Notice number: 5.003

COMMISSION OF INQUIRY INTO DNA PROJECT 13

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

STATEMENT OF THOMAS NURTHEN

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

1. On 25 October 2023, I provided a written statement to this Commission responding to Notice 5.001 "Requirement to Given Information in a Written Statement".

2. This statement provides my written response to Notice 5.003.

Identification

Question 1(a) - State your full name:

3. My full name is Thomas Edmund Kersey Nurthen.

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

4. Please see my statement dated 25 October 2023.

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.

5. Please see my statement dated 25 October 2023.

DNA IQ protocol

Question 2 - Describe each iteration of the DNA IQ™ protocol used by Queensland Health Forensic and Scientific Services from 24 October 2007 to the present date, including but not limited to, in respect of each iteration:

(a) in seriatim, all steps comprising the protocol;

(b) the resin and reagent volumes used for extraction of DNA;

(c) the volume of the sample containing the DNA at the end of the extraction;

(d) the temperature used during the lysis step of the extraction process;

(e) the number of 'washes' employed following the lysis step of the extraction process.

6. In answering this question, I have considered

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Thomas Nurthen Witness

(a) MultiPROBE® II Protocols as detailed in the standard operating procedures (**SOPs**) listed below.

(b) Manual DNA IQ in Project 11, Report on the Validation of a manual method for Extracting DNA using the DNA IQ System (**Manual DNA IQ in Project 11**) **Attached and marked TN-15** is a copy of the Manual DNA IQ in Project 11;

(c) A Modified DNA IQ Method Consisting of Off-Deck Lysis to Allow Supernatant Retention for Presumptive Identification of a-Amylase (**Project 21 Report**). **Attached and marked TN-16** is a copy of the Project 21 Report.

(d) Project 22. A Modified DNA IQ Method for Off-Deck Lysis Prior to Performing Automated DNA Extraction (**Project 22 Report**). **Attached and marked TN-17** is a copy of the Project 22 Report.

(e) 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 (**Re-implementing the automated DNA IQ™ extraction protocol**). **Attached and marked TN-18** is a copy of the Re-implementing the automated DNA IQ™ extraction protocol.

7. I have not considered the Maxwell 16 DNA IQ™ extraction methods or Maxwell FSC DNA IQ™ extraction methods.

8. I have not considered the Manual Method (for DNA IQ) specified in The QIAsymphony® Instrument SOPs as this process is only used for recovery of DNA after a failed QIAsymphony batch.

9. **Attached and marked Exhibit TN-01** is a table of my responses to Question 2.

10. **Attached and marked Exhibit TN-03** is a copy of version 1 of the SOP relevant to the DNA IQ protocol dated 24 October 2007.

11. **Attached and marked Exhibit TN-04** is a copy of version 2 of the SOP relevant to the DNA IQ protocol dated 11 January 2008.

12. **Attached and marked Exhibit TN-05** is a copy of version 3 of the SOP relevant to the DNA IQ protocol dated 27 March 2008.

13. **Attached and marked Exhibit TN-06** is a copy of version 4 of the SOP relevant to the DNA IQ protocol dated 21 May 2008.

14. **Attached and marked Exhibit TN-07** is a copy of version 5 of the SOP relevant to the DNA IQ protocol which is dated 29 June 2009.

15. **Attached and marked Exhibit TN-08** is a copy of version 6 of the SOP relevant to the DNA IQ protocol dated 13 August 2009.

16. **Attached and marked Exhibit TN-09** is a copy of version 7 of the SOP relevant to the DNA IQ protocol dated 9 November 2010.

17. **Attached and marked Exhibit TN-10** is a copy of version 8 of the SOP relevant to the DNA IQ protocol dated 27 June 2012.

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18. **Attached and marked Exhibit TN-11** is a copy of version 9 of the SOP relevant to the DNA IQ protocol dated 3 January 2014.

19. **Attached and marked Exhibit TN-12** is a copy of version 10 of the SOP relevant to the DNA IQ protocol dated 12 June 2015.

20. **Attached and marked Exhibit TN-13** is a copy of version 11 of the SOP relevant to the DNA IQ protocol dated 30 January 2017.

21. **Attached and marked Exhibit TN-14** is a copy of version 1 of the SOP relevant to the DNA IQ protocol dated 15 June 2017.

Question 3 - To the extent that the DNA IQ™ protocol changed at any point in time, describe the reasons for the change.

22. In answering this question, I have considered:

    (a) MultiPROBE® II Protocols as detailed in the SOPs listed above;

    (b) Manual DNA IQ in Project 11 (**TN-15**);

    (c) Project 21 Report (**TN-16**);

    (d) Project 22 Report (**TN-17**); and

    (e) Re-implementing the automated DNA IQ™ extraction protocol (**TN-18**).

23. I have not considered the Maxwell 16 DNA IQ™ extraction methods or Maxwell FSC DNA IQ™ extraction methods. The Maxwell 16 and the Maxwell FSC are different instruments from the MultiPROBE® II with their own DNA IQ protocols.

24. I have not considered the Manual Method (for DNA IQ) specified in The QIAsymphony® Instrument SOPs as this process is only used for recovery of DNA after a failed QIAsymphony batch.

25. I have reviewed the amendment history for each SOP to answer this question. The SOP amendment history is a brief outline of the overall changes to the process. I am not able to accurately describe the changes or the reasons for the changes to the instrument protocol based on the SOP amendment history. The SOP amendment history does not provide detail about, for example, changes to the pipette aspiration and dispense speeds and mixing etc which may affect the final DNA result.

26. The SOPs are a series of instructions to direct the user how to prepare samples before putting them on the instrument whereas the WinPrep software protocols stores the instrument processes. The instrument protocols tell the instrument (i.e. the robot) how to process those samples.

27. It is necessary to open the WinPrep software protocols to understand every change to every instrument process at any point in time. I am not able to open and read the instrument protocol without the instrument protocol software (WinPrep).

28. With respect to the Manual Method, on review of the SOPs it would appear there has only been one change to this method. This is to the volume of lysis buffer added with resin, as

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stated in versions QIS24897V3 (550 µL), QIS#24897V4 (500 µL) and QIS#24897V5 (550 µL) of Table 2. I do not know why this volume was changed and subsequently changed back. It may have been a typographical error corrected in subsequent SOPs.

29. Further to my statement in response to Notice 5.001 dated 25 October 2023, the Laboratory has now located WinPrep installations discs but they are not installed on the Laboratory's systems. When the software was installed on or around 2006, PerkinElmer Engineers performed this task as part of their installation qualification.

30. **Attached and marked Exhibit TN-02** is a table of my responses to Question 3.

Question 4 - To the extent that the DNA IQ™ protocol changed in any respect from the DNA IQ™ protocol issued by the manufacturer, describe the reasons for the change.

31. As stated above, to give an accurate answer to this question requires an expert in WinPrep software to determine all of the differences in MultiPROBE® II Protocol versions compared to the CFS automated protocol (PerkinElmer, 2004).

Project 13

Question 5 - Were you the author of the Abstract appearing in 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)?

32. I refer and rely on my answers provided at paragraphs 72-77 of my statement dated 25 October 2023 in response to question 8 of Notice 5.001.

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*.

TAKEN AND DECLARED before me at Brisbane on 27 October 2023.

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Thomas Nurthen Witness

EXHIBITS INDEX

Exhibits Index – Thomas Nurthen Statement

Question	Exhibit	Document Title
2	TN-01	Table of Thomas Nurthen responses to question 2 in Notice 5.003.
3	TN-02	Table of Thomas Nurthen responses to question 3 in Notice 5.003.
2 & 3	TN-03	Version 1 of the SOP relevant to the DNA IQ protocol dated 24 October 2007.
2 & 3	TN-04	Version 2 of the SOP relevant to the DNA IQ protocol dated 11 January 2008.
2 & 3	TN-05	Version 3 of the SOP relevant to the DNA IQ protocol dated 27 March 2008.
2 & 3	TN-06	Version 4 of the SOP relevant to the DNA IQ protocol dated 21 May 2008.
2 & 3	TN-07	Version 5 of the SOP relevant to the DNA IQ protocol dated 29 June 2009.
2 & 3	TN-08	Version 6 of the SOP relevant to the DNA IQ protocol dated 13 August 2009.
2 & 3	TN-09	Version 7 of the SOP relevant to the DNA IQ protocol dated 9 November 2010.
2 & 3	TN-10	Version 8 of the SOP relevant to the DNA IQ protocol dated 27 June 2012.
2 & 3	TN-11	Version 9 of the SOP relevant to the DNA IQ protocol dated 3 January 2014.
2 & 3	TN-12	Version 10 of the SOP relevant to the DNA IQ protocol dated 12 June 2015.
2 & 3	TN-13	Version 11 of the SOP relevant to the DNA IQ protocol dated 30 January 2017.
2 & 3	TN-14	Version 1 of the SOP relevant to the DNA IQ protocol dated 15 June 2017.

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2 & 3	TN-15	Project 11. Report on the Validation of a manual method for Extracting DNA using the DNA IQ System dated August 2008.
2 & 3	TN-16	Project 21. A Modified DNA IQ Method Consisting of Off-Deck Lysis to Allow Supernatant Retention for Presumptive Identification of a-Amylase.
2 & 3	TN-17	Project 22. A Modified DNA IQ Method for Off-Deck Lysis Prior to Performing Automated DNA Extraction.
2 & 3	TN-18	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.

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Table of Thomas Nurthen's responses to Question 2 in Notice 5.003

DNA IQ protocol

Question 2 - Describe each iteration of the DNA IQ™ protocol used by Queensland Health Forensic and Scientific Services from 24 October 2007 to the present date, including but not limited to, in respect of each iteration:

Iteration of protocol	MultiProbe II Protocol version No.	Project No.	(a) In seriatim, all steps comprising the protocol	(b) The resin and reagent volumes used for extraction of DNA	(c) The volume of the sample containing the DNA at the end of the extraction	(d) The temperature used during the lysis step of the extraction process	(e) The number of 'washes' employed following the lysis step of the extraction process
On deck lysis protocol							
QIS#24897V1 SOP relevant to the DNA IQ protocol dated 24 October 2007	1.1	13	See section 7	500 µL extraction buffer 50 µL of resin 957 µL of lysis buffer See Figure 1	100 µL See section 3	37°C See Figure 1	1x lysis buffer wash 3x wash buffer wash See Figure 1
QIS#24897V2 SOP relevant to the DNA IQ protocol dated 11 January 2008	1.3	N/A	See section 7	500 µL extraction buffer See section 7, point 1 50 µL of resin 957 µL of lysis buffer See section 7, point 3	100 µL See section 3	37°C See section 7, point 1	1x lysis buffer wash 3x wash buffer wash See section 7, point 5
Off deck lysis protocol							
QIS#24987V3 SOP relevant to the DNA IQ protocol dated 27 March 2008	2.0 ODL No retained supernatant	22	See sections 7 & 9	500 µL extraction buffer See section 7, point 8	100 µL See section 3	First incubation - 37°C See section 7, point 9	1x lysis buffer wash 3x wash buffer wash

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Iteration of protocol	MultiProbe II Protocol version No.	Project No.	(a) In seriatim, all steps comprising the protocol	(b) The resin and reagent volumes used for extraction of DNA	(c) The volume of the sample containing the DNA at the end of the extraction	(d) The temperature used during the lysis step of the extraction process	(e) The number of 'washes' employed following the lysis step of the extraction process
				50 µL of resin 957 µL of lysis buffer See section 9, point 1		Second incubation – 65°C See section 7, point 16	See section 9, point 3
	2.0 ODL Retained supernatant	21	See sections 8 & 9	650 µL TNE See section 8, point 7 25 µL of Pro K & 12.5 µL of SDS See section 8, point 12 50 µL of resin 957 µL of lysis buffer See section 9, point 1	As above	First incubation - Room temperature See section 8, point 8 Second incubation - 37°C See section 8, point 12 Third incubation - 65°C See section 8, point 20	As above
QIS#24897V4 SOP relevant to the DNA IQ protocol dated 21 May 2008	4.1 ODL No retained supernatant	N/A	See sections 7 & 9	500 µL extraction buffer See section 7, point 8 50 µL of resin 957 µL of lysis buffer See section 9, point 1	100 µL See section 3	First incubation - 37°C See section 7, point 9 Second incubation - 65°C See section 7, point 15	1x lysis buffer wash 3x wash buffer wash See section 9, point 3

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Iteration of protocol	MultiProbe II Protocol version No.	Project No.	(a) In seriatim, all steps comprising the protocol	(b) The resin and reagent volumes used for extraction of DNA	(c) The volume of the sample containing the DNA at the end of the extraction	(d) The temperature used during the lysis step of the extraction process	(e) The number of 'washes' employed following the lysis step of the extraction process
	4.1 ODL Retained supernatant	N/A	See sections 8 & 9	650 µL TNE See section 8, point 7 25 µL of Pro K & 12.5 µL of SDS See section 8, point 11 50 µL of resin 957 µL of lysis buffer See section 9, point 1	As above	First incubation - Room temperature See section 8, point 8 Second incubation - 37°C See section 8, point 12 Third incubation - 65°C See section 8, point 18	As above
Post reimplementation							
QIS#24897V5 SOP relevant to the DNA IQ protocol dated 29 June 2009	6.4 ODL No retained supernatant	Re-implementing the automated DNA IQ™ extraction protocol	See sections 8.1 & 9.5	300 µL extraction buffer See section 8.1, point 6 53 µL of resin 2 volumes of lysis buffer See section 9.5, point 2	100 µL See section3	First incubation - 37°C See section 8.1, point 7 Second incubation – 65°C See section 8.1, point 11	1x lysis buffer wash 3x wash buffer wash See section 9.5, point 5
	6.4 ODL Retained supernatant	As above	See sections 8.2 & 9.5	450 µL TNE See section 8.2, point 5	As above	First incubation - Room temperature	As above

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Iteration of protocol	MultiProbe II Protocol version No.	Project No.	(a) In seriatim, all steps comprising the protocol	(b) The resin and reagent volumes used for extraction of DNA	(c) The volume of the sample containing the DNA at the end of the extraction	(d) The temperature used during the lysis step of the extraction process	(e) The number of 'washes' employed following the lysis step of the extraction process
				14 µL of Pro K & 7 µL of Sarcosyl See section 8.2, point 9 53 µL of resin 2 volumes of lysis buffer See section 9.5, point 2		See section 8.2, point 6 Second incubation - 37°C See section 8.2, point 10 Third incubation - 65°C See section 8.2, point 14	
QIS#24897V6 SOP relevant to the DNA IQ protocol dated 13 August 2009	6.5 ODL No retained supernatant	Re-implementing the automated DNA IQ™ extraction protocol	See sections 8.1 & 9.5	300 µL extraction buffer See section 8.1, point 6 53 µL of resin 2 volumes of lysis buffer See section 9.5, point 2	100 µL See section3	First incubation - 37°C See section 8.1, point 7 Second incubation – 65°C See section 8.1, point 11	1x lysis buffer wash 3x wash buffer wash See section 9.5, point 5
	6.5 ODL Retained supernatant	As above	See sections 8.2 & 9.5	450 µL TNE See section 8.2, point 5 14 µL of Pro K & 7 µL of Sarcosyl	As above	First incubation - Room temperature See section 8.2, point 6 Second incubation - 37°C	As above

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Iteration of protocol	MultiProbe II Protocol version No.	Project No.	(a) In seriatim, all steps comprising the protocol	(b) The resin and reagent volumes used for extraction of DNA	(c) The volume of the sample containing the DNA at the end of the extraction	(d) The temperature used during the lysis step of the extraction process	(e) The number of 'washes' employed following the lysis step of the extraction process
				See section 8.2, point 9 53 µL of resin 2 volumes of lysis buffer See section 9.5, point 2		See section 8.2, point 10 Third incubation - 65°C See section 8.2, point 14	
QIS#24897V7 SOP relevant to the DNA IQ protocol dated 9 November 2010	6.6 ODL No retained supernatant	N/A	See sections 7.1 & 8.6	300 µL extraction buffer See section 7.1, point 6 53 µL of resin 2 volumes of lysis buffer See section 8.6.2	100 µL See section 3.2	First incubation - 37°C See section 7.1, point 7 Second incubation – 65°C See section 7.1, point 11	1x lysis buffer wash 3x wash buffer wash See section 8.6.5
	6.6 ODL Retained supernatant	N/A	See section 7.2 & 8.6	450 µL TNE See section 7.2, point 6 14 µL of Pro K & 7 µL of Sarcosyl See section 7.2, point 10 53 µL of resin 2 volumes of lysis buffer	As above	First incubation – Room temperature See section 7.2, point 7 Second incubation – 37°C See section 7.2, point 11 Third incubation - 65°C	As above

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Iteration of protocol	MultiProbe II Protocol version No.	Project No.	(a) In seriatim, all steps comprising the protocol	(b) The resin and reagent volumes used for extraction of DNA	(c) The volume of the sample containing the DNA at the end of the extraction	(d) The temperature used during the lysis step of the extraction process	(e) The number of 'washes' employed following the lysis step of the extraction process
				See section 8.6.2		See section 7.2, point 15	
QIS#24897V8 SOP relevant to the DNA IQ protocol dated 27 June 2012	6.6 ODL	N/A	See section 8	300 µL extraction buffer See section 8, point 6 53 µL of resin 2 volumes of lysis buffer See section 9.3.2	100 µL	First incubation - 37°C See section 8, point 7 Second incubation – 65°C See section 8, point 11	1x lysis buffer wash 3x wash buffer wash See section 9.3.5
QIS#24897V9 SOP relevant to the DNA IQ protocol dated 3 January 2014	6.6 ODL	N/A	See section 8	300 µL extraction buffer See section 8, point 6 53 µL of resin 2 volumes of lysis buffer See section 9.3.2	100 µL	First incubation - 37°C See section 8, point 7 Second incubation – 65°C See section 8, point 11	1x lysis buffer wash 3x wash buffer wash See section 9.3.5
QIS#24897V10 SOP relevant to the DNA IQ protocol dated 12 June 2015	6.7 ODL		See section 8	300 µL extraction buffer See section 8, point 5 53 µL of resin 2 volumes of lysis buffer See section 9.3.2	100 µL	First incubation - 37°C See section 8, point 6 Second incubation – 65°C See section 8, point 11	1x lysis buffer wash 3x wash buffer wash See section 9.3.5

