considerable contamination risk when adhesive seals are being removed from the plate.

7.7 Adhesive seal testing using NaI

The NaI test was used to assess the sealing capacity of a pierceable adhesive aluminium seal of the ABgene 2.2ml DWP. Briefly, 100µl of bleach was dispensed into each well containing a 6mm x 6mm piece of cardboard. To wells D4, F4 and D7 300µL of Extraction buffer and 500µL of 3M sodium iodide was added. Wells D4, F4 and D7 are the positive sample wells and all surrounding wells are "blanks". The ABgene plate was sealed with an adhesive aluminium seal, mixed on an Eppendorf MixMate for 5 minutes at 1100rpm and centrifuged at 3000rpm for 2 minutes. Visual examination of the wells surrounding the positive sample wells did not show any indication of a bleach and NaI reaction. A negative result indicates no contamination has occurred during the mixing or centrifuging process of the ABgene 2.2ml DWP using an adhesive aluminium seal.

The sealing ability of the adhesive aluminium seal was evaluated further by briefly turning the plate upside down followed by centrifuging. A single contamination event was observed from a positive sample to a blank control, see Figure 11.

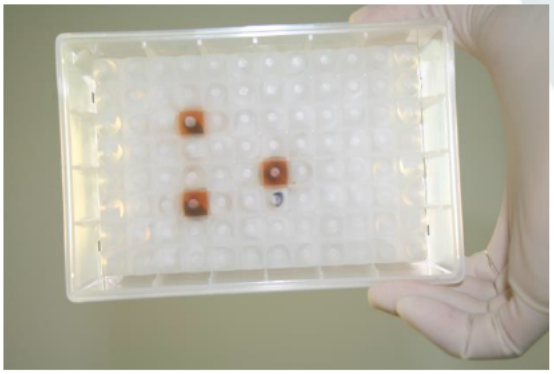

Figure 11 Contamination present after inverting the plate and centrifuging. Contamination is identified by the dark colour in a previously blank well.

This is not part of the routine extraction procedure. The occurrence of contamination while the plate is inverted was tested further by placing the plate upside down on the bench for 5 minutes prior to centrifuging the plate. After 5 minutes eight contamination events occurred, see Figure 12.

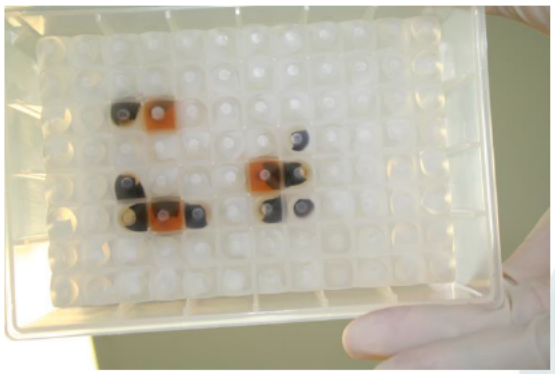

Figure 12 Plate left sitting upside on bench for 5 minutes and then centrifuged.

No cross contamination events were observed when the plate was handled according to the standard operating procedure. It was concluded that the adhesive aluminium seal is fit for purpose to seal the ABgene 2.2ml DWP.

Due to the removal of the adhesive seal being noted as a potential cause for contamination events during the opportunity for quality improvement investigations it was concluded that seal piercing was a fit for purpose method for sample access. An Axygen 96 well half skirted PCR Microplate with single notch has the same well spacing and notch size as an ABgene 96 2.2ml DWP, the aluminium seal was pierced using the underside of the PCR plate. This creates holes in the seal adequate for the automated extraction to continue. This method for sample access was employed for the anti-contamination plates using purified genomic DNA from Promega in the plate layouts presented in section 6.10 and results are presented in section 7.8. The extraction process using a pierceable seal is referred to as process version 6.4 - 6.5.

7.8 Anti-contamination checks using purified genomic DNA

Using extraction platform B, nine plates consisting of purified genomic DNA were extracted in Soccerball, Zebra stripe and Checkerboard format. Every sample was reviewed for a quantification value and visible peaks in the profile. All profiles were viewed below threshold and below the LOD for possible indications of peaks. If a quantification value was obtained above or below the LOD the sample was re-quantified to ensure the quantification value was reproducible. Samples that on the initial run had allelic peaks or indications of peaks below threshold were re-quantified and subsequently re-amplified. These samples were made according to section 6.3.