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Iteration of protocol	MultiProbe II Protocol version No.	Project No.	(a) In seriatim, all steps comprising the protocol	(b) The resin and reagent volumes used for extraction of DNA	(c) The volume of the sample containing the DNA at the end of the extraction	(d) The temperature used during the lysis step of the extraction process	(e) The number of 'washes' employed following the lysis step of the extraction process
QIS#24897V11 SOP relevant to the DNA IQ protocol dated 30 January 2017	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Manual Protocol

Iteration of protocol	Manual version type	Project No.	(a) In seriatim, all steps comprising the protocol	(b) The resin and reagent volumes used for extraction of DNA	(c) The volume of the sample containing the DNA at the end of the extraction	(d) The temperature used during the lysis step of the extraction process	(e) The number of 'washes' employed following the lysis step of the extraction process
Manual Protocol							
QIS#24897V1 SOP relevant to the DNA IQ protocol dated 24 October 2007	N/A	N/A	N/A	N/A	N/A	N/A	N/A
QIS#24897V2 SOP relevant to the DNA IQ protocol dated 11 January 2008	No retained supernatant	11	See section 16.2.2	300 µL extraction buffer See section 16.2.2, point 5 50 µL of resin See 16.2.2, point 11 550 µL of lysis buffer See section 16.2.2, point 9	~95 µL See section 16.2.2, points 23 & 24	Incubation - 37°C See section 16.2.2, point 5	1x lysis buffer wash See section 16.2, point 15 3x wash buffer wash See section 16.2.2, points 16 & 17

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Iteration of protocol	Manual version type	Project No.	(a) In seriatim, all steps comprising the protocol	(b) The resin and reagent volumes used for extraction of DNA	(c) The volume of the sample containing the DNA at the end of the extraction	(d) The temperature used during the lysis step of the extraction process	(e) The number of 'washes' employed following the lysis step of the extraction process
QIS#24987V3 SOP relevant to the DNA IQ protocol dated 27 March 2008	No retained supernatant	N/A	See section 18.4.2	300 µL extraction buffer See section 18.4.2, point 5 50 µL of resin See 18.4.2, point 11 500 µL of lysis buffer See section 18.4.2, point 9	~95 µL See section 18.4.2, points 23 & 24	Incubation - 37°C See section 18.4.2, point 5	1x lysis buffer wash See section 18.4.2, point 15 3x wash buffer wash See section 18.4.2, points 16 & 17
QIS#24987V4 SOP relevant to the DNA IQ protocol dated 21 May 2008	No retained supernatant	N/A	See section 18.4.2	300 µL extraction buffer See section 18.4.2, point 5 50 µL of resin See 18.4.2, point 11 500 µL of lysis buffer See section 18.4.2, point 9	~95 µL See section 18.4.2, points 23 & 24	Incubation - 37°C See section 18.4.2, point 5	1x lysis buffer wash See section 18.4.2, point 15 3x wash buffer wash See section 18.4.2, points 16 & 17
QIS#24987V5 SOP relevant to the DNA IQ protocol dated 29 June 2009	No retained supernatant	N/A	See section 17.1.4	300 µL extraction buffer See section 17.1.4, point 5 50 µL of resin See 17.1.4, point 11 550 µL of lysis buffer	~95 µL See section 17.1.4, point 24	Incubation - 37°C See section 17.1.4, point 6	1x lysis buffer wash See section 17.1.4, point 15 3x wash buffer wash See section 17.1.4, points 17 & 18

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Iteration of protocol	Manual version type	Project No.	(a) In seriatim, all steps comprising the protocol	(b) The resin and reagent volumes used for extraction of DNA	(c) The volume of the sample containing the DNA at the end of the extraction	(d) The temperature used during the lysis step of the extraction process	(e) The number of 'washes' employed following the lysis step of the extraction process
				See section 17.1.4, point 10			
	Retained supernatant	N/A	See section 17.1.5	450 µL TNE See section 17.1.5, point 7 14 µL of Pro K & 7 µL of Sarcosyl See section 17.1.5, point 11 50 µL of resin See 17.1.5, point 17 550 µL of lysis buffer See section 17.1.5, point 16	~95 µL See section 17.1.5, point 30	First incubation – Room temperature See section 17.1.5, point 8 Second incubation – 37°C See section 17.1.5, point 12	1x lysis buffer wash See section 17.1.5, point 21 3x wash buffer wash See section 17.1.5, points 23 & 24
QIS#24987V6 SOP relevant to the DNA IQ protocol dated 13 August 2009	No retained supernatant	N/A	See section 17.1.4	300 µL extraction buffer See section 17.1.4, point 6 50 µL of resin See 17.1.4, point 11 550 µL of lysis buffer See section 17.1.4, point 10	~95 µL See section 17.1.4, point 24	Incubation - 37°C See section 17.1.4, point 6	1x lysis buffer wash See section 17.1.4, point 15 3x wash buffer wash See section 17.1.4, points 17 & 18

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Iteration of protocol	Manual version type	Project No.	(a) In seriatim, all steps comprising the protocol	(b) The resin and reagent volumes used for extraction of DNA	(c) The volume of the sample containing the DNA at the end of the extraction	(d) The temperature used during the lysis step of the extraction process	(e) The number of 'washes' employed following the lysis step of the extraction process
	Retained supernatant	N/A	See section 17.1.5	450 µL TNE See section 17.1.5, point 6 14 µL of Pro K & 7 µL of Sarcosyl See section 17.1.5, point 10 50 µL of resin See 17.1.5, point 17 550 µL of lysis buffer See section 17.1.5, point 16	~95 µL See section 17.1.5, point 30	First incubation – Room temperature See section 17.1.5, point 8 Second incubation – 37°C See section 17.1.5, point 12	1x lysis buffer wash See section 17.1.5, point 21 3x wash buffer wash See section 17.1.5, points 23 & 24
QIS#24987V7 SOP relevant to the DNA IQ protocol dated 9 November 2010	No retained supernatant	N/A	See section 9.4	300 µL extraction buffer See section 9.4, point 5 50 µL of resin See 9.4, point 12 550 µL of lysis buffer See section 9.4, point 11	~95 µL See section 9.4, point 25	Incubation - 37°C See section 9.4 point 6	1x lysis buffer wash See section 9.4 point 16 3x wash buffer wash See section 9.4, points 18 & 19
	Retained supernatant	N/A	See section 9.5	450 µL TNE See section 9.5, point 7 14 µL of Pro K & 7 µL of Sarcosyl	~95 µL See section 9.5, point 31	First incubation – Room temperature See section 9.5, point 8	1x lysis buffer wash See section 9.5, point 22 3x wash buffer wash

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Witness

Iteration of protocol	Manual version type	Project No.	(a) In seriatim, all steps comprising the protocol	(b) The resin and reagent volumes used for extraction of DNA	(c) The volume of the sample containing the DNA at the end of the extraction	(d) The temperature used during the lysis step of the extraction process	(e) The number of 'washes' employed following the lysis step of the extraction process
				See section 9.5, point 11 50 µL of resin See 9.5, point 18 550 µL of lysis buffer See section 9.5, point 17		Second incubation – 37°C See section 9.5, point 12	See section 9.5, points 24 & 25
QIS#24987V8 SOP relevant to the DNA IQ protocol dated 27 June 2012	No retained supernatant	N/A	See section 7.1	300 µL extraction buffer See section 7.1, point 5 50 µL of resin See 7.1, point 12 550 µL of lysis buffer See section 7.1, point 11	~95 µL See section 7.1, point 25	Incubation - 37°C See section 7.1 point 6	1x lysis buffer wash See section 7.1 point 16 3x wash buffer wash See section 7.1, points 18 & 19
	Retained supernatant	N/A	See section 7.2	450 µL TNE See section 7.2, point 5 14 µL of Pro K & 7 µL of Sarcosyl See section 7.2, point 9 50 µL of resin See 7.2, point 16 550 µL of lysis buffer	~95 µL See section 7.2, point 29	First incubation – room temperature See section 7.2, point 6 Second incubation – 37°C See section 7.2, point 10	1x lysis buffer wash See section 7.2 point 20 3x wash buffer wash See section 7.2, points 22 & 23

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Witness

Iteration of protocol	Manual version type	Project No.	(a) In seriatim, all steps comprising the protocol	(b) The resin and reagent volumes used for extraction of DNA	(c) The volume of the sample containing the DNA at the end of the extraction	(d) The temperature used during the lysis step of the extraction process	(e) The number of 'washes' employed following the lysis step of the extraction process
				See section 7.2, point 15			
QIS#24987V9 SOP relevant to the DNA IQ protocol dated 3 January 2014	No retained supernatant	N/A	See section 7.1	300 µL extraction buffer See section 7.1, point 5 50 µL of resin See 7.1, point 12 550 µL of lysis buffer See section 7.1, point 11	~95 µL See section 7.1, point 25	Incubation - 37°C See section 7.1, point 6	1x lysis buffer wash See section 7.1, point 16 3x wash buffer wash See section 7.1, points 18 & 19
	Retained supernatant	N/A	See section 7.2	450 µL TNE See section 7.2, point 5 14 µL of Pro K & 7 µL of Sarcosyl See section 7.2, point 9 50 µL of resin See 7.2, point 16 550 µL of lysis buffer See section 7.2, point 15	~95 µL See section 7.2, point 29	First incubation – room temperature See section 7.2, point 6 Second incubation – 37°C See section 7.2, point 10	1x lysis buffer wash See section 7.2 point 20 3x wash buffer wash See section 7.2, points 22 & 23
QIS#24987V10 SOP relevant to the DNA	No retained supernatant	N/A	See section 7.1	300 µL extraction buffer	~95 µL See section 7.1, point 25	Incubation - 37°C See section 7.1, point 6	1x lysis buffer wash See section 7.1, point 16

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Thomas Nurthen

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Witness

Iteration of protocol	Manual version type	Project No.	(a) In seriatim, all steps comprising the protocol	(b) The resin and reagent volumes used for extraction of DNA	(c) The volume of the sample containing the DNA at the end of the extraction	(d) The temperature used during the lysis step of the extraction process	(e) The number of 'washes' employed following the lysis step of the extraction process
IQ protocol dated 12 June 2015				See section 7.1, point 5 50 µL of resin See 7.1, point 12 550 µL of lysis buffer See section 7.1, point 11			3x wash buffer wash See section 7.1, points 18 & 19
	Retained supernatant	N/A	See section 7.2	450 µL TNE See section 7.2, point 5 14 µL of Pro K & 7 µL of Sarcosyl See section 7.2, point 9 50 µL of resin See 7.2, point 16 550 µL of lysis buffer See section 7.2, point 15	~95 µL See section 7.2, point 29	First incubation – room temperature See section 7.2, point 6 Second incubation – 37°C See section 7.2, point 10	1x lysis buffer wash See section 7.2, point 20 3x wash buffer wash See section 7.2, points 22 & 23
QIS#24987V11 SOP relevant to the DNA IQ protocol dated 30 January 2017	No retained supernatant	N/A	See section 7.1	300 µL extraction buffer See section 7.1, point 5 50 µL of resin See 7.1, point 12 550 µL of lysis buffer	~95 µL See section 7.1, point 25	Incubation - 37°C See section 7.1, point 6	1x lysis buffer wash See section 7.1, point 16 3x wash buffer wash See section 7.1, points 18 & 19

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Thomas Nurthen

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Witness

Iteration of protocol	Manual version type	Project No.	(a) In seriatim, all steps comprising the protocol	(b) The resin and reagent volumes used for extraction of DNA	(c) The volume of the sample containing the DNA at the end of the extraction	(d) The temperature used during the lysis step of the extraction process	(e) The number of 'washes' employed following the lysis step of the extraction process
				See section 7.1, point 11			
	Retained supernatant	N/A	See section 7.2	450 µL TNE See section 7.2, point 5 14 µL of Pro K & 7 µL of Sarcosyl See section 7.2, point 9 50 µL of resin See 7.2, point 16 550 µL of lysis buffer See section 7.2, point 15	~95 µL See section 7.2, point 29	First incubation – room temperature See section 7.2, point 6 Second incubation – 37°C See section 7.2, point 10	1x lysis buffer wash See section 7.2, point 20 3x wash buffer wash See section 7.2, points 22 & 23
QIS#34043V1 SOP relevant to the DNA IQ protocol dated 15 June 2017	No retained supernatant	N/A	See section 7.1	300 µL extraction buffer See section 7.1, point 8 50 µL of resin See 7.1, point 17 550 µL of lysis buffer See section 7.1, point 16	~95 µL See section 7.1, point 30	Incubation - 37°C See section 7.1, point 9	1x lysis buffer wash See section 7.1, point 21 3x wash buffer wash See section 7.1, points 23 & 24
	Retained supernatant	N/A	See section 7.2	450 µL TNE See section 7.2, point 8	~95 µL See section 7.2, point 34	First incubation – room temperature	1x lysis buffer wash See section 7.2, point 25

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Thomas Nurthen

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Witness

ME_215031159_2

Iteration of protocol	Manual version type	Project No.	(a) In seriatim, all steps comprising the protocol	(b) The resin and reagent volumes used for extraction of DNA	(c) The volume of the sample containing the DNA at the end of the extraction	(d) The temperature used during the lysis step of the extraction process	(e) The number of 'washes' employed following the lysis step of the extraction process
				14 µL of Pro K & 7 µL of Sarcosyl See section 7.2, point 12 50 µL of resin See 7.2, point 21 550 µL of lysis buffer See section 7.2, point 20		See section 7.2, point 9 Second incubation – 37°C See section 7.2, point 13	3x wash buffer wash See section 7.2, points 27 & 28

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Thomas Nurthen

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Witness

Table of Thomas Nurthen's responses to Question 3 in Notice 5.003

DNA IQ protocol

Question 3 - To the extent that the DNA IQ™ protocol changed at any point in time, describe the reasons for the change.

Iteration of protocol	MultiProbe II Protocol version No.	Project No.	SOP amendment history	Reasons for change
QIS#24897V1 SOP relevant to the DNA IQ protocol dated 24 October 2007	1.1	13	First Issue	N/A
QIS#24897V2 SOP relevant to the DNA IQ protocol dated 11 January 2008	1.3	N/A	Reviewed and updated after initial training	I do not know the reason for the changes based on the SOP's amendment history. I have no independent recollection of the reasons for the changes.
QIS#24987V3 SOP relevant to the DNA IQ protocol dated 27 March 2008	2.0 ODL No retained supernatant	22	Addition of Off-deck Lysis procedure, Retention of fully automated method as Appendix, addition of reagent record tables into Appendix	Introduction of Off-deck Lysis procedure to allow for varying sample sizes, substrate material type and requirement for retaining supernatant for presumptive screening.
	2.0 ODL Retained supernatant	21		Introduction of Off-deck Lysis (Retained supernatant) procedure to allow for varying sample sizes, substrate material type and requirement for retaining supernatant for presumptive screening.
QIS#24897V4 SOP relevant to the DNA IQ	4.1 ODL No retained supernatant	N/A	Headers and Footers changed to new CaSS format. Amended Business references from QHSS to FSS, QHPSS to CaSS and QHPS to Pathology Queensland	Replacement of SDS detergent with Sarcosyl detergent due to gel formation.

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Thomas Nurthen

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Witness

ME_215083326_3

Iteration of protocol	MultiProbe II Protocol version No.	Project No.	SOP amendment history	Reasons for change
protocol dated 21 May 2008	4.1 ODL Retained supernatant	N/A	Version incremented by one on migration to QIS2	Replacement of SDS detergent with Sarcosyl detergent due to gel formation.
QIS#24897V5 SOP relevant to the DNA IQ protocol dated 29 June 2009	6.4 ODL No retained supernatant	Re-implementing the automated DNA IQ™ extraction protocol	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.	To address contamination previously identified.
	6.4 ODL Retained supernatant	As above		To address contamination previously identified.
QIS#24897V6 SOP relevant to the DNA IQ protocol dated 13 August 2009	6.5 ODL No retained supernatant	Re-implementing the automated DNA IQ™ extraction protocol	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	Dispense height for DNA IQ resin solution optimised.
	6.5 ODL Retained supernatant	As above		Dispense height for DNA IQ resin solution optimised.
QIS#24897V7 SOP relevant to the DNA IQ protocol dated 9 November 2010	6.6 ODL No retained supernatant	N/A	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.	I do not know the reason for the changes based on the SOP's amendment history. I have no independent recollection of the reasons for the changes.
	6.6 ODL Retained supernatant	N/A		I do not know the reason for the changes based on the SOP's amendment history. I have no independent recollection of the reasons for the changes.

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Thomas Nurthen

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Witness

Iteration of protocol	MultiProbe II Protocol version No.	Project No.	SOP amendment history	Reasons for change
QIS#24897V8 SOP relevant to the DNA IQ protocol dated 27 June 2012	6.6 ODL	N/A	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.	I do not know the reason for the changes based on the SOP's amendment history. I have no independent recollection of the reasons for the changes.
QIS#24897V9 SOP relevant to the DNA IQ protocol dated 3 January 2014	6.6 ODL	N/A	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.	I do not know the reason for the changes based on the SOP's amendment history. I have no independent recollection of the reasons for the changes.
QIS#24897V10 SOP relevant to the DNA IQ protocol dated 12 June 2015	6.7 ODL	N/A	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	I do not know the reason for the changes based on the SOP's amendment history. I have no independent recollection of the reasons for the changes.
QIS#24897V11 SOP relevant to the DNA IQ protocol dated 30 January 2017	N/A	N/A	Removed Automated Extraction	I do not know the reason for the changes based on the SOP's amendment history. I have no independent recollection of the reasons for the changes.

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Thomas Nurthen Witness

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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.

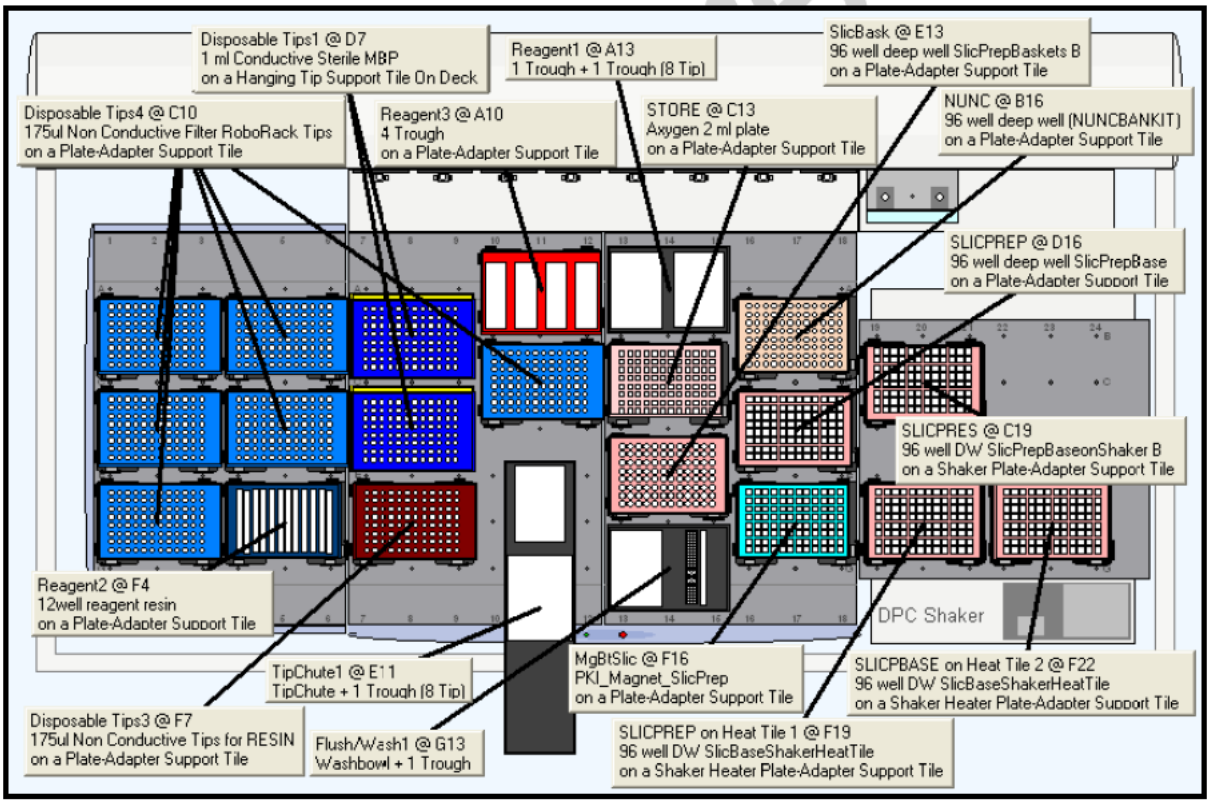


Figure 3. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

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 65^0C 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]**.

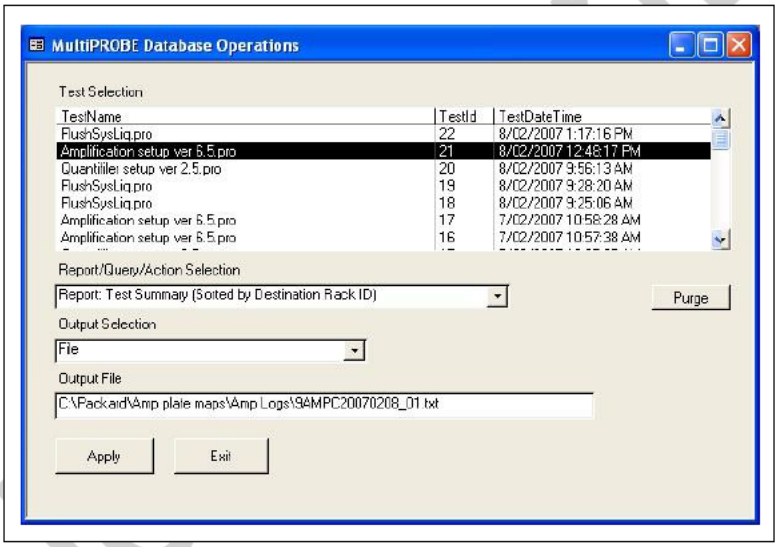
Finalising the MP II run

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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11. PerkinElmer, Automated DNA IQ™ System for Mixed Casework Sample DNA Isolation. MultiPROBE II Liquid Handling - Forensic Workstation Application Guide, 2004: p. 1-25.
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13. Promega, Protocols & Applications Guide. Chapter 9. rev. 7/06.
14. Promega, DNA IQ™ System -Small Casework Protocol. Promega Technical Bulletin #TB296 2006. Rev 4/06: p. 1-14.
15. Promega, DNA IQ™ System-Database Protocol. Promega Technical Bulletin #TB297, 2006. Rev 4/06: p. 1-14.
16. 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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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.

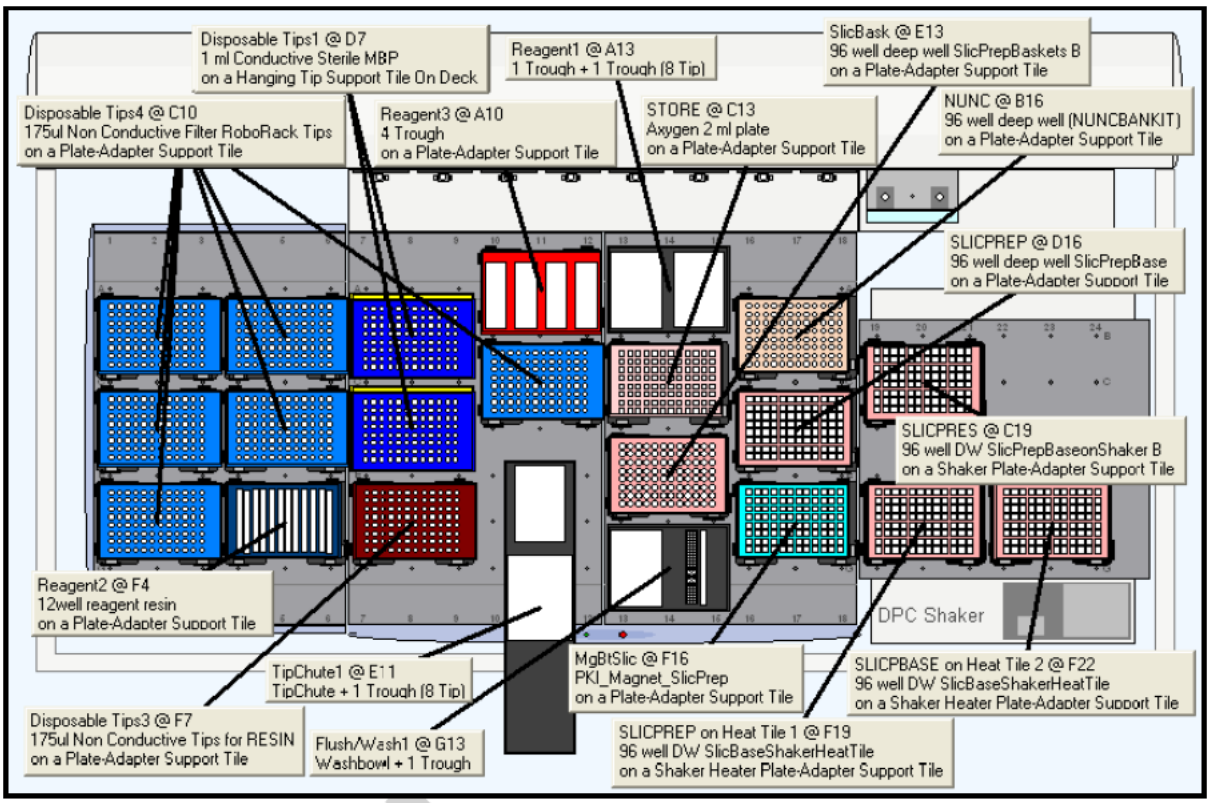


Figure **2**. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

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)

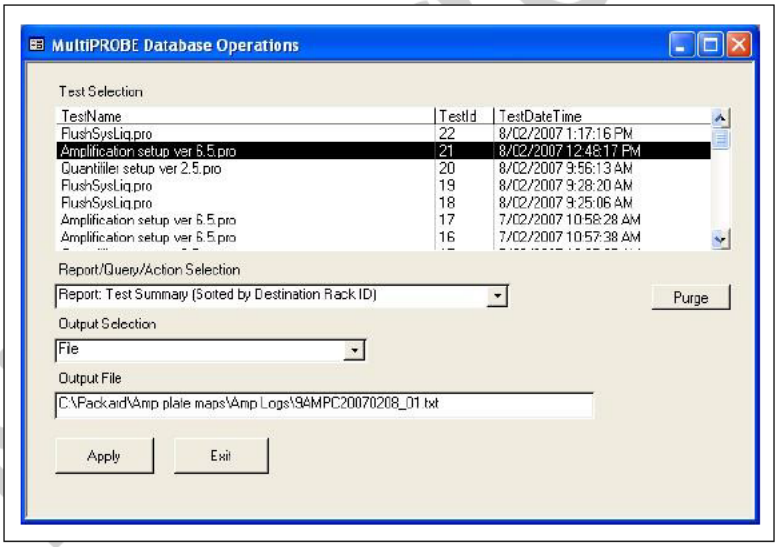


Figure 4. The MultiPROBE log database for collecting MP II run information

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.

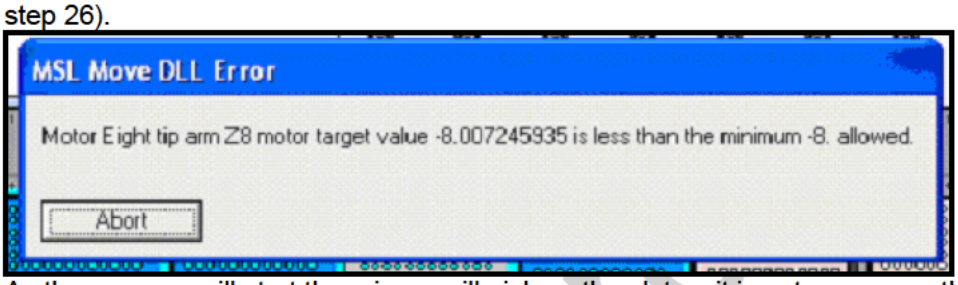
9 TROUBLESHOOTING

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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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**.

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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.

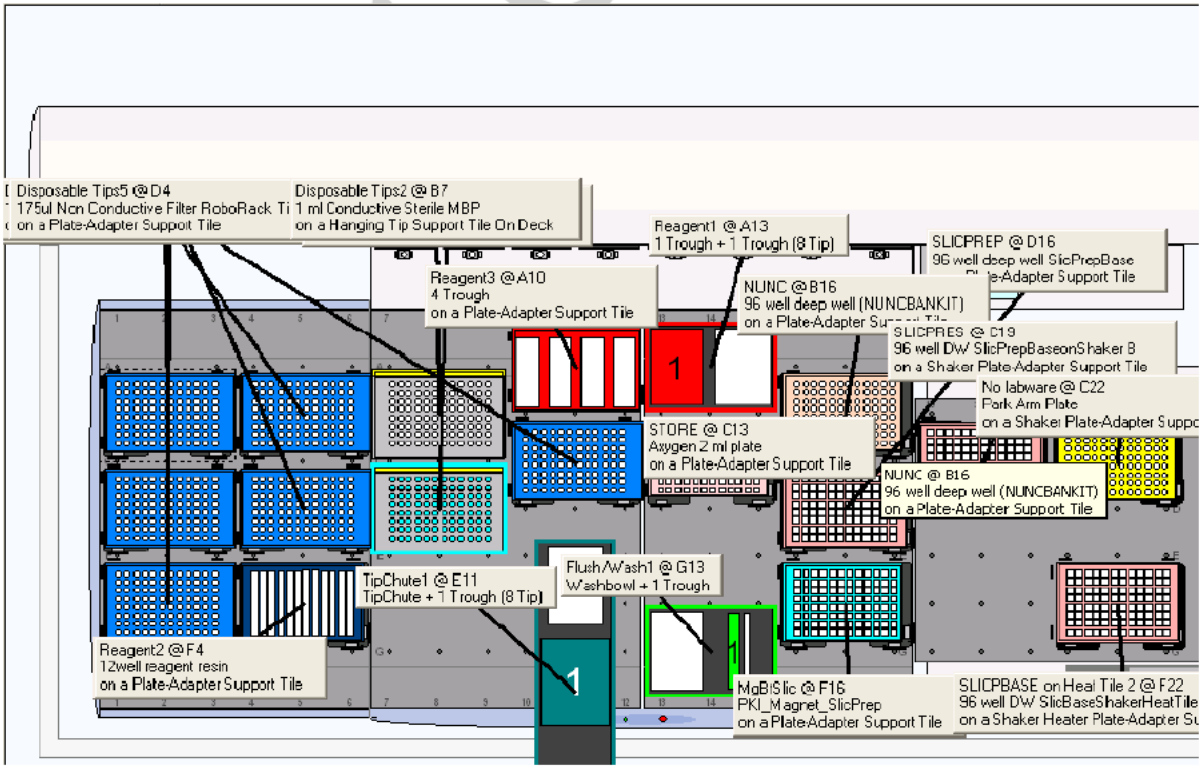

Figure 2. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

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):

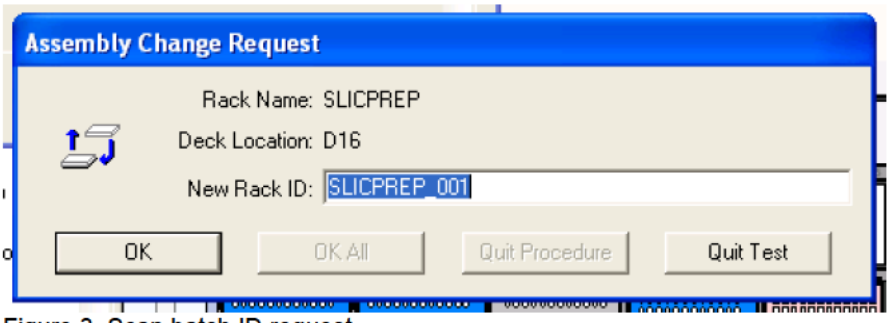

Figure 3. Scan batch ID request

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)

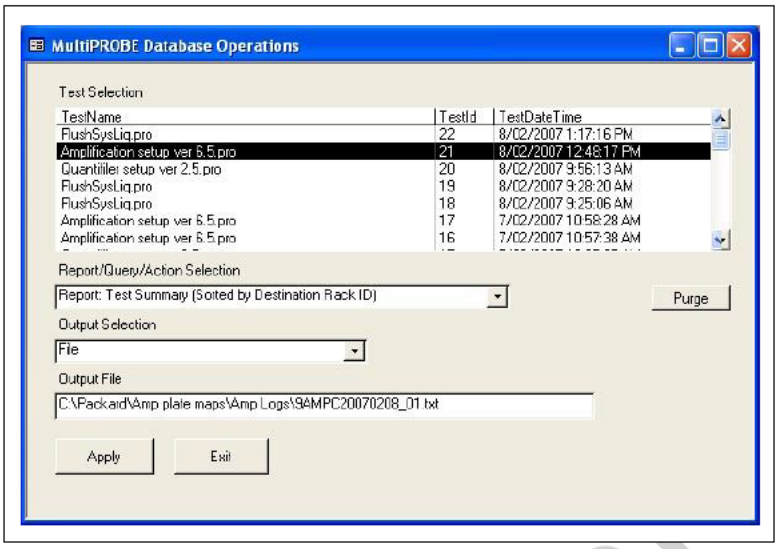

Figure 4. The MultiPROBE log database for collecting MP II run information

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).

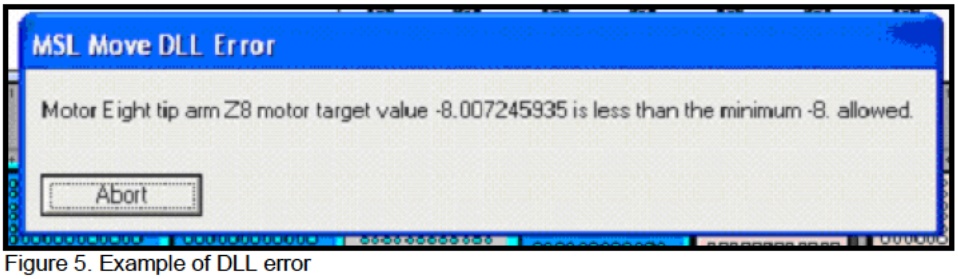

Figure 5. Example of DLL error

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:

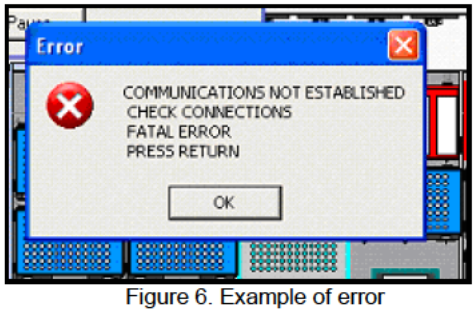

Figure 6. Example of error

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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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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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.