The positive (DNA) samples returned yields that were comparable between each run. With an input of 1125ng of purified human DNA, the system retained a mean total yield of 501.52ng, resulting in an approximate recovery rate of 44.58% which is expected due to saturation of resin with DNA. As noted earlier, DNA profiles were obtained and found to be concordant across the various plates, although some exhibited less alleles compared to other replicates. This is due to the DNA used, as it contains a complex mixture of various individuals, and minor fluctuations in DNA template amounts may cause some alleles to amplify at a lower efficiency rate compared to others. No instances of cross-contamination between the male and female DNA samples were encountered after interrogating the alleles that are unique to each sample.

7.8.1. Soccer ball 1 (FBOT 433)

Two negative controls yielded quantification values. One sample had a quantification value below the LOD and was not reproducible. There were no indications of peaks in the profiles.

The second negative control had an initial quantification value of 0.0127ng/uL. This sample was amplified at maximum template addition volume and yielded a full single source profile. This sample was re-amplified and the profile was shown to be reproducible from the extract. Both the quantification value and the profile were reproducible. The profile in the negative extraction control is not a contamination event caused by the automated extraction process as all samples within the extraction batch were either negative controls or Promega DNA samples with known profiles. Contamination from a "sample" within the batch would present as a mixture with multiple contributors. This is a spurious profile that does not indicate sample to sample contamination within the batch.

It is hypothesised that the profile has been extracted from a component of the labware used during the extraction. The profile does not match to any profiles contained within the DNA Analysis staff database. The database includes technicians that have serviced or come in contact with the extraction platform. The profile also does not match any previously obtained profiles within the laboratory. Contaminating DNA profiles obtained from labware have been reported in the literature previously, Sullivan et al 2004.

7.8.2. Soccer ball 2 (FBOT 436)

All negative control samples yielded undetermined quantification values and no indications of peaks were observed in the profiles.

7.8.3. Soccer ball 3 (FBOT 440)

One negative control sample yielded a quantification value below the LOD. The quantification value was not reproduced when the sample was re-quantified. This indicates that there is no quantifiable DNA present in the extract. None of the negative controls yielded peaks or identifiable below threshold peaks.

7.8.4. Zebra Stripe 1 (FBOT 432)

All negative control samples yielded undetermined quantification values and no indications of peaks were observed in the profiles.

7.8.5. Zebra Stripe 2 (FBOT 435)

Four negative control samples yielded a quantification value. Three of these were below the LOD and were not reproducible when the sample was re-quantified. One sample yielded a quantification value above the LOD, re-quantification of this sample yielded an undetermined quantification value. The quantification plot indicates a spike in fluorescence occurred.
None of the negative control profiles yielded any indication of above or below threshold peaks.

7.8.6. Zebra Stripe 3 (FBOT 439)

All negative control samples yielded undetermined quantification values and no indications of peaks were observed in the profiles.

7.8.7. Checkerboard 1 (FBOT 434)

Two negative controls yielded a quantification value below the LOD. The quantification values were not reproducible when the samples were re-quantified. This indicates no quantifiable DNA is present in the extract.
None of the negative controls yielded identifiable peaks above or below threshold when the profiles were viewed.

7.8.8. Checkerboard 2 (FBOT 437)

One of the negative controls yielded a quantification value below the LOD. The quantification value was not reproducible when the sample was re-quantified. This indicates no quantifiable DNA is present in the extract. None of the negative controls yielded identifiable peaks above or below threshold.

7.8.9. Checkerboard 3 (FBOT 447)

Three negative controls yielded a quantification value below the LOD. Results are presented below in Table 2.

Table 2 Checkerboard 3 negative controls investigated

Sample description	QUANT	9PLEX	REQC	REQC EPG	MICFCW
403159394	0.000417	NSD	Undet	-	-
403159460	0.000358	NSD	0.00218	?PBT	NSD
403159644	0.000692	PBT	0.00524	PBT	PBT

* PBT = Peaks Below Threshold

The initial quantification value for sample 403159394 is well below the LOD for quantification. The quantification value was also not repeatable and no indications of peaks were observed when the profile was viewed. There are no indications that the sample is contaminated. It is concluded that the quantification value was a baseline measurement error.