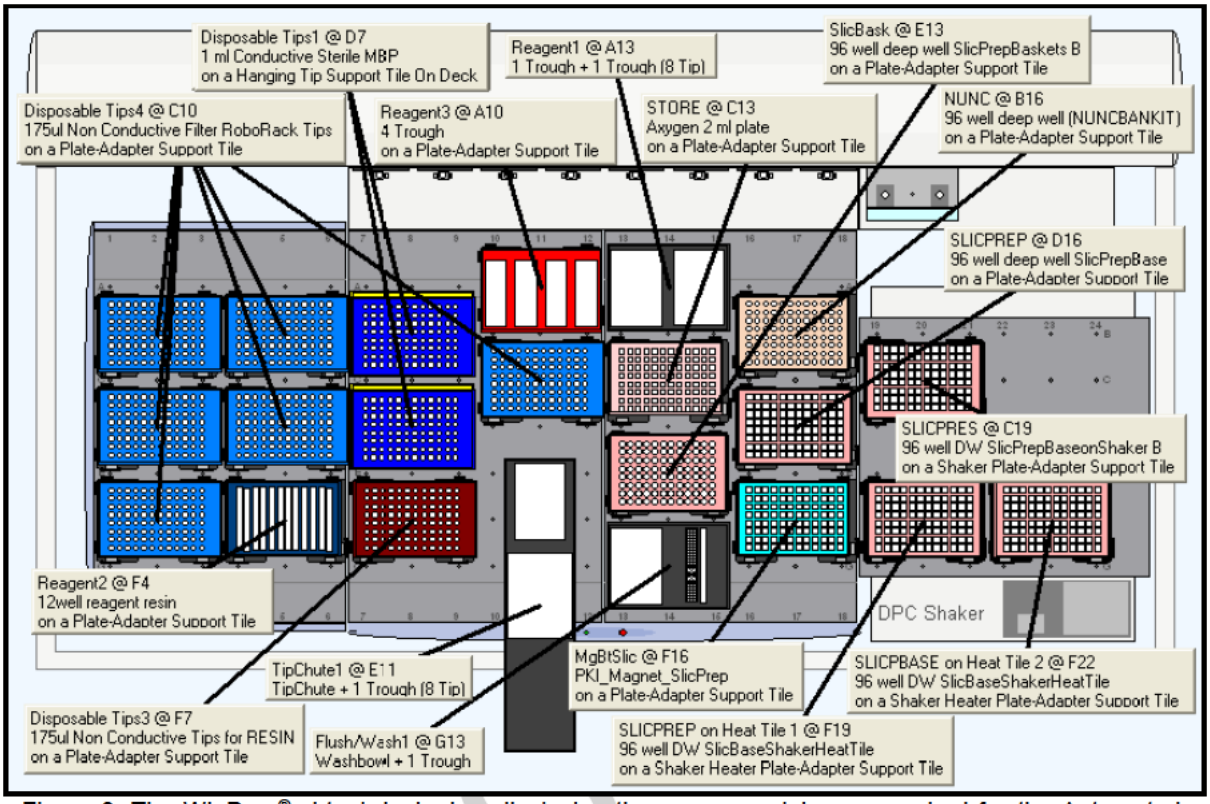

Figure 8. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

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^{0}C (real temp 65^{0}C). **DO NOT PRESS CONTINUE** it will continue automatically when temperature has reached 85^{0}C.

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)

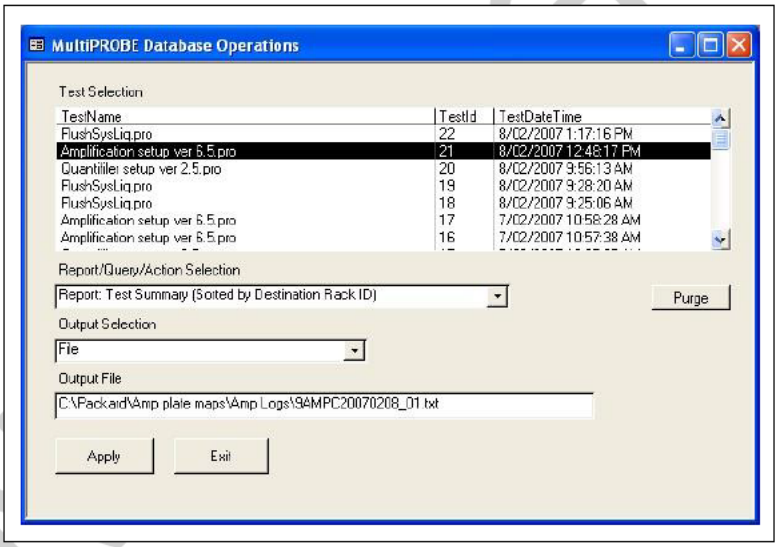

Figure 9. The MultiPROBE log database for collecting MP II run information

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

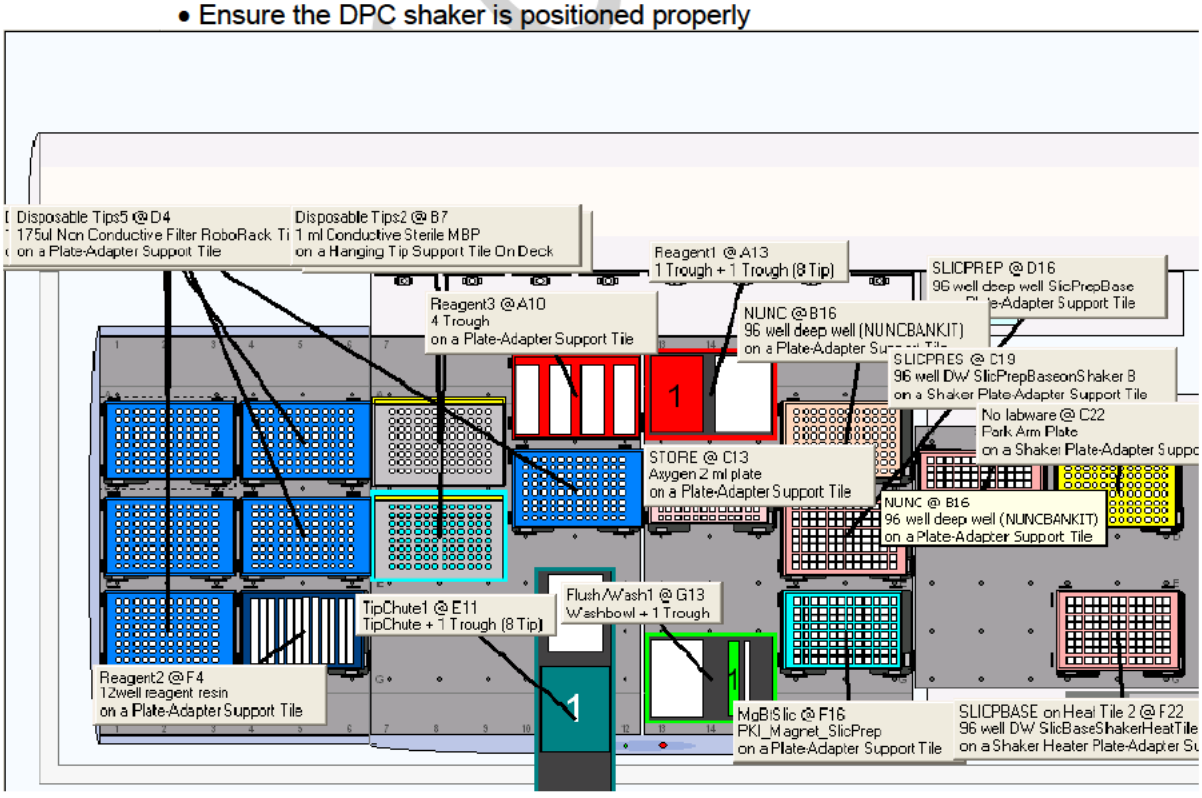

Figure 2. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

14. Ensure that the DPC shaker and Heater Controller Box are switched on.
    For EP-A: Tile 1 at F22 (85^0C).
    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.

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):

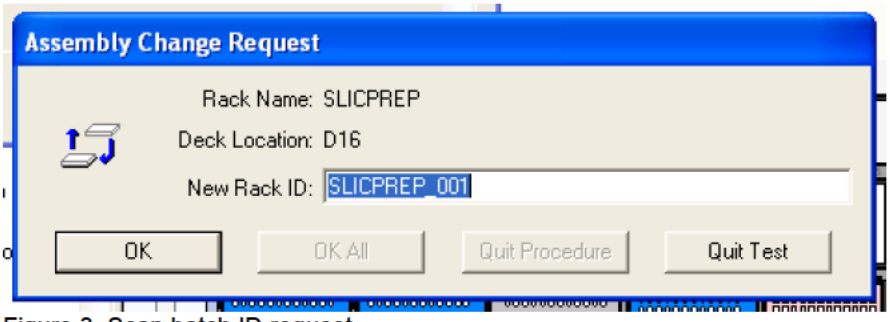

Figure 3. Scan batch ID request

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)

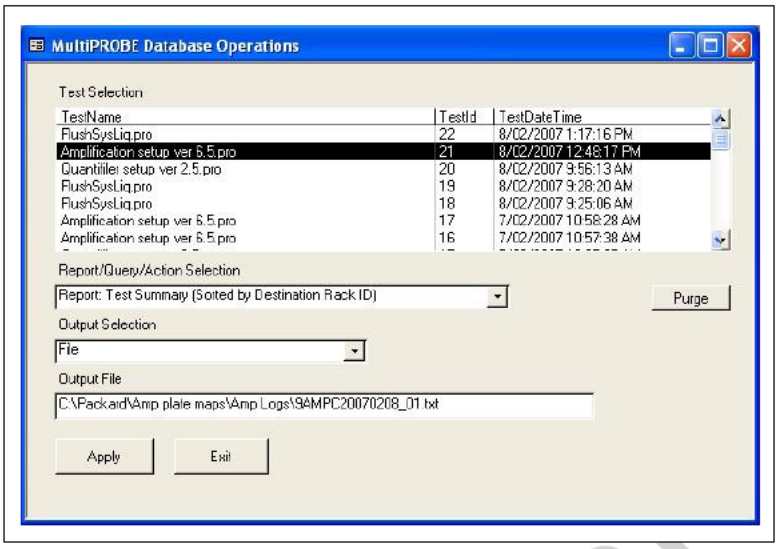

Figure 4. The MultiPROBE log database for collecting MP II run information

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).

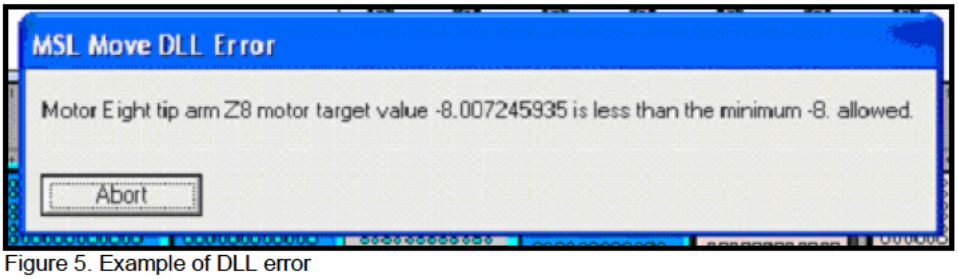

Figure 5. Example of DLL error

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.
8. Mandrekar, P.V., Kreneke, B. E., & Tereba, A., DNA IQ™: The Intelligent Way to Purify DNA. Profiles in DNA, 2001: p. 16.

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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, 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).

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 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.

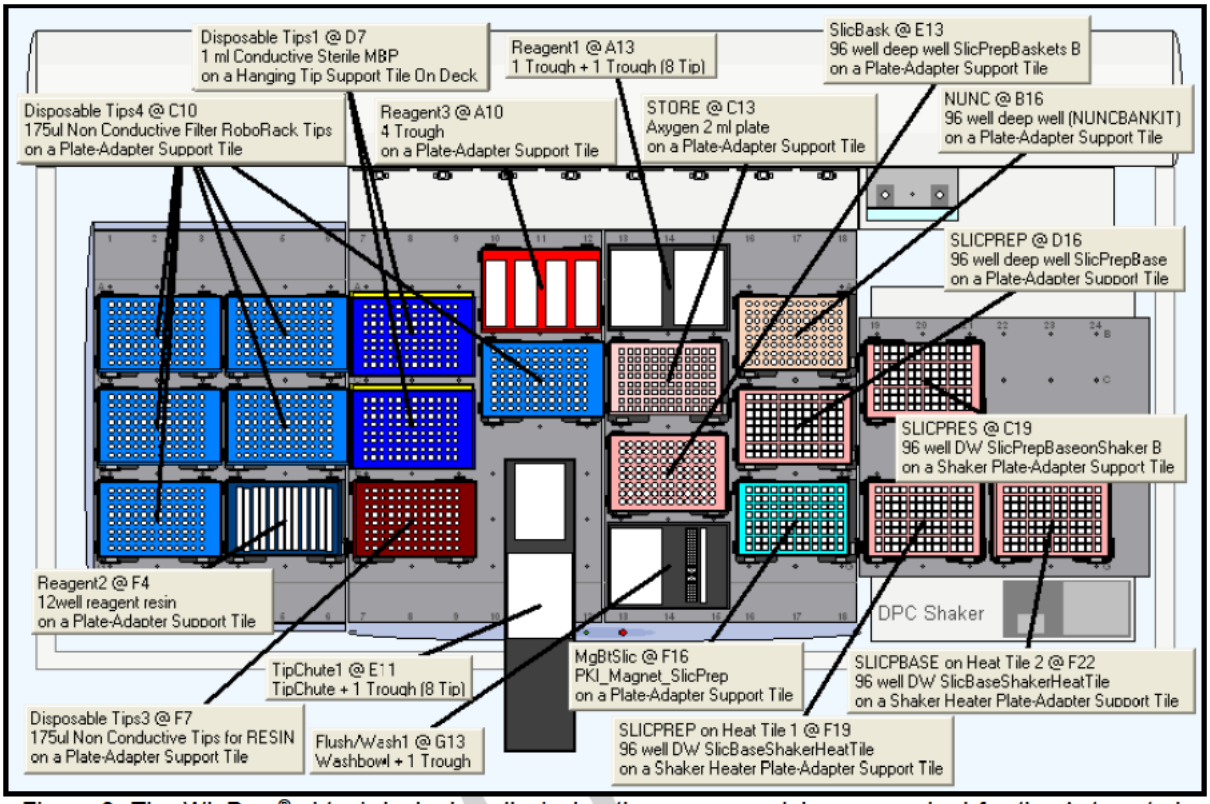

Figure 8. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

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 50^0C (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^{0}C (real temp 65^{0}C). **DO NOT PRESS CONTINUE** it will continue automatically when temperature has reached 85^{0}C.

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)

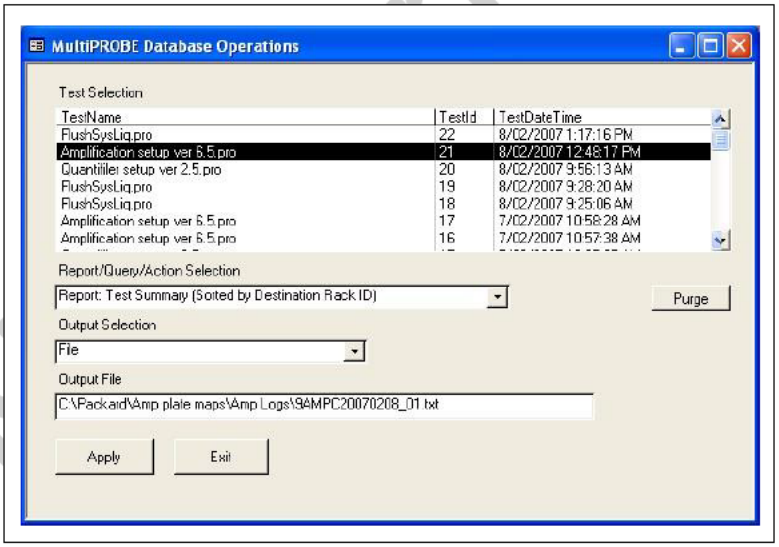

Figure 9. The MultiPROBE log database for collecting MP II run information

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 37°C.

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 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 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 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
   - 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 vis ble 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):

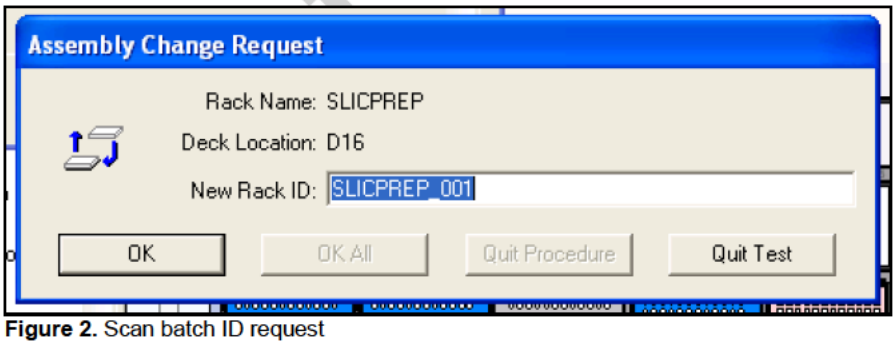

Figure 2. Scan batch ID request

    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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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 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 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 inh bit 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 37°C. 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 inh bit 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):

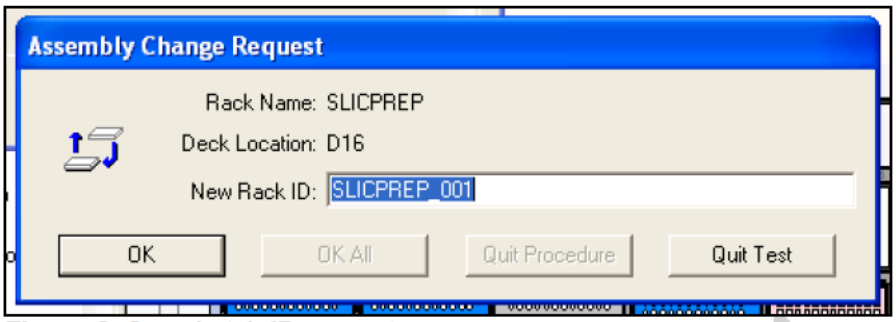

Figure 2. Scan batch ID request

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 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.

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, Manual DNA IQ Retain Supernatant	Negative Extraction control
Positive Control	Off-Deck Lysis Manual DNA IQ Retain Supernatant	Positive Extraction control – dried blood swab from a known donor
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):

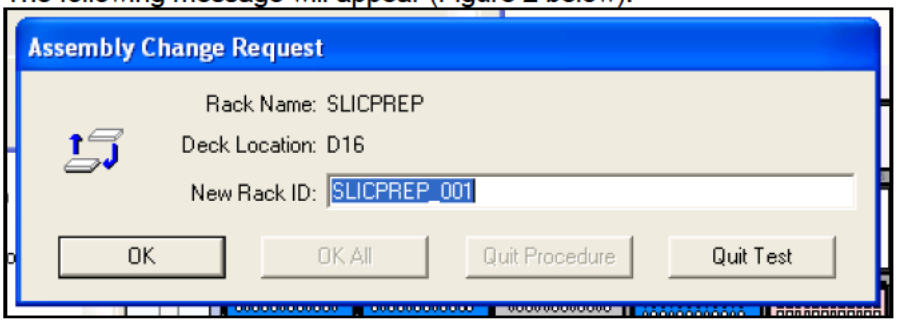

Figure 2. Scan batch ID request

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 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**".

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

- 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

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):

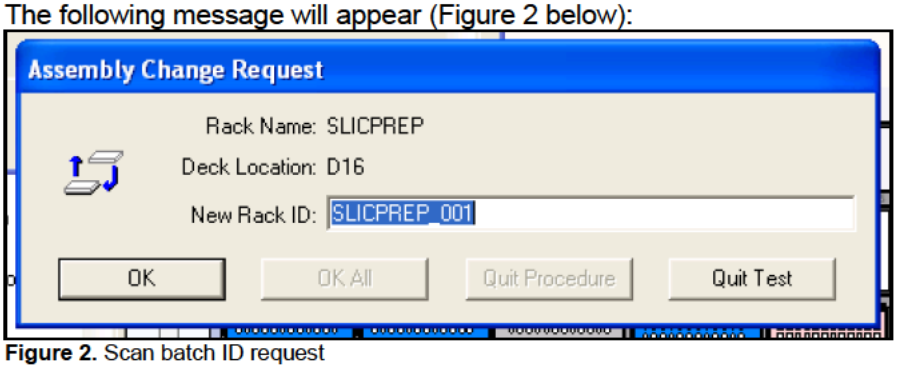

Figure 2. Scan batch ID request

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.
- 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

11.3 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.

11.4 REFERENCES

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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):

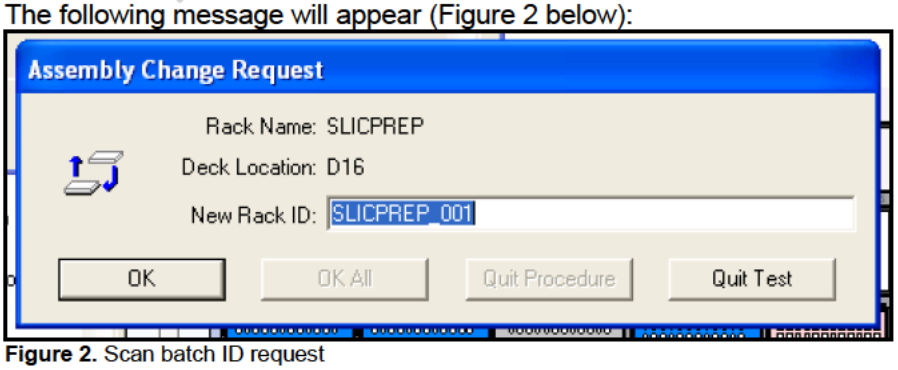

Figure 2. Scan batch ID request

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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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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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. Ne 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 wa k-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 Exh bit 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).

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 6.7_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):

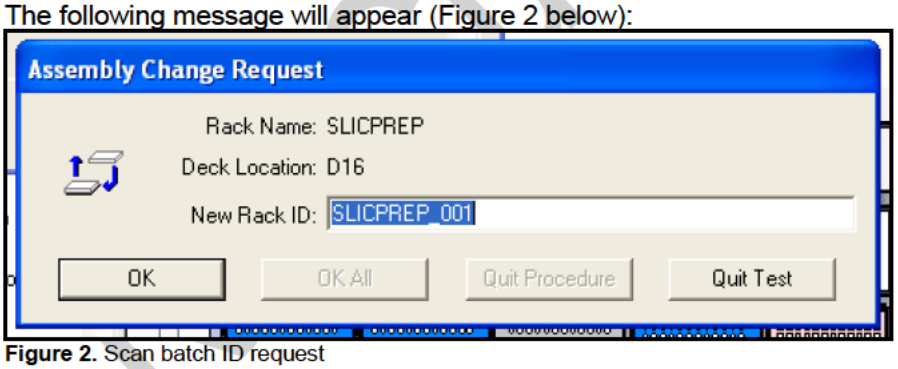

Figure 2. Scan batch ID request

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

- 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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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.

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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 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).

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 wa k-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, Protocols & Applications Guide. Chapter 9. rev. 7/06.
- 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.

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DNA IQ™ Method of Extracting DNA from Casework and Reference Samples

1 Purpose and Scope

This document outlines the manual procedures for extracting DNA from reference and casework samples using the DNA IQ™ kit (Promega Corp., Madison, WI, USA) within the Forensic DNA Analysis Unit.

Reference samples and casework samples must be extracted on separate batches. If casework and reference batches are to be extracted on the same extraction bench, the biological safety cabinet and all associated lab-ware and pipettes must be decontaminated between operations.

This procedure applies to all staff members who are required to extract DNA from samples using manual DNA IQ™ methods

2 Definitions

DTT	1,4 Dithiothreitol
EDTA	Ethylenediaminetetraacetatic acid
Extracts	Samples that have undergone DNA extraction
FR	Forensic Register
Lysates	Samples that have undergone pre-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. 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 100 ng 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 80 bp.

4 Reagents, Equipment and Consumables

4.1 Reagents

Table 1 outlines all the reagents and their storage locations required for DNA IQ™ extraction.

Table 1: Reagents with storage room and location

Reagent	Room	Location
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
1,4 Dithiothreitol (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
Proteinase K (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 fresh Extraction Buffer in the biosafety cabinet in room 3188 just prior to commencing the Manual DNA IQ™ (No Retain Supernatant) procedure.

1. Determine reagent volumes required by referring to Appendix 14.1 Table 6.
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 biological safety cabinet, aliquot the required amount of TNE buffer into a 50 mL tube. Do not pipette 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 with **"Extraction Buffer"**, initials and date.

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™ procedure.

1. Determine reagent volumes required by referring to Appendix 14.1 Table 6 (No Retain Supernatant) or Appendix 14.1 Table 7 (Retain Supernatant).

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 50 mL tube.

4. Add the required volume of DTT to the Lysis Buffer and gently swirl to mix.

5. Label with **"Lysis Buffer + DTT"**, initials and date.

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) in the biosafety cabinet in room 3188 just prior to commencing the Manual procedure.

Note: Ensure the resin is properly mixed by vortex mixing prior to use.

1. Determine reagent volumes required by referring to Appendix 14.1 Table 6 (No Retain Supernatant) or Appendix 14.1 Table 7 (Retain Supernatant).

2. In the biohazard cabinet, aliquot the required amount of Lysis Buffer with DTT solution (from 4.1.2) into a 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 with **"Resin"**, initials and date.

4.1.4 1x Wash Buffer

Note: 2x Wash Buffer is supplied with the DNA IQ™ kit. A 1x Wash Buffer is required for the procedure.

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. Refer to Receipt, Storage and Preparation of

Chemicals, Reagents and Test Kits (34103). Update the 1x Wash Buffer reagent log in FR by:

- At the FR main menu click the **Reference** tab and the **Supply** button.
- Type **DNA IQ** into the **Category** field followed by the enter key.
- Click the **Received Date** of the wash buffer that is being prepared.
- Click the **Edit** icon at the top right of the screen.
- Click in the **Comments** box and specify the 1x Wash Buffer aliquot (e.g. A1) and the lot numbers of the ethanol and isopropyl alcohol used.
- Click the **Save** icon at the top right of the screen.

Note: 1x Wash Buffer can be prepared and stored at room temperature prior to use in the manual DNA IQ™ procedure.

4. Determine reagent volumes required by referring to Appendix 14.1 Table 6 (No Retain Supernatant) or Appendix 14.1 Table 7 (Retain Supernatant).

5. In the biological safety cabinet, aliquot the required amount of 1x Wash Buffer into a 5 or 10 mL tube.

6. Label with **"Wash Buffer"**, initials and date.

4.1.5 Elution Buffer

Elution Buffer is supplied with the DNA IQ™ kit.

1. Determine reagent volumes required by referring to Appendix 14.1 Table 6 (No Retain Supernatant) or Appendix 14.1 Table 7 (Retain Supernatant).

2. In the biohazard cabinet, aliquot the required amount of 1x Wash Buffer into a 2 or 5 mL tube.

3. Label with **"Elution Buffer"**, initials and date.

4.2 Equipment

Table 2 outlines the equipment and their locations required for Manual DNA IQ™ extraction.

Table 2: Location of required equipment

Equipment	Location
Fridge	3189
Freezers	3189 / 3190
Biological safety cabinet class II x 4	3188 x 1, 3189 x 4
96 well tube racks	3189
Vortex x 4	3189
Hot block x 4	3189 / 3191 / 3194
Centrifuge x 4	3189 / 3194
Mini/Micro centrifuges x 4	3189
Finnpipettes 100 – 1000 µL	3189
Finnpipette 20 – 200 µL	3189
Thermomixer x 4	3189
Magnetic rack	3189
Multitube Shaker	3191

4.3 Consumables

Table 3 outlines the consumables and their location required for Manual DNA IQ™ extraction.

Table 3: Location of required consumables

Consumables	Location
Racks	3189
Spin baskets and 2mL collection tubes	3189
1.5mL and/or 2 mL tubes	3188 / 3189
Nunc™ tubes	3189
Nunc™ caps	3189 / 3194
5 mL, 10 mL and 50 mL sterile tubes	3188
Sharps bins	3189
20 µL pipette tips	3189
300 µL pipette tips	3188 / 3189
1000 µL pipette tips	3188 / 3189
Inoculation loops	3189

Additional consumables can be found in the Store Room (3184).

4.4 Entering Reagents, Equipment, Consumables and Locations into FR

1. Access the batch according to the Forensic DNA Analysis Workflow Procedure (34034).
2. Click the **Edit/Update Batch** icon.
3. Using the relevant dropdown menu enter consumables and reagents.
4. Scan the equipment, location and reagents (Figure 1).
5. Click the **Save Batch** icon.

Note: Fields should be filled out contemporaneously while processing the batch. These steps can be performed at any stage prior to batch completion, and entries can be modified after saving.

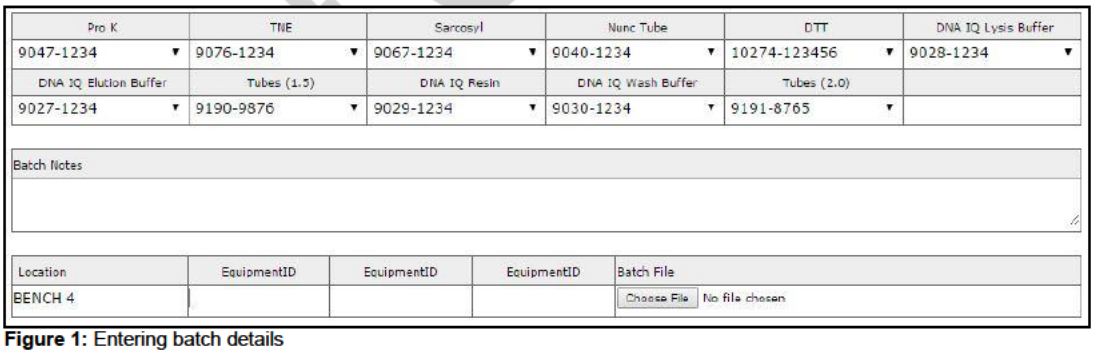

Figure 1: Entering batch details

5 Safety

As per the Anti-Contamination Procedure (22857), PPE is to be worn by all staff when performing this procedure. This includes the use of safety glasses.

Work benches and non-metallic equipment should be wiped with 0.5 % v/v bleach, followed by 70 % v/v ethanol. Metallic surfaces such as biological safety cabinets are to be wiped with 5% v/v Trigene **only**, followed by 70% v/v ethanol.

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 on to PPE, (e.g. gloves, gowns) remove and discard the contaminated item/s in a biohazard bin.

6 Sample Location and Batch Preparation

6.1 Electronic Workflow Diary

Within the electronic workflow diary (I:\AAA Electronic Workflow Diary) record details of the batch sample retrieval and processing.

6.2 Batch Creation

Create extraction batches according to the Forensic DNA Analysis Workflow Procedure (34034).

6.3 QC Samples

One negative control and one positive control will be automatically registered by the FR when creating Manual DNA IQ™ batches according to the Forensic DNA Analysis Workflow Procedure (34034).

6.4 Sample Location and Locating Samples

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

Table 4: Sample storage locations

Sample type	Storage Device	Storage Location
Urgent/High/Medium Priority Samples	Freezer	3190 or Block 6 wa k-in freezer*

* Some storage boxes containing samples are located in Block 6, within the Exhibit Room (6106).

Locate samples according to Forensic DNA Analysis Workflow Procedure (34034).

6.5 Analytical Notes

1. Access the batch according to the Forensic DNA Analysis Workflow Procedure (34034).

2. On the batch **Exhibit Analysis** page, if any samples are coloured half orange, hover the cursor over it to check for analytical notes that request for specific processing comments (e.g. Extract and hold) (Figure 2).

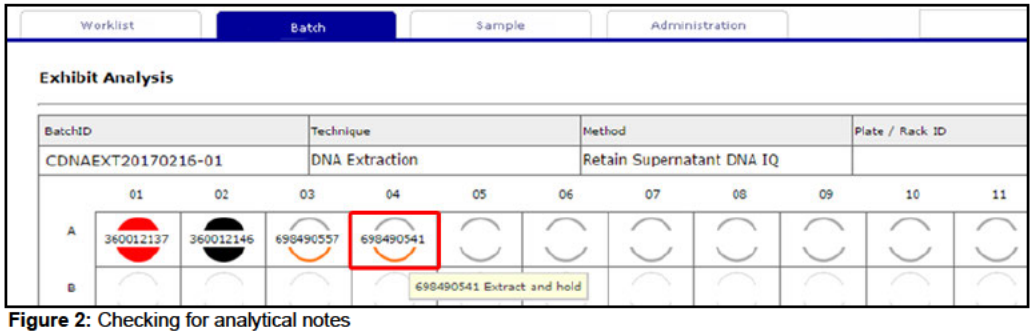

Figure 2: Checking for analytical notes

6.6 Creating and Printing Sample Labels

1. Access the batch according to the Forensic DNA Analysis Workflow Procedure (34034).
2. Click the **Subsamples** icon to create additional barcodes for samples and positive extraction controls requiring a spin basket label (substrates that need to be stored permanently in spin basket storage & substrates that need to be retained for 1 month in discard storage). Refer to Table 5 for subsample labels required for each type of extraction batch.

Note: Substrates that require a spin basket include absorbant substrates such as swabs and fabric. Substrates that do not require a spin basket are: tapelifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles. Small absorbant substrates such as paper and cigarette butt papers can be spun in spin baskets at the operators discretion.

Table 5: Subsample labels required for Manual DNA IQ™ batches

Method	SPIN	SUPNAT	EFRAC	SLIDE	DILN
Manual IQ	Yes	No	No	No	No
Retain Supernatant	Yes	Yes	No	No	No

3. For samples not requiring substrate storage (e.g. negative controls or hair), uncheck the corresponding **SPIN** box (Figure3).
4. For positive controls not requiring supernatant storage uncheck the corresponding **SUPNAT** box (this is only for retain supernatant batches).
5. Click the **Create Subsamples** icon (Figure 3).

Note: Once subsamples have been created and saved, this option is no longer available for the batch.

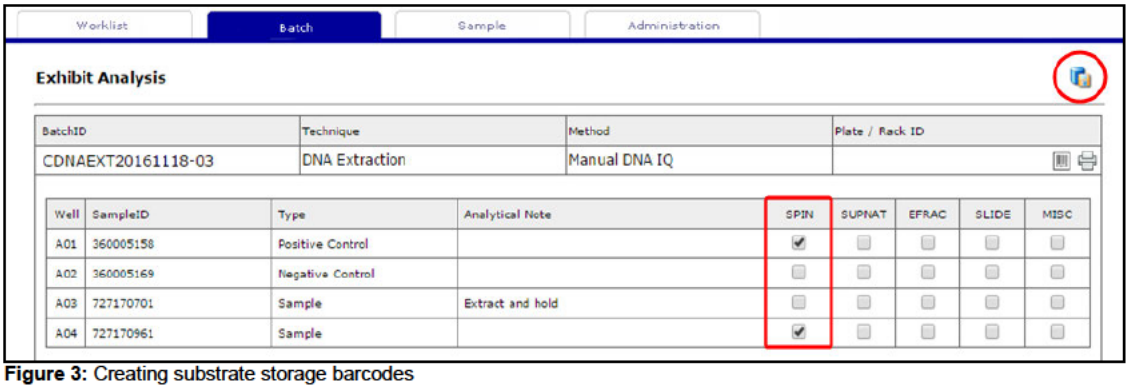

Figure 3: Creating substrate storage barcodes

6. Click the **Batch Labels** icon to display the sample labels. Print the labels to the designated label printer.

6.7 Sequence Checking

1. Perform a sequence check of all tubes for the batch by clicking the **Sequence Check & Lock** icon.
2. Scan all tubes in the order they are positioned in the rack corresponding with the FR plate map. The parent barcode must be the first barcode scanned.