The quantification value for sample 403159460 is below the LOD, however it was reproducible when the sample was re-quantified. No peaks were observed in the initial amplification. The second amplification as part of the re-quantification showed below threshold (16 RFU) indications of peaks. The sample was subsequently processed using the Microcon® procedure. The amplification of the Microcon® product did not yield any peaks. This sample did not yield sufficient information to confirm that contamination had occurred from a sample within the extraction batch. The amplifiable DNA present in the extract may have been extracted from the labware. It is not possible to identify the labware/consumable that has contaminated the negative control. All labware and consumables used during the extraction process are guaranteed to be DNA free by the suppliers/manufacturers. Contaminating DNA profiles from labware/consumables have been observed to occur sporadically within the laboratory.

With the limited interpretable data available for this sample it is not possible to conclude how the negative control was contaminated with amplifiable DNA. If contamination from a positive sample within the batch had occurred it is expected that contamination would be clear due to the high DNA concentration of the samples and the relatively small amount of contaminating sample required to amplify a DNA profile and the presence of a mixed DNA profile has many alleles.

The below LOD quantification values for sample 403159644 indicate that DNA may be present in the extract. The two amplifications and the amplification of the Microcon® product for this sample yielded identifiable peaks confirming the extract contains amplifiable DNA.

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Table 3 Peaks identified for sample 403159644

Sample description	D3	vWA	FGA	Amel	D8	D21	D18	D5	D13	D7
403159644_9PLEX	NSD	17,NR	NSD	X,NR	NSD	NSD	19,NR	?7,NR	NSD	NSD
403159644_REQC	NSD	17,NR	21,NR	X,NR	NSD	NSD	NSD	12,NR	NSD	NSD
403159644_MICFCW	15,16	17,NR	NSD	X,NR	NSD	NSD	NSD	7,NR	NSD	NSD

As presented in Table 3 contaminating peaks are clearly identifiable in each of the three amplifications for the negative control. Comparison of the obtained alleles to the alleles represented in the Promega DNA positive control profiles identifies the D5S818 allele 7 as a clear difference. This allele difference is sufficient to conclude that the contaminating DNA within the extract for sample 403159644 is extraneous DNA and does not indicate a cross contamination event from another sample within the batch. The most likely explanation for the obtained profile/peaks is that DNA contaminated the labware during the manufacturing process. The obtained profile does not match staff members or any other profiles obtained within DNA analysis indicating that contamination has not occurred from a sample processed within the laboratory.

7.8.10. Conclusion

The nine anti-contamination plates extracted using purified genomic DNA in three different plate orientations did not indicate that cross contamination of samples was occurring. On the basis of these results the decision was made to return to use of the automated extraction procedure for reference samples in a checkerboard format on extraction platform B.

7.9 Assessment of the 4TITUDE 4Seal heat sealer

Due to the force required to pierce the adhesive aluminium seal using the underside of a PCR plate a heat sealer was investigated for routine sealing during the extraction procedure. The verified seal was a 4titude Pierce Seal (Cat No. 4ti-0531). The seal integrity of the 2ml DWP was assessed using the NaI test as per the test used for the adhesive seals. Each test was performed in duplicate.

The 2.2ml DWP was sealed with an aluminium film on the 4Seal heat sealer at 175°C for 2.5 seconds. The plate was then mixed for 5 minutes at 1100rpm on an Eppendorf MixMate and centrifuged at 3000rpm for 2 minutes in an Eppendorf centrifuge. No contamination events were observed at this point.

The plate was briefly inverted and then centrifuged in an Eppendorf centrifuge at 3000rpm for 2 minutes. No contamination events were observed.

The plate was inverted and left on the bench for 5 minutes before centrifuging at 3000rpm for 2 minutes. No contamination events were observed.

The plate was dropped from shoulder height (~1.5m) and then centrifuged at 3000rpm for 2 minutes. No contamination events were observed.

At no stage was cross contamination observed in any of the tests used to evaluate the heat seal. The seal integrity is strong to the point that limited contamination can be expected when the plate is dropped, although this is not recommended as part of the standard operating procedure. If a plate is dropped it should be noted that a cross contamination event may have occurred.

The Pierce Seal heat seal is easily pierced using the underside of the Axygen PCR microplate. The contamination risk of piercing the seal using the PCR plate was assessed

using the NaI test. NaI was placed in four wells of the 2ml DWP and then the plate was sealed, mixed and centrifuged. The PCR plate was used to pierce the seal and the underside of the PCR plate was pressed against cardboard sprayed with bleach. Reactions occurred for three positions that contained NaI, see Figure 13.

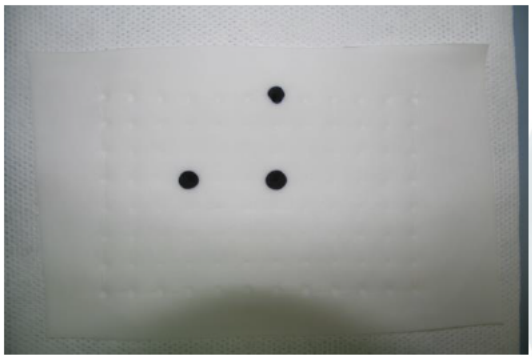

Figure 13 NaI reactions with bleach on the underside of a PCR plate after it has been used to pierce a heat seal.

It is hypothesised that due to the sensitivity of the NaI method the PCR plate does not contact the main part of the sample but rather contacts a small amount of sample adhering to the seal, or a small meniscus bubble present above sample. Bubbles have been observed to remain in the 2ml DWP after the plate has been centrifuged, this is due to the detergents contained in the extraction and lysis buffers. The positive reaction may also be caused by the NaI vapour contacting the PCR plate. No drips, drops or moisture were observed adhering to the PCR plate therefore this is considered a low risk of contamination.

7.10 Anti-contamination checks using reference buccal cell samples on FTA® Classic Card

With the nine anti-contamination plates indicating the automated extraction procedure is free of contamination the automated re-implementation program moved to extractions using reference samples that could be confirmed with a previously obtained profile. FTA RPT and LINK samples were included for automated reference sample extractions in checkerboard format. Blanks consisted of DNA IQ™ Extraction buffer without Proteinase K.

Using samples with single source profiles aids troubleshooting if a profile in a blank occurs and can also assist in detecting cross contamination from sample to sample as mixtures are not expected. Extra samples were included by ordering LINKs on reference samples that may in the future require a confirmatory profile. In total, seventeen plates were processed on extraction platform B using the automated extraction procedure.

7.10.1. Reference Plate 1 (RFIQEXT20090526_05)

All negative controls yielded undetermined quantification values. None of the negative control profiles yielded indications of peaks. All positive samples yielded profiles concordant to the previous results for each individual sample. No indications of extra peaks or mixed profiles were observed in the single source samples.

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7.10.2. Reference Plate 2 (RFIQEXT20090527_01)

All negative controls yielded undetermined quantification values. Two of the negative controls yielded possible peaks in the initial amplification. The peaks for both samples were reproducible when the amplification product was re-prepared and re-run through capillary electrophoresis. The profiles obtained for sample 403170904 are presented in Table 4.

Table 4 Profile results for negative control 403170904

Sample description	D3	vWA	FGA	Amel	D8	D21	D18	D5	D13	D7
403170904_9PLEX	NSD	NSD	NSD	NR,Y	NSD	NSD	NSD	NSD	NSD	NSD
403170904_ReGS	NSD	NSD	NSD	?Y	NSD	NSD	NSD	NSD	NSD	NSD
403173903 (2µL ReGS)	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
403170904_REQR	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
403170904_MICFCW	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD

The peak obtained in the first amplification for sample 403170904 was not reproducible above the LOD in subsequent reworks. The re-run of the initial amplification product reproduced the single peak at amelogenin, however the peak was below the LOD. The interpretation of peaks below the LOD is not reliable and can easily be misinterpreted. The further reworks of this sample did not produce any further allelic peaks, including below threshold peaks.

The inability to reproduce allelic peaks from the extract with the second amplification and the microcon procedure indicates that no amplifiable DNA is present within the extract. It is hypothesised that the single peak at amelogenin reproducible from the first amplification product may be due to drop in. It is concluded that a contamination event did not occur during the extraction of this sample using the automated extraction procedure.

The profiles obtained for the second sample (403170764) are presented in Table 5. The designated alleles are above the 16 RFU LOD. The highest allele reported was 24 RFU. All peaks are below the LOR and need to be interpreted with caution.

Table 5 Profile results for negative control 403170764

Sample description	D3	vWA	FGA	Amel	D8	D21	D18	D5	D13	D7
403170764_9PLEX	17,NR	NSD	NSD	X,NR	NSD	30,NR	NSD	NSD	NSD	NSD
403170764_RRREF	NSD	NSD	NSD	NSD	11,NR	30,NR	NSD	NSD	NSD	NSD
403173914 (2µL ReGS)	17,NR	NSD	NSD	NSD	11,NR	30,NR	NSD	NSD	NSD	NSD
403170764_REQR	NSD	NSD	25,NR	NSD	NSD	NSD	NSD	NSD	NSD	NSD
403170764_MCREF	NSD	18,NR	25,NR	NR,Y	NSD	30,NR	NSD	NSD	NSD	NSD

A number of the alleles obtained for sample 403170764 were reproducible from the first amplification product and from the extract in further amplifications. This indicates the presence of contaminating DNA in the extract.