Note: If a barcode is scanned incorrectly during the sequence check an error message will appear above the virtual rack. Check the position the error message indicates and rescan with the correct barcode.

3. To complete the sequence check, check the **Confirm Sequence Check** box. (Figure 4). The **Confirm Sequence Check** box should not be checked if there are any errors showing on the sequence check screen.

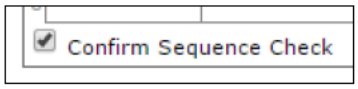

Figure 4: Confirm Sequence Check box

4. Click the **Lock Batch** icon.

7 Procedure

7.1 Manual DNA IQ™ (No Retain Supernatant)

1. In the Clean Reagent Room (3188) prepare/aliquot Extraction Buffer, Lysis Buffer-DTT, Resin, Wash Buffer and Elution Buffer. Refer to Section 4.1 for preparation of reagents. Use Appendix 14.1 for reagent volumes.
2. All extractions are to be performed within a biological safety cabinet in room 3189.
3. Turn on the Thermomixer and set to 37˚C and 1400 rpm.
4. Check for Analytical notes as per Section 6.5 and action as required.
5. Print labels required as per Section 6.6
6. For each sample label::
    - Original sample tube (use **parent** barcode if a control, use **spin** if used for substrate storage)
    - Spin basket collection tube assembly as required (**parent** barcode)
    - 1.5 ml or 2.0 mL tube (**spin** barcode)
    - Nunc™ tube (**parent** barcode)

Note: If the substrate is to be stored in the original tube post extraction, ensure that the **spin** label is applied over the barcode of the original tube making sure the original tube label number is still visible.

7. Ensure a sequence check is performed as per Section 6.7 prior to continuing.

8. Add 300 µL of Extraction Buffer to each sample. Ensure that large substrates including tape-lifts are fully submerged.

9. Vortex and 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.

10. Remove from the Thermomixer and increase the temperature on the Thermomixer to 65 °C for use in the elution steps.

11. Transfer substrates requiring a spin basket to the spin basket assembly.

12. Centrifuge the spin basket at 15800 rcf for 2 minutes.

13. For samples not requiring a spin basket transfer the lysate to a new and appropriately labelled 2.0 mL tube. Retain the original tube containing the substrate if no spin basket is used.

14. 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.

15. Transfer the substrate from the spin basket to a 1.5mL or 2mL tube labelled with a **SPIN** label and retain.

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.

16. Add 550 µL of Lysis Buffer-DTT solution to the lysate.

17. 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.

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 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.

21. Add 125 µL of Lysis Buffer-DTT solution. Vortex and place into the magnetic stand.

22. Once separation has occurred, remove and discard the liquid. Remove the tube from the magnetic stand.

23. 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.

24. 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.

25. 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.

26. Add 50 µl 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 is available, incubate samples within a hot block and vortex mix at the start and end of the second 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™ tube.

30. Remove from the magnetic stand and repeat steps 22-25 once. The final volume after the second elution is approximately 95 µL of DNA extract.

31. Store the DNA extracts in temporary storage located within the Pre-PCR sorting room 3194-A in the upright freezer as per Storage Guidelines for Forensic DNA Analysis (23959).

32. Store tubes containing substrates in spin basket or discard boxes located within the Pre-PCR sorting room in the upright freezer (Room 3194 A) as per Storage Guidelines for Forensic DNA Analysis (23959).

7.2 Manual DNA IQ™ Retain Supernatant

1. In the Clean Reagent Room (3188) prepare/aliquot TNE, Pro K, Sarcosyl, Lysis Buffer-DTT, Resin, Wash Buffer and Elution Buffer. Refer to Section 4.1 for preparation of reagents. Use Appendix 14.2 for reagent volumes.

2. All extractions are to be performed within a biological safety cabinet in room 3189.

3. Turn on the Thermomixer and set to 37˚C and 1400 rpm.

4. Check for Analytical notes as per Section 6.5 and action as required.

5. Print labels required as per Section 6.6.

6. Label for each sample:
   - Original sample tube (use **parent** barcode if a control, use **spin** barcode if used for substrate storage)

- 2.0 mL tube for sample transfer and processing (**parent** barcode)
- 1.5 mL tube (**supnat** barcode)
- Spin basket assembly if required (use **parent** barcode)
- Nunc™ tube (**parent** barcode)

7. Ensure a sequence check is performed as per Section 6.7 prior to continuing.

8. Add 450 µL of TNE buffer to the sample and vortex.

9. Incubate at room temperature for 30 minutes.

10. Vortex, then centrifuge at 15800 rcf for 3 minutes.

11. Remove 150 µL of supernatant and place into the respective 1.5 mL tube labelled with **SUPNAT** (for further testing).

12. Add 14 µL of 20 mg/mL Proteinase K and 7 µL 40 % (w/v) Sarcosyl to the original sample tube containing substrate and TNE buffer, then vortex.

13. 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.

14. Remove from the Thermomixer and increase the temperature on the Thermomixer to 65 °C (for use in the Elution steps).

15. Transfer substrates requiring a spin basket to the spin basket assembly.

16. Centrifuge the spin basket at 15800 rcf for 2 minutes.

17. For samples not requiring a spin basket transfer the lysate to a new and appropriately labelled 2.0 mL tube. Retain the original tube containing the substrate if no spin basket is used.

18. 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.

19. Transfer the substrate from the spin basket to a 1.5mL or 2mL tube labelled with a **SPIN** label and retain.

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.

20. Add 550 µL of Lysis-DTT Buffer solution.

21. Add 50 µL of DNA IQ™ Resin-Lysis solution. Pipette mix the Resin-Lysis solution between each addition to keep the resin suspended in solution.

22. Vortex and place on the Multitube shaker set at 1200 rpm for 5 minutes.

23. 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.

24. 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.

25. Add 125 µL of Lysis-DTT Buffer solution. Vortex and place into the magnetic stand.

26. Once separation has occurred, remove and discard the Lysis-DTT Buffer. Remove from the magnetic stand.

27. Add 100 µL 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.

28. Repeat the Wash Buffer step (step 22) 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.

29. 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.

30. Add 50 µl of Elution Buffer by carefully pipetting the solution to the side of the tube, above the pellet. **Do not mix**.

31. 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.

32. Vortex and immediately place into the magnetic stand while hot to ensure maximum DNA yield during elution.

33. Carefully transfer the DNA extract to the corresponding labelled Nunc™ tube.

34. Remove from the magnetic stand and repeat the Elution Buffer steps (step 25-28). The final volume after the double elution is approximately 95 µL of DNA extract.

35. Store the DNA extracts in temporary storage located within the Pre-PCR sorting room in the upright freezer as per Storage Guidelines for Forensic DNA Analysis (23959).

36. Store tubes containing substrates in Spin Basket boxes located within the Pre-PCR sorting room in the upright as per Storage Guidelines for Forensic DNA Analysis (23959).

37. Store supernatants in the S/N Retention boxes located within the Extraction sorting room freezer as per Storage Guidelines for Forensic DNA Analysis (23959).

8 Batch Finalisation

1. Ensure all reagents, equipment, consumables and locations are selected against the batch as per Section 4.4

2. Click the **Sample Transition** icon and select the appropriate technique and method from the dropdown menus (Figure 5 and Figure 6 for retain supernatant). If samples have an **Extract & Hold** or **NWQPS** analytical note, the **Technique** and **Method** fields need to be left blank.

3. Click the **Complete Batch** icon.

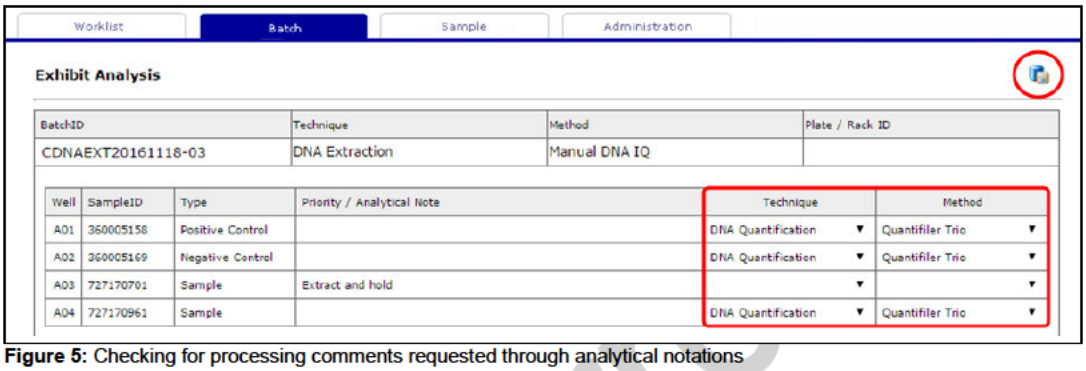

Figure 5: Checking for processing comments requested through analytical notations

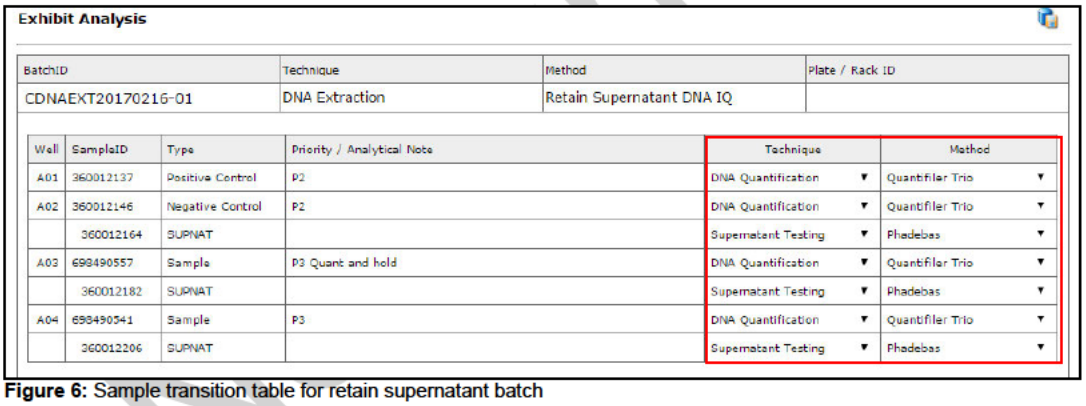

Figure 6: Sample transition table for retain supernatant batch

4. Click the **Edit/Update Batch** icon.

5. Complete the **Run Date & Run Time** fields (Figure 7) (manually enter date and time, or select the current date from the calender which autofills the time field upon batch completion).

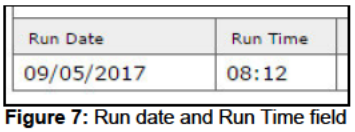

Figure 7: Run date and Run Time field

6. Click the **Complete Batch** icon to complete the batch.

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.

10 Quality Assurance/Acceptance Criteria

Positive and negative controls are included in each extraction batch and are processed as normal samples through to completion.
If any results are obtained from the negative control, 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 Capillary Electrophoresis (CEQ) Quality Check (34131) and Miscellaneous Analytical Section Tasks (34064).

11 References

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12 Associated Documents

QIS 22857 Anti-Contamination procedure
QIS 23959 Storage Guidelines for Forensic DNA Analysis
QIS 34034 Forensic DNA Analysis Workflow Procedure
QIS 34064 Miscellaneous Analytical Section Tasks
QIS 34103 Receipt, Storage and Preparation of Chemicals, Reagents and Test Kits

13 Amendment History

Version	Date	Author/s	Amendments
1	26 April 2016	Adam Kaity, Megan Mathieson, Lisa Farrelly	Remove all references to off-deck lysis procedure and automated IQ extraction. Changeover from AUSLAB to Forensic Register – update FR related sections.

14 Appendices

14.1 Reagent Volumes for Manual DNA IQ™ Extractions

14.1 Reagent Volumes for Manual DNA IQ™ Exatrctions

Reagent Volumes for Manual DNA IQ™ Extraction

Table 6: Reagent volumes required for manual extraction (no retain supernatant)

	Reagent	Volume for 12 samples (µL)	Volume for 24 samples (µL)
Extraction Buffer	TNE buffer	4000	8000
	Proteinase K (20 mg/mL)	216	432
	Sarcosyl (40 %)	108	216
Lysis Buffer-DTT	DNA IQ™ Lysis Buffer	10000	20000
	DTT	100	200
Resin	Lysis Buffer-DTT	645	1290
	DNA IQ™ Resin	105	210
Wash Buffer	DNA IQ™ 1x Wash Buffer	4000	8000
Elution Buffer	DNA IQ™ Elution Buffer	1400	2800

Note: The volume of Lysis Buffer-DTT calculated includes the volume used in the Resin-Lysis solution preparation

Reagent Volumes for Retain Supernatant Manual DNA IQ™ Extraction

Table 7: Reagent volumes required for Retain Supernatant manual extraction

	Reagent	Volume per sample (µL)	Volume for 24 samples (µL)
TNE	TNE buffer	450	4000
Pro K	Proteinase K (20 mg/mL)	14	216
Sarcosyl	Sarcosyl (40 %)	7	108
Lysis Buffer-DTT	DNA IQ™ Lysis Buffer	880	10000
	DTT	8.8	100
Resin	Lysis Buffer-DTT	43	645
	DNA IQ™ Resin	7	105
Wash Buffer	DNA IQ™ 1x Wash Buffer	300	4000
Elution Buffer	DNA IQ™ Elution Buffer	100	1400

Note: The volume of Lysis Buffer-DTT 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.

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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

CaSS | Forensic and Scientific Services
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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 inhibitors used in the inh bition 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.

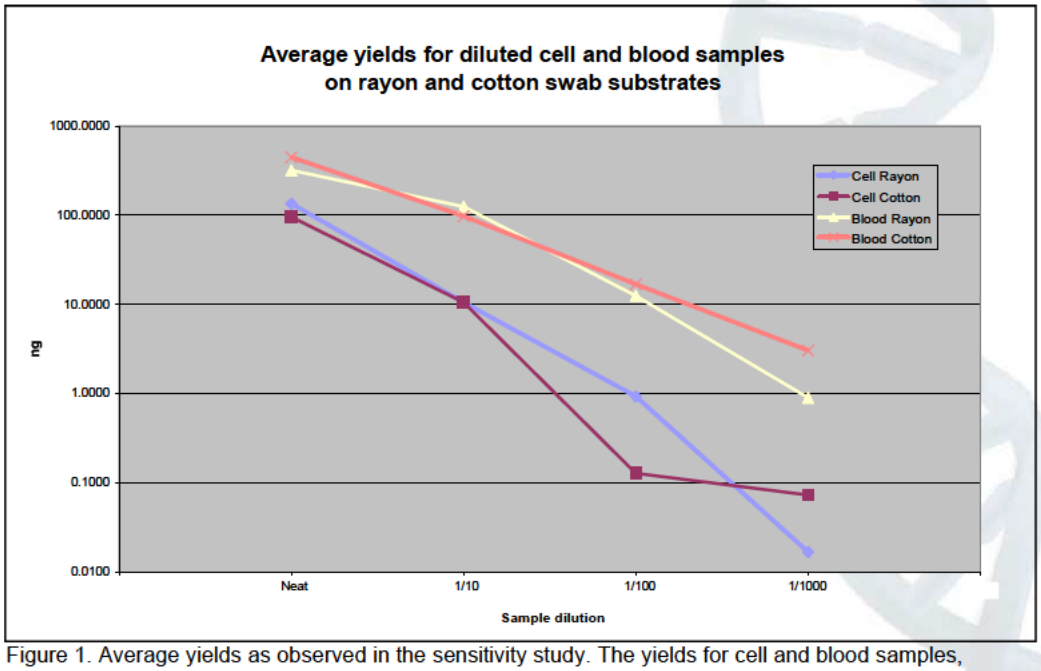

Figure 1. Average yields as observed in the sensitivity study. The yields for cell and blood samples, on two different swab types, were comparable as indicated by overlapping lines on the graph.

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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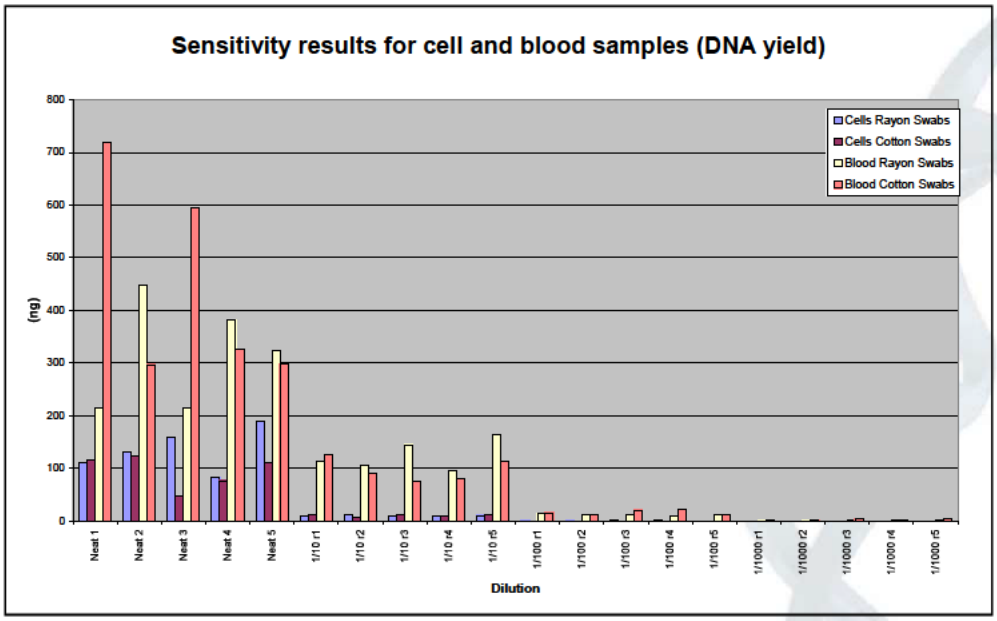

Figure 2. DNA yields (ng) observed for the sensitivity study. As expected, neat samples provided the highest yields. Yields were obtained down to 1/1000 for blood samples and 1/100 for cell samples.