A copy of the profiles as viewed in GeneMapper® *ID-X,* are attached as Appendix 1. The sample position on the extraction batch is presented in Figure 14.

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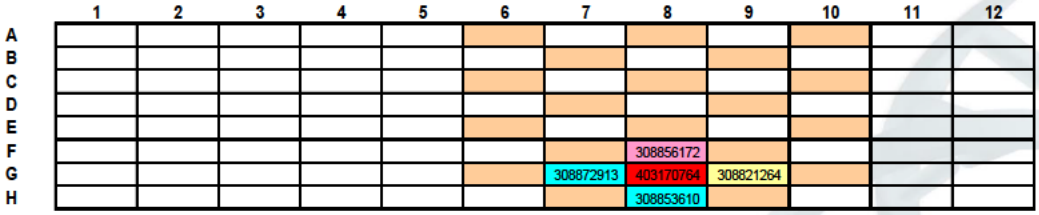

Figure 14 Plate map indicating position of sample 403170764 on the extraction batch. All alleles obtained for the contaminated negative control are represented in the profile of sample 308821264.

Sample 308821264 shares the five alleles obtained from negative control 403170764. From the limited alleles obtained it has been concluded that sample 308821264 contaminated the negative control. See Table 6 for the profile comparison.

Table 6 Profile comparison of Negative control and adjacent sample from Reference batch 2

Sample	D3	vWA	FGA	Amel	D8	D21	D18	D5	D13	D7
403170764	17,NR	18,NR	25,NR	X,Y	11,NR	30,NR	NSD	NSD	NSD	NSD
308821264	14,17	17,18	23,25	X,Y	11,13	30,31	13,15	10,11	8,11	10,11

No drips or drops, with the potential to cause contamination, have been observed with the automated extraction procedure version 6.4-6.5. Therefore, it is hypothesised that cross contamination occurred during a manual process within the extraction procedure. The manual intervention steps are key to the increased yield of the automated extraction procedure. Investigations were conducted to optimise the manual steps. For the results of these investigations refer to section 7.11. It is possible that a labware or consumable fault allowed for a small amount of sample transfer to the negative control. For example, a small chip in the side of the well at the sealing surface would allow for sample transfer.

All positive samples yielded profiles concordant to the previous results for each individual sample. No indications of extra peaks or mixed profiles were observed in the single source samples within the batch.

7.10.3. Reference Plate 3 (RFIQEXT20090529_01)

All negative controls yielded undetermined quantification values. One negative control on the initial amplification and capillary electrophoresis run yielded one peak above the LOD and indications of peaks below the LOD. The re-run of this sample did not yield any indications of peaks. The profile from the re-quantification and the Microcon also did not yield any indications of peaks. No quantification value was obtained for this negative control. Due to the reworking of this control not yielding any further results it is concluded that no quantifiable or amplifiable DNA was present in the final extract. It is hypothesised that the initial peaks may have contaminated the control during capillary electrophoresis preparation or carryover within the genetic analyzer capillary. The profile obtained is not due to a contamination event during the extraction procedure.

All positive samples yielded profiles concordant to the previous results for each individual sample. No indications of extra peaks or mixed profiles were observed in the single source samples within the batch.

7.10.4. Reference Plate 4 (RFIQEXT20090529_02)

All negative controls yielded undetermined quantification values. None of the negative control profiles yielded indications of peaks. All positive samples yielded profiles

concordant to the previous results for each individual sample. No indications of extra peaks or mixed profiles were observed in the single source samples within the batch.

7.10.5. Reference Plate 5 (RFIQEXT20090609_03)

All negative controls yielded undetermined quantification values. A negative control yielded indications of peaks below the LOD on the initial amplification and capillary electrophoresis run. The re-run of this sample yielded an indication of a peak at amelogenin below the LOD. The profile from the re-quantification and the Microcon did not yield any indications of peaks. No quantification value was obtained for this negative control. Due to the reworking of this control not yielding any further results it is concluded that no quantifiable or amplifiable DNA was present in the final extract. It is hypothesised that the DNA responsible for the initial peaks may have contaminated the control at some point after the extraction. The below LOD peaks were a result of drop in. The profile obtained is not due to a contamination event during the extraction procedure.

7.10.6. Reference Plate 6 (RFIQEXT20090610_02)

All negative controls yielded undetermined quantification values. None of the negative control profiles yielded indications of peaks. All positive samples yielded profiles concordant to the previous results for each individual sample. No indications of extra peaks or mixed profiles were observed in the single source samples within the batch.

7.10.7. Reference Plate 7 (RFIQEXT20090612_02)

A negative control yielded an initial quantification value. This was re-quantified and yielded an undetermined quantification value. The initial amplification and the re-run of the initial amplification product yielded indications of peaks at amelogenin below the LOD. No peaks were yielded with the re-quantification and Microcon® reworks of the extract. It is hypothesised that the indications of peaks yielded in the initial amplification and re-run of the initial amplification product are due to allele drop in.

A negative control yielded an initial undetermined quantification value. The control was re-quantified due to a peak at amelogenin above the LOD and identifiable peaks below the LOD for the initial amplification. The re-quantification yielded a value of 0.0006, below the LOD. The re-run of the product from the initial amplification yielded a peak at amelogenin below the LOD. No indications of peaks were reproduced in the re-quantification profile or the microcon of the extract. It is hypothesised that the initial amplification peaks were caused by environmental drop in. The quantification value yielded in the re-quantification is below the LOD and therefore is classified as indistinguishable from background fluorescence.

It is concluded that the quantification values and the peaks yielded for the two negative controls are not a result of contamination during the extraction procedure.

All positive samples yielded profiles concordant to the previous results for each individual sample. No indications of extra peaks or mixed profiles were observed in the single source samples within the batch.

7.10.8. Reference Plate 8 (RFIQEXT20090616_01)

Two negative control samples yielded a quantification value below 0.0006 ng/µL, this is below the LOD. The re-quantification for these samples was undetermined. The initial profile and the re-quantification profile did not yield any identifiable peaks. The Microcon® rework of these samples did not yield any identifiable peaks. It is concluded that the quantification values were background fluorescence. This can be expected for quantification values below the LOD.

All positive samples yielded profiles concordant to the previous results for each individual sample. No indications of extra peaks or mixed profiles were observed in the single source samples within the batch.

7.10.9. Reference Plate 9 (RFIQEXT20090617_02)

All negative controls yielded undetermined quantification values. None of the negative control profiles yielded indications of peaks. All positive samples yielded profiles concordant to the previous results for each individual sample. No indications of extra peaks or mixed profiles were observed in the single source samples within the batch.

7.10.10. Reference Plate 10 (RFIQEXT20090623_05)

All negative controls yielded undetermined quantification values. None of the negative control profiles yielded indications of peaks. All positive samples yielded profiles concordant to the previous results for each individual sample. No indications of extra peaks or mixed profiles were observed in the single source samples within the batch.

7.10.11. Reference Plate 11 (RFIQEXT20090623_07)

All negative controls yielded undetermined quantification values. None of the negative control profiles yielded indications of peaks. All positive samples yielded profiles concordant to the previous results for each individual sample. No indications of extra peaks or mixed profiles were observed in the single source samples within the batch.

7.10.12. Reference Plate 12 (RFIQEXT20090629_09)

All negative controls yielded undetermined quantification values. Two negative controls were reworked further due to indications of peaks in the initial amplification. No peaks were reproducible when the initial amplification was re-run. The re-quantification of the controls yielded an undetermined result. No peaks were observed in the re-quantification profile or after the samples were processed through the Microcon® procedure. It is hypothesised that the initial peaks may have contaminated the control during capillary electrophoresis preparation. The profile obtained is not due to a contamination event during the extraction procedure. The profile was not reproducible from the control extract.

All positive samples yielded profiles concordant to the previous results for each individual sample. No indications of extra peaks or mixed profiles were observed in the single source samples within the batch.

7.10.13. Reference Plate 13 (RFIQEXT20090629_11)

All negative controls yielded undetermined quantification values. None of the negative control profiles yielded indications of peaks. All positive samples yielded profiles concordant to the previous results for each individual sample. No indications of contaminating extra peaks or mixed profiles were observed in the single source samples within the batch.

7.10.14. Reference Plate 14 (RFIQEXT20090706_01)

One negative control yielded a quantification value. The re-quantification of this sample reproduced the quantification value indicating quantifiable DNA was present in the extract. The initial amplification produced a below LOR peak at amelogenin. The profile also had an indication of a below LOD peak at D21S11. The re-run through capillary electrophoresis of the amplification product increased the peak heights of the peaks at amelogenin and D21S11. The profiles obtained for this negative control are presented in **Table 7** and attached as Appendix 2.