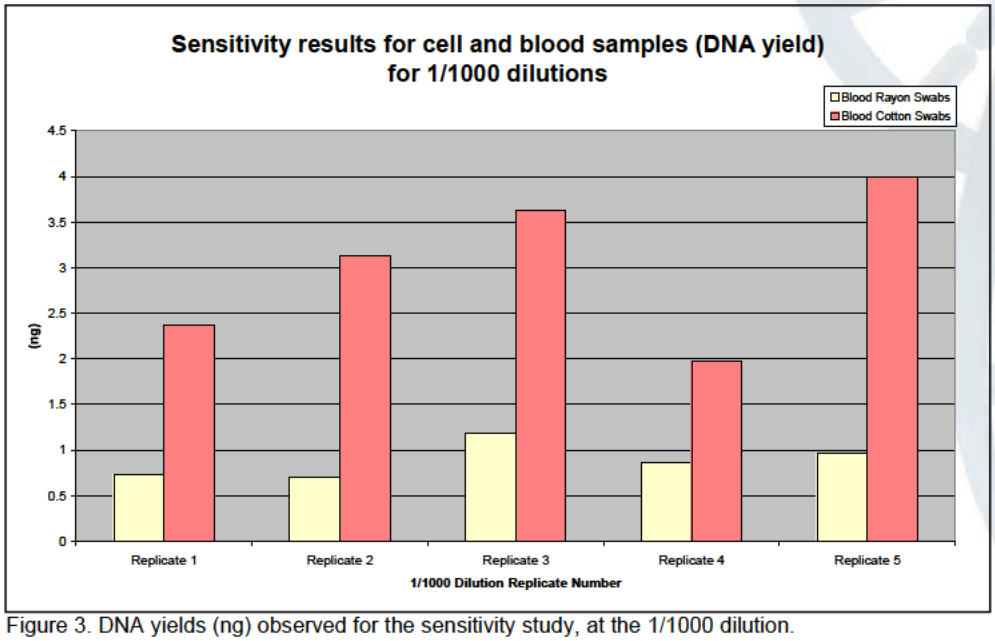

Figure 3. DNA yields (ng) observed for the sensitivity study, at the 1/1000 dilution.

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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.

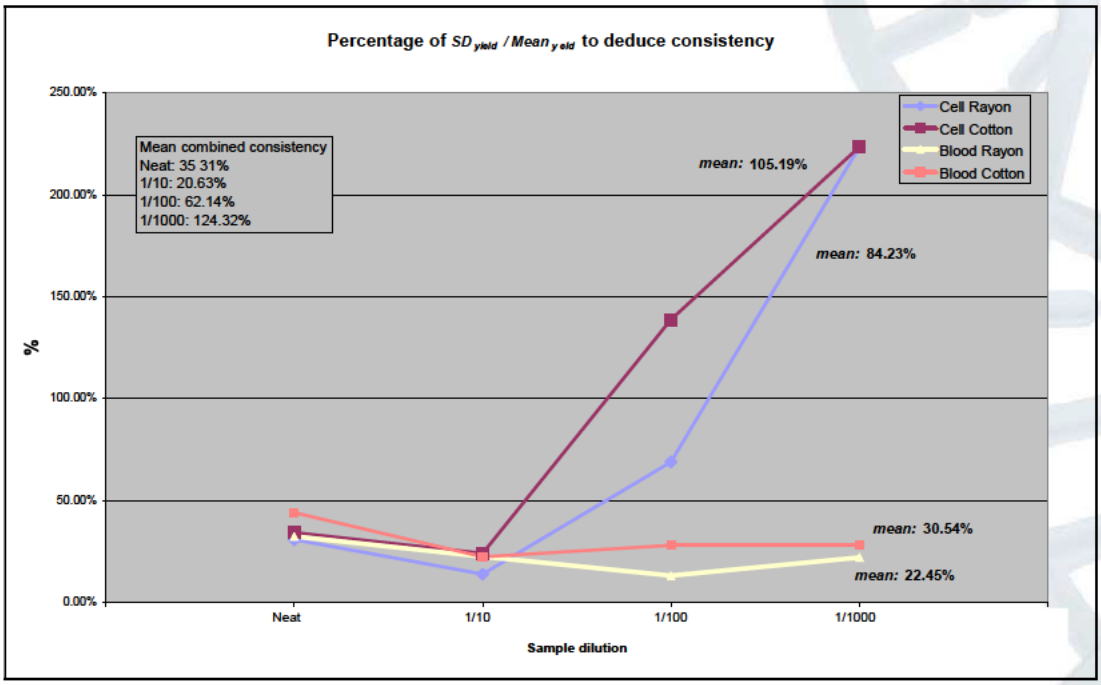

Figure 4. Reproducibility between replicates for cell and blood samples diluted down to 1/1000.

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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.

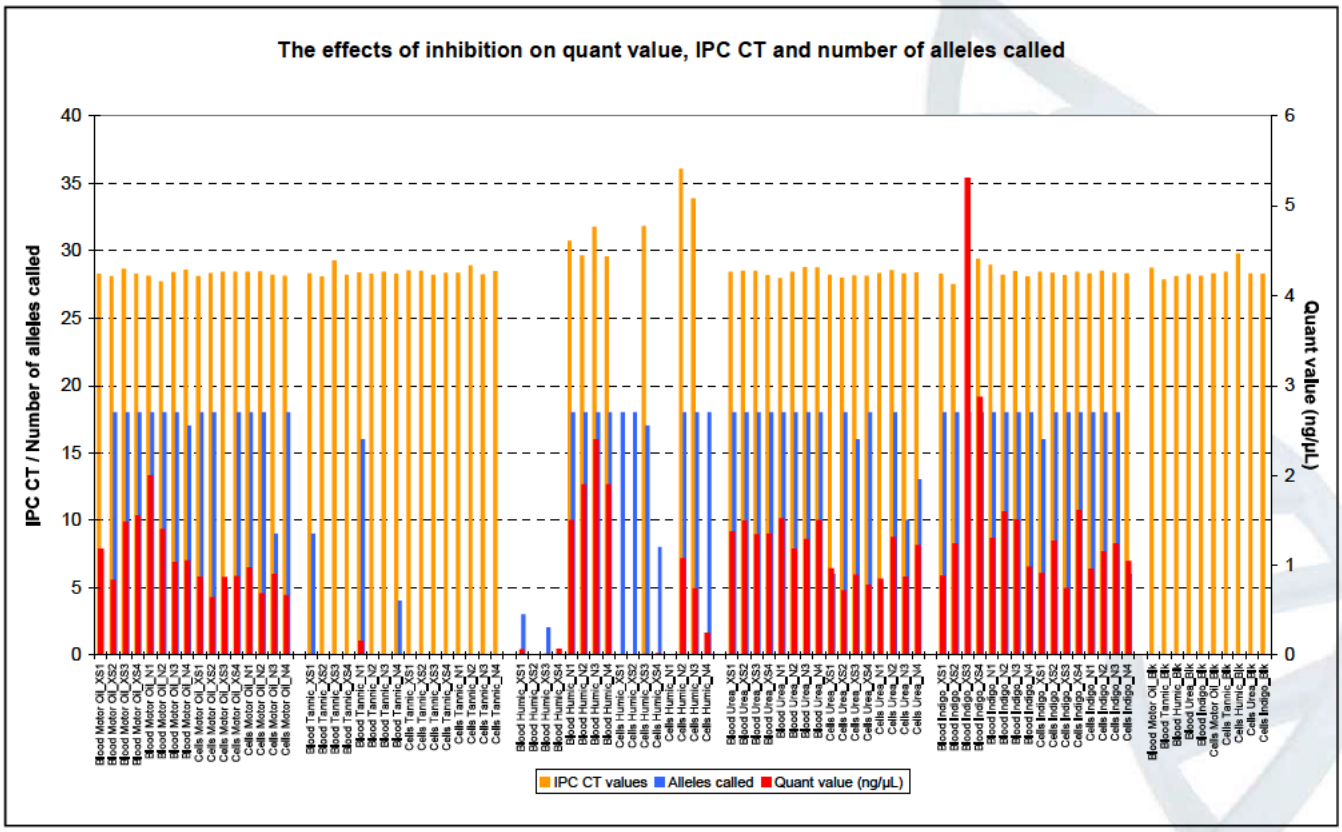

Figure 5. Effects of various inhibitors on quant value, IPC CT and number of resolved alleles.

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.

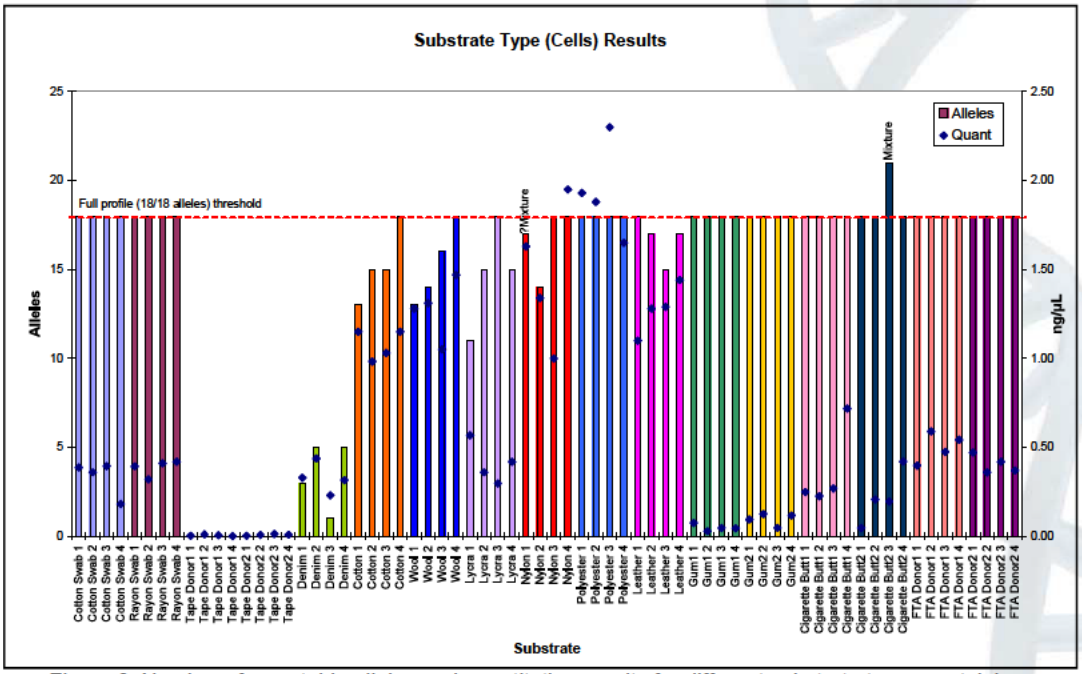

Figure 6. Number of reportable alleles and quantitation results for different substrate types containing cellular material.

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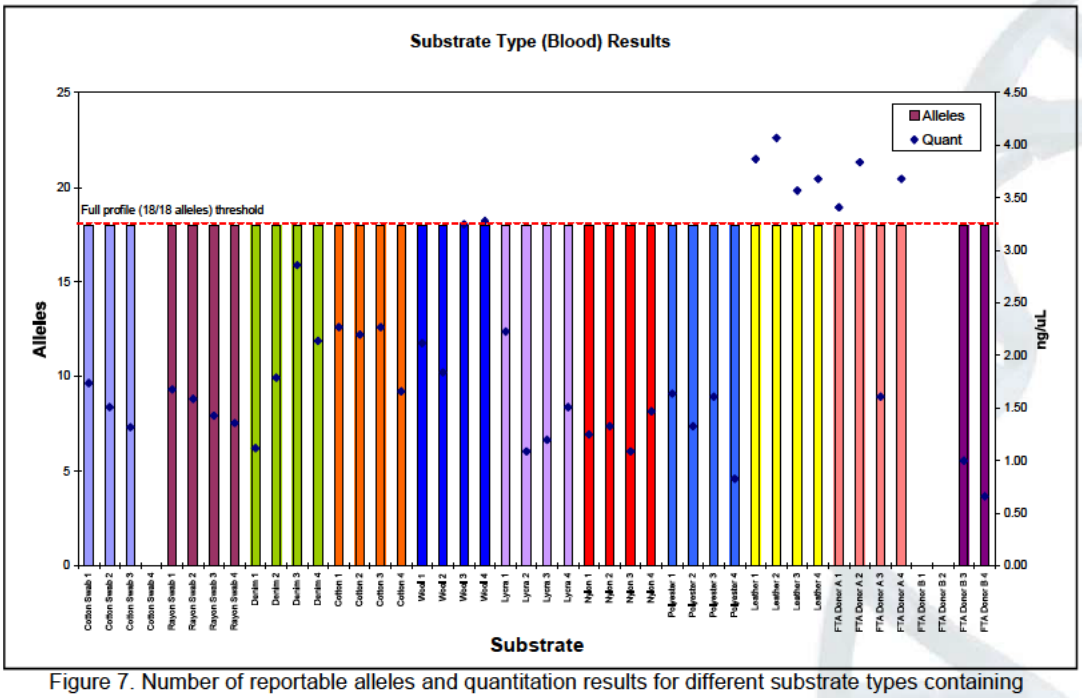

Figure 7. Number of reportable alleles and quantitation results for different substrate types containing blood material.

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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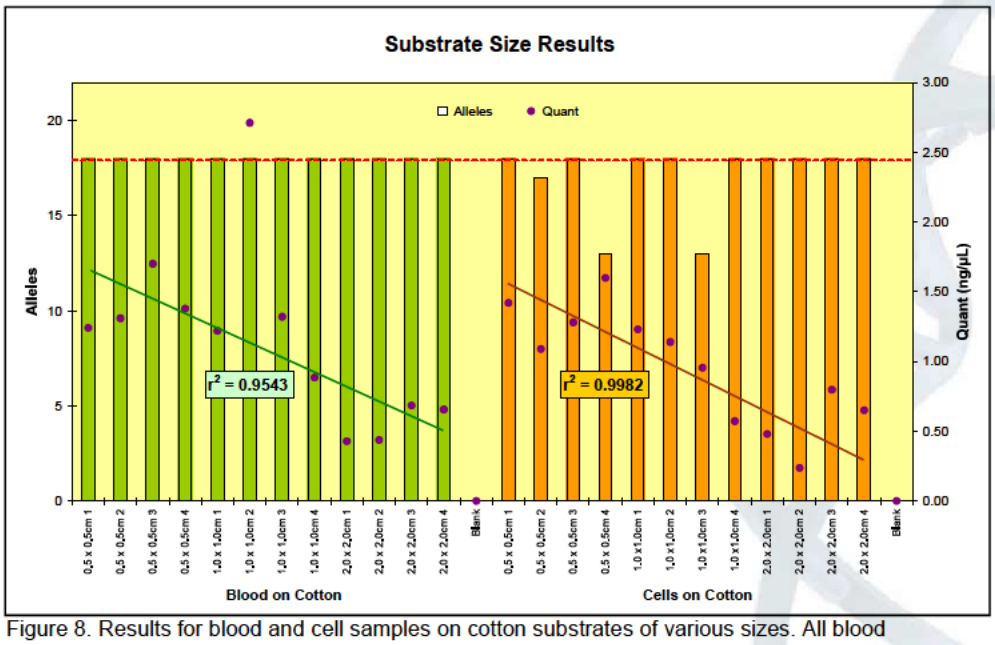

Figure 8. Results for blood and cell samples on cotton substrates of various sizes. All blood samples generated full profiles, but cell samples were more variable. The quantitation results for 0.5 x 0.5cm samples were higher than those for 2.0 x 2.0cm samples (blood r^2 = 0.9543*; cell r^2 = 0.9982;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

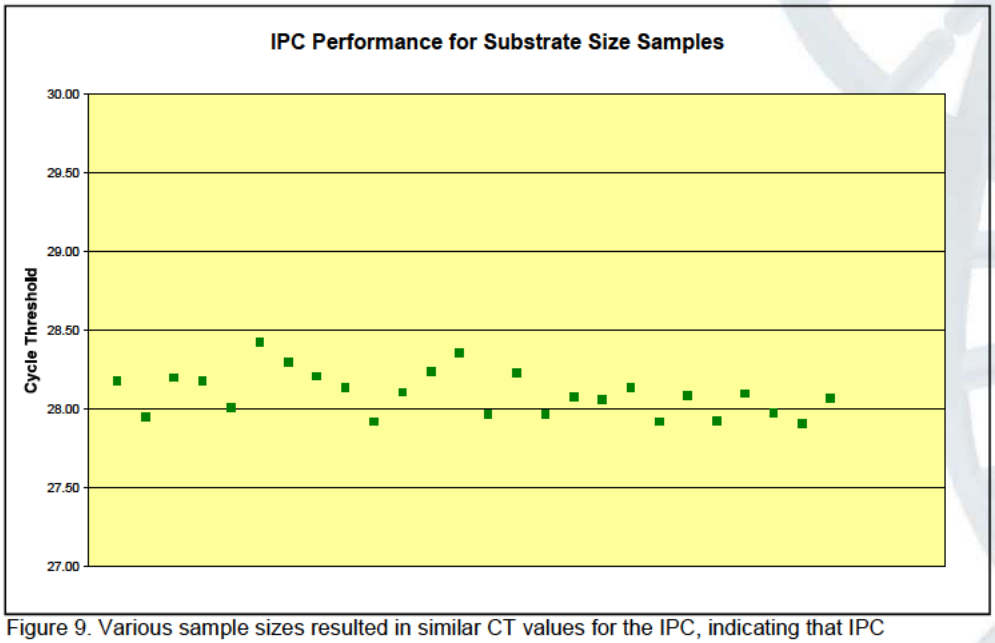

Figure 9. Various sample sizes resulted in similar CT values for the IPC, indicating that IPC performance is not affected by sample size, and that one sample size does not display a level of inhibition that is different to another sample size.