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Table 7 Profile results for negative control 403176028

Sample description	D3	vWA	FGA	Amel	D8	D21	D18	D5	D13	D7
403176028_9PLEX	NSD	NSD	NSD	X,NR	NSD	NSD	NSD	NSD	NSD	NSD
403176028_ReGS	NSD	NSD	NSD	X,NR	NSD	30,NR	NSD	NSD	NSD	NSD
403176028_REQR	15,NR	18,NR	NSD	X,NR	10,NR	30,NR	NSD	NSD	NSD	NSD
403176028_MICFCW	15,NR	NSD	NSD	X,NR	10,NR	30,NR	NSD	13,NR	NSD	NSD

A number of the alleles obtained were reproducible in subsequent amplifications indicating that amplifiable DNA is present in the extract. Interrogation of the alleles for all samples extracted on the automated extraction batch and the alleles obtained in the negative control 403176028, was conducted and no matches were observed. It is hypothesised that the DNA contaminating the negative control is not cross contamination from a sample or due to staff contamination. The inability to match the alleles obtained indicates contamination of the labware from an external source. This does not indicate contamination due to the extraction procedure itself.

7.10.15. Reference Plate 15 (RFIQEXT20090707_02)

All negative controls yielded undetermined quantification values. None of the negative control profiles yielded indications of peaks. All positive samples yielded profiles concordant to the previous results for each individual sample. No indications of extra peaks or mixed profiles were observed in the single source samples within the batch.

7.10.16. Reference Plate 16 (RFIQEXT20090710_06)

All negative controls yielded undetermined quantification values. None of the negative control profiles yielded indications of peaks. All positive samples yielded profiles concordant to the previous results for each individual sample. No indications of extra peaks or mixed profiles were observed in the single source samples within the batch.

7.10.17. Reference Plate 17 (RFIQEXT20090714_02)

All negative controls yielded undetermined quantification values. None of the negative control profiles yielded indications of peaks. All positive samples yielded profiles concordant to the previous results for each individual sample. No indications of extra peaks or mixed profiles were observed in the single source samples within the batch.

7.11 Adverse event manual process investigations

7.11.1. Nunc Bank-It™ de-capping

The manual de-capping of the Nunc Bank-It™ tubes containing lysates before presentation of the samples to the MPII was identified as a risk of contamination. Bubbles forming a meniscus above samples within the tube have been noted. These bubbles remained after extensive centrifuging. The manual de-capper was also noted to be faulty with the caps not adhering to the shaft of the de-capper.

It was noted that some operators at the time de-capped the tubes from top left to bottom left, column by column. The Nunc Bank-It™ caps were being discarded in a biohazard bin behind the samples. Therefore, caps from samples at the bottom of the plate were being moved over open samples before being discarded. Due to the faulty tip on the de-capper caps were occasionally dropped on the bench. This has the potential to produce small aerosols of sample adhering to the cap. The faulty de-capper tip has been fixed.

The small meniscus in the tube may also produce aerosols as the cap is removed from the tube. The risk of contamination during the de-capping of lysate Nunc tubes was investigated using the NaI test.

Briefly, 200µL of extraction buffer and 100µL of 3M NaI was dispensed into 16 Nunc Bank-It™ tubes in a Nunc rack. The tubes were then briefly mixed and centrifuged as per the standard operating procedure. The tubes were de-capped and pressed against cardboard sprayed with bleach. A positive reaction indicates that sample remained adhering to the cap.

The first rack of 16 Nunc tubes was de-capped after 2 minutes of centrifuging. The samples were at room temperature. All 16 lids reacted with the bleach and cardboard. This indicates a trace amount of NaI was adhering to the cap.

A second rack of 16 Nunc tubes with extraction buffer and NaI was stored in the fridge overnight and de-capped after mixing and centrifuging the next morning. Three of the sixteen lids reacted with the bleach and cardboard. This indicates a trace amount of NaI was adhering to the cap.

There is a clear variation between the samples de-capped immediately and the samples stored in the fridge. It is hypothesised that this is due to NaI vapour reacting with the bleach for the samples at room temperature. This highlights the sensitivity of the test. No caps appeared moist, or had adhering droplets. None of the samples contained the bubble meniscus.

De-capping of tubes from the bottom left, to the top left hand corner finishing in the top right hand corner has been included in the procedure. This will minimise the risk of contamination associated with the de-capping of the Nunc lysate tubes. De-capping of Nunc Bank-It™ tubes in this order ensures caps are not carried across open tubes.

It is also hypothesised that some lysate may have become stuck in the thread of the tube when the 300µL of lysate was dispensed into the Nunc Bank-It™ tubes. It has been reported that the lysate forms bubbles when dispensed due to the detergents in the extraction buffer. Operators have been informed to take care when dispensing the lysate into Nunc Bank-It™ tubes. If all processes are adhered to the risk of contamination from solution adhering to the cap thread is considered low. The risk does however increase if caps are dropped off the end of the de-capper.

7.11.2. Seal Piercing investigation

In section 7.9 it is noted that sample contacts the underside of the PCR plate during the piercing process. The piercing process was highlighted as a risk for well to well contamination. The risks were assessed using the NaI test. Briefly, 3M NaI in extraction buffer was dispensed into selected wells across the plate and all other wells filled with 300uL bleach and cardboard. The plate was mixed, centrifuged and then pierced using the underside of a PCR plate. The 2ml DWP was heat sealed a second time after the initial seal had been pierced. The plate was then centrifuged to ensure small droplets that may have contaminated surrounding wells made contact with bleach solution.

The plate was examined for any indications of NaI cross contamination. No indications of cross contamination during the piercing procedure were observed. This test was performed in duplicate and identical results were obtained.

It is concluded that the use of a PCR plate to pierce the heat seal for sample access is fit for purpose. The PCR plate can be easily discarded between batches limiting the need for decontamination of a sample piercer. A manual piercing press is currently being

manufactured for routine use in the laboratory. The device will be thoroughly tested prior to implementation.

8. Conclusion

In total 26 anti-contamination plates have been extracted through the automated DNA IQ™ procedure using process version 6.4 – 6.5. This is the version that includes sealing and off-deck mixing of the lysates in contact with resin, the seal is pierced rather than peeled. Peeling or removing a seal has been identified as a contamination risk due to adhering condensation forming aerosols. Piercing the aluminium seal is proven to be a fit for purpose method for sample access by the extraction platform.

No indications of cross contamination in the 9 purified genomic DNA Plates in 3 plate layout configurations were observed. On the basis of this result the verification progressed to extraction of single source reference samples in checkerboard format. The 17 checkerboard reference sample plates contained a number of blank controls that exhibited quantification values below the LOD and indications of allelic peaks in the initial amplification. The quantification values were not reproducible in re-quantifications. The allelic peaks were not reproducible from further amplifications indicating the final extract did not contain amplifiable DNA. This does not indicate cross contamination has occurred during the extraction procedure.

A possible cross contamination event has been observed in a negative control in reference plate 2. The 5 alleles obtained for the negative control cannot exclude the sample adjacent as the source of contamination. Indications of peaks below the LOD obtained for the negative control also match the adjacent sample. The manual processes in the extraction procedure were hypothesised to be the cause of contamination. The NaI investigations identified the risks in the manual process. Procedures limiting the risks of contamination were implemented. No further batches indicated cross contamination occurring during the extraction process.

The spurious single source profiles observed during both the purified genomic DNA and reference sample extraction plates are hypothesised to be labware or consumable contamination that has occurred at the manufacturer or during transport. Labware contamination has been observed in the laboratory for non-automated procedures and reported by other laboratories. All labware used during the automated DNA IQ™ extraction is guaranteed to be DNA free by the manufacturers. Spurious profiles have been identified in some of the plates extracted on extraction platform A. One of the profiles matches the profile obtained on extraction platform B. It is not possible to identify the labware or consumable responsible for the spurious profiles therefore feedback cannot be given to the manufacturer. Developments are being undertaken to create a DNA elimination database of staff from all manufacturers that provide products to DNA Analysis, Forensic and Scientific Services.

It is concluded that the automated DNA IQ™ extraction procedure is fit for routine extractions within DNA Analysis on extraction platform B. Extraction platform A is currently undergoing the same testing process. This report will be updated once all results are complete.

9. Recommendations

- The automated DNA IQ™ extraction procedure can be re-implemented as a routine extraction procedure within DNA Analysis for volume crime samples in checkerboard format.

- The automated DNA IQ™ extraction procedure should be monitored closely for wear or loss of calibration and issues reported immediately to the Analytical Senior Scientist.
- An audit be conducted 6 – 8 months post implementation of volume crime casework sample extractions in checkerboard format.
- Extraction process review following the audit to assess the implementation of automated DNA IQ™ extractions in full batches.

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