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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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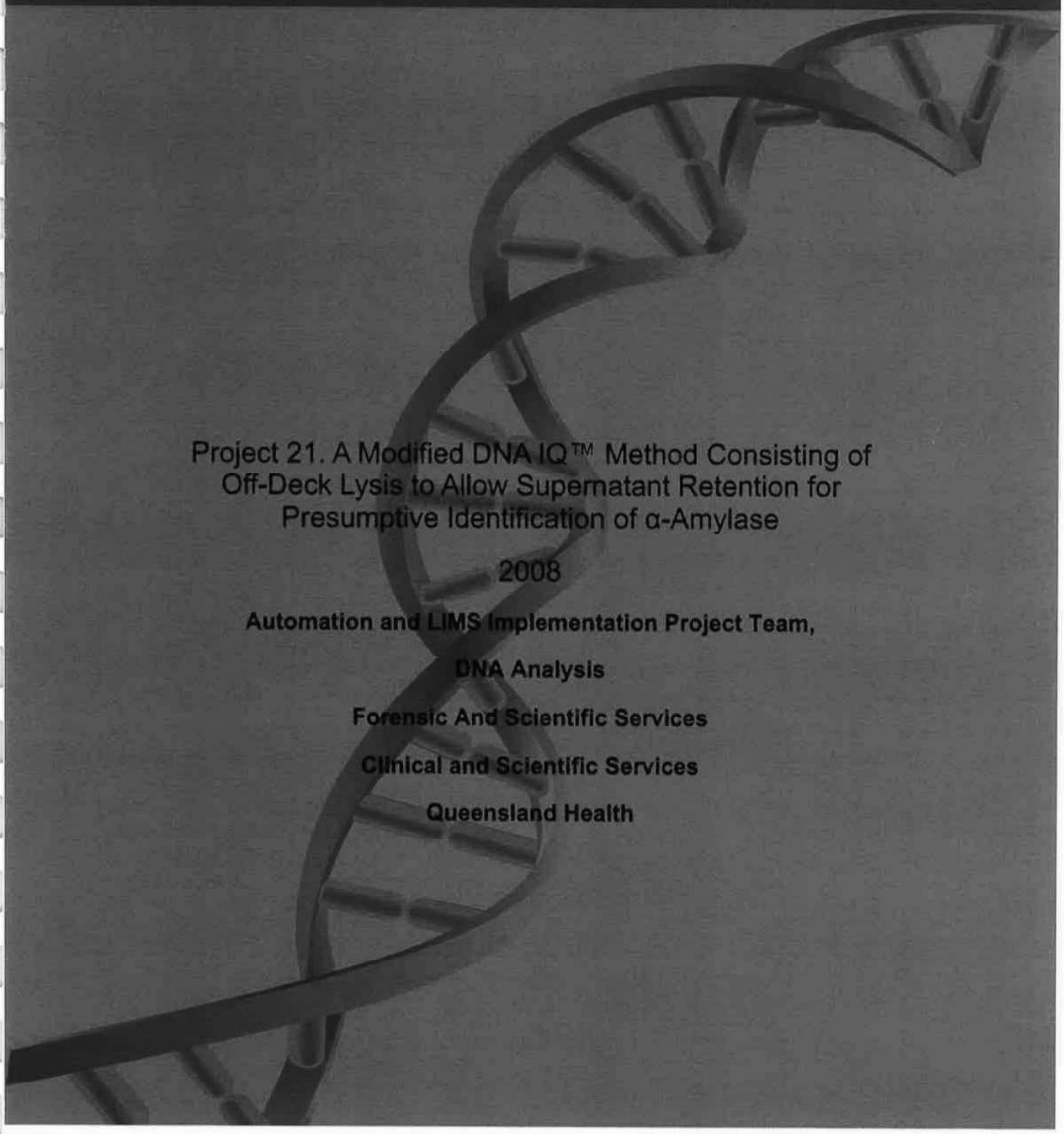

Project 21. A Modified DNA IQ™ Method Consisting of Off-Deck Lysis to Allow Supernatant Retention for Presumptive Identification of α-Amylase

2008

Automation and LIMS Implementation Project Team,

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Project 21. A Modified DNA IQ™ Method Consisting of Off-Deck Lysis to Allow Supernatant Retention for Presumptive Identification of α-Amylase

Iman Muharam, Vojtech Hlinka, Breanna Gallagher, Generosa Lundie, Cecilia Iannuzzi, Thomas Nurthen, Allan McNevin, Vanessa Ientile
Automation/LIMS Implementation Project, DNA Analysis FSS (February 2008)

1. Abstract

The current in-house DNA IQ™ method for extracting forensic DNA samples of different types in the Slicprep™-96 Device does not allow for some sample types to be processed, e.g. fluffy swab heads or materials with static attraction. Furthermore, sample preparation for the Slicprep™ 96 Device is labour intensive and time consuming. In addition, the current DNA IQ™ method does not allow for samples where presumptive testing on the supernatant is required, such as for α-amylase testing.

We have investigated a modified DNA IQ™ protocol that incorporates off-deck lysis of forensic samples in 1.5mL tubes prior to automated extraction on the MultiPROBE® II PLUS HT EX platform. The off-deck lysis method allows for an increase in the range of sample types that can be processed using the automated DNA IQ™ method. The off-deck lysis method also incorporates the option to retain supernatant for use in presumptive identification procedures.

DNA samples where supernatant was or was not retained for presumptive testing generated comparable results. Importantly, the retained supernatant could be used to perform presumptive testing for α-amylase and produced the expected presumptive and DNA profile results.

We recommend the use of a modified DNA IQ™ method, incorporating an off-deck lysis protocol, to increase the range of sample types that can be extracted using the automated DNA IQ™ protocol, and to allow supernatant retention for presumptive testing.

2. Aim

- To investigate an off-deck lysis method that is compatible with the in-house automated DNA IQ™ protocol.
- To investigate the option of retaining supernatant to allow α-amylase presumptive testing, without compromising the ability to obtain DNA profile results from the same DNA extract.

3. Equipment and Materials

- DNA IQ™ System (Promega Corp., Madison, WI, USA)
- DNA IQ™ Spin Baskets (Promega Corp., Madison, WI, USA)
- TNE buffer (10mM Tris, 100mM NaCl, 1mM EDTA, pH 8.0)
- Proteinase K (20ng/μL)
- 20% w/v SDS
- Rayon swabs (Copan)

- Eppendorf 5415C centrifuge

4. Methods

4.1 Sample collection

Two donors were selected to provide saliva samples on swabs. The specific donors were selected based on the reactivity of their saliva to the presumptive α-amylase test using Phadebas: one donor was known to generate strong positive readings, while the other donor was known to generate weak positive readings.

A total of eight swabs were taken from each donor, collected over 2 days (4 swabs per person, per day, with two swabs collected from the left check and two from the right cheek).

Two samples each of negative controls, QC cells and QC blood sample types were also included in the test for quality control purposes.

All samples were split equally into two batches, to be processed under slightly different methods, designated Method 1 and Method 2.

4.2 Method 1: Off-deck lysis followed by automated DNA IQ™

All samples were placed in sterile 1.5mL tubes, and 500µL of TNE buffer was aliquoted into each tube and vortexed gently. To each sample, 25µL of 20ng/µL Proteinase K and 12.5µL 20% w/v SDS was added and vortexed briefly, before incubating at 37°C on a Thermomixer (Eppendorf) at 1000 rpm for 45 minutes. The sample substrate material was transferred to a DNA IQ™ Spin Basket and centrifuged for 2 minutes at room temperature at maximum speed (15800g). The centrifuged lysate, and the lysate in the original tube, were transferred and combined into a fresh 1.5mL tube. The samples were then incubated at 65°C on a Thermomixer (Eppendorf) at 1100 rpm for 10 minutes. After incubation, the lysate was added into a Slicprep™ 96 Device (without basket) using the STORstar instrument. Automated DNA IQ™ was then performed (without the automated addition of Extraction Buffer).

4.3 Method 2: Off-deck lysis with supernatant retention, followed by automated DNA IQ™

650µL of TNE buffer was aliquoted into each tube and vortexed gently. The sample was allowed to incubate at room temperature for 30 minutes, prior to vortexing and centrifuging at maximum speed for 3 minutes (15800g). From this tube, 150µL of supernatant was transferred to a fresh sterile 1.5mL tube and stored at -20°C to be used for presumptive testing. To each sample, 25µL of 20ng/µL Proteinase K and 12.5µL 20% w/v SDS was added and vortexed briefly, before incubating at 37°C on a Thermomixer (Eppendorf) at 1000 rpm for 45 minutes. The remainder of the off-deck lysis protocol was performed on all samples as per Method 1 above.

4.4 α-Amylase presumptive screening

Presumptive screening for the presence of α-amylase was performed as per QIS 17193.

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4.5 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.

4.6 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.

4.7 Capillary electrophoresis and fragment analysis

PCR product was prepared for capillary electrophoresis using the manual 9+1 protocol as per 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%.

5. Results and Discussion

The differences between the two off-deck lysis methods that were assessed are outlined in Table 1 below.

Table 1. Differences between two off-deck lysis methods.

	Method 1: Off-deck lysis	**Method 2: Off-deck lysis with supernatant retention**
Sample format	In 1.5mL tube	In 1.5mL tube
Volume of TNE buffer added to sample	500µL	650µL
Incubation at room temperature		30 minutes
Supernatant transfer		150µL
Proteinase K	25µL (20ng/µL)	25µL (20ng/µL)
20% w/v SDS	12.5µL	12.5µL
Sample lysis 37°C	37°C, 45 minutes at 1100 rpm	37°C, 45 minutes at 1100 rpm
Substrate transfer	DNA IQ™ Spin Basket, all lysate transferred to fresh 1.5mL tube	DNA IQ™ Spin Basket, all lysate transferred to fresh 1.5mL tube
Inactivate Proteinase K	65°C, 10 minutes at 1100 rpm	65°C, 10 minutes at 1100 rpm
Automated protocol	Transfer lysate to Slicprep™ using STORstar, then present to MP II	Transfer lysate to Slicprep™ using STORstar, then present to MP II

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The primary difference between both methods is the addition of 150µL extra TNE buffer in Method 2 that allows 150µL of supernatant to be retained. The sample is incubated at room temperature for 30 minutes with the additional TNE buffer, which is then transferred to a fresh tube for use in presumptive testing procedures. The remainder of the Method 2 protocol from this point onwards is identical to Method 1 in order to achieve off-deck lysis, with identical lysis volumes for both methods of 500µL. The off-deck lysis protocol differs from the automated DNA IQ™ lysis protocol in the following aspects:

- Lysis at 37°C is performed on a Thermomixer at 1100 rpm, instead of the DPC shaker with heater tiles controlled by the automated heater controller.
- Sample substrates in each individual tube are separated from the lysate using the DNA IQ™ Spin Basket, instead of using the Slicprep™ basket with collar attached.
- Proteinase K is inactivated at 65°C on a Thermomixer at 1100 rpm, instead of the DPC shaker.

Quantitation results (ng/µL) for Method 2, where the supernatant was retained, was comparable to the results for Method 1, where supernatant was not retained (Table 2). Importantly, α-amylase presumptive screening was able to be performed on the retained supernatant and generated the expected results (Table 2).

Table 2. Quantitation (ng/µL) and α-amylase presumptive testing results for samples extracted using Method 1 (off-deck lysis) and Method 2 (off-deck lysis with retained supernatant option).

Sample	Method 1 Q'filer	Method 2 Q'filer	Phadebas Test	Saliva kit 5 min	Saliva kit 10 min
NegCtl	0.0000	0.0000	Negative	Neg	Neg
QC Cells	0.1030	0.0582	Negative	Neg	Weak
QC Blood	0.0700	0.0991	Negative	Neg	Neg
Donor 1 Right Cheek Day 1†	0.9190	1.2600	3+	V. Strong	V. Strong
Donor 1 Right Cheek Day 2†	0.6990	1.7000	2+	V. Strong	V. Strong
Donor 1 Left Cheek Day 1†	1.9700	0.9350	1+	V. Strong	V. Strong
Donor 1 Left Cheek Day 2†	3.0600	1.7000	1+	V. Strong	V. Strong
Donor 2 Right Cheek Day 1‡	0.6860	1.4300	1+	V. Strong	V. Strong
Donor 2 Right Cheek Day 2‡	2.0300	1.4000	3+	V. Strong	V. Strong
Donor 2 Left Cheek Day 1‡	0.7290	1.9800	2+	V. Strong	V. Strong
Donor 2 Left Cheek Day 2‡	0.7630	1.7300	2+	V. Strong	V. Strong

†Donor 1: strong positive α-amylase
‡Donor 2: weak positive α-amylase

DNA profiles were obtained for all samples (Table 3). In general, full profiles were generated by all samples, but less allelic imbalance was observed in samples processed using Method 2.

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Table 3. DNA profile results for all samples processed using Method 1 and Method 2.

Sample	Method 1	Method 2
NegCtl	NSD	NSD
QC Cells	OK	PA*
QC Blood	AI @ D13 ~69%	OK
Donor 1 Right Cheek Day 1†	OK	OK
Donor 1 Right Cheek Day 2†	OK	OK
Donor 1 Left Cheek Day 1†	OK	OK
Donor 1 Left Cheek Day 2†	AI @ D18 ~63%	OK
Donor 2 Right Cheek Day 1‡	OK	OK
Donor 2 Right Cheek Day 2‡	AI @ D7 ~66%	OK
Donor 2 Left Cheek Day 1‡	OK	OK
Donor 2 Left Cheek Day 2‡	OK	OK

†Donor 1: strong positive α-amylase
‡Donor 2: weak positive α-amylase
*Resolved alleles were consistent with the expected profile

6. Conclusion and Recommendations

The processing of forensic samples using Method 2, where supernatant is retained, was satisfactory for both α-amylase testing and for obtaining quantitation and DNA profile results. A volume of 650µL of TNE buffer is recommended for addition to samples where supernatants are retained for testing.

7. Acknowledgements

Phadebas testing was performed by Rhys Parry, Rebecca Gregory and Kirsten Scott (DNA Analysis, FSS). Experiments were performed by the Automation/LIMS Implementation Project team, with the assistance of the Analytical Section.

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Managing Scientist
DNA Analysis
Forensic and Scientific Services
PO Box 594, Archerfield QLD Australia 4108

Or by telephone (07) 3274 9169

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Project 22. A Modified DNA IQ™ Method for Off-Deck Lysis Prior to Performing Automated DNA Extraction

Iman Muharam, Vojtech Hlinka, Breanna Gallagher, Generosa Lundie, Cecilia Iannuzzi, Thomas Nurthen, Allan McNevin, Vanessa Ientile
Automation/LIMS Implementation Project, DNA Analysis FSS (February 2008)

1. Abstract

Some forensic sample types that were submitted for DNA extraction using the current in-house automated DNA IQ™ protocol could not be efficiently processed due to varying sample sizes, substrate material type and the requirement for retaining supernatant for presumptive screening. An off-deck lysis protocol was investigated as an option to automated lysis on the MultiPROBE® II PLUS HT EX platform, and includes the option to retain supernatant for presumptive testing (see Project 21).

Further testing was performed to investigate the amount of time that samples can be stored in Extraction Buffer after off-deck lysis, prior to presenting the sample lysate to the MultiPROBE® II PLUS HT EX platforms. A comparison of off-deck lysis performed on the Eppendorf Thermomixer Comfort and Ratek hot blocks was also performed.

We found that samples where off-deck lysis was performed could be stored in the fridge for up to 4 days, or frozen, prior to automated DNA IQ™ extraction. Performing off-deck lysis on the hot blocks produced results that were comparable to samples that were incubated on the Thermomixer.

2. Aim

- To test the effect of storage in Extraction Buffer, in the fridge or freezer, for samples where off-deck lysis has been performed.
- To investigate any differences between performing off-deck lysis on the Eppendorf Thermomixer Comfort or Ratek hot blocks.

3. Equipment and Materials

- DNA IQ™ System (Promega Corp., Madison, WI, USA)
- DNA IQ™ Spin Baskets (Promega Corp., Madison, WI, USA)
- TNE buffer (10mM Tris, 100mM NaCl, 1mM EDTA, pH 8.0)
- Proteinase K (20ng/µL)
- 20% w/v SDS
- Thermomixer Comfort (Eppendorf)
- Hot blocks (Ratek)
- Eppendorf 5415C centrifuge
- Rayon swabs (Copan)
- Buccal cell suspension (donor CJA), collected as described previously
- Whole blood (donor VKI), collected as described previously

4. Methods

4.1 Off-deck lysis: storage of lysate prior to automated DNA IQ™ extraction

4.1.1. Sample creation for testing storage time

A total of 25 fresh cell swabs and 25 blood swabs were created. For cell samples, 10µL of donor buccal cell suspension was spotted on to pre-cut rayon swab heads in 1.5mL tubes. For blood samples, 30µL of donor whole blood was spotted onto pre-cut rayon swab heads in 1.5mL tubes. Both sample types were dried on a Thermomixer at 56°C for 2 hours.

In addition to the fresh samples, 25 blood QC swabs were included in the experiments.

4.1.2. Off-deck lysis protocol

Samples that were created for testing were divided equally into 5 sets for testing over 4 days. The off-deck lysis protocol for all samples was identical, except for the storage conditions and storage time for samples after off-deck lysis was performed (Table 1). 500µL of Extraction Buffer was added to each tube. For spiked samples, 30µL of 1M urea was added to each fresh blood sample on rayon swab. Each tube was vortexed, before incubating at 37°C on a Thermomixer Comfort (Eppendorf) at 1000 rpm for 45 minutes. The sample substrate material was transferred to a DNA IQ™ Spin Basket and centrifuged for 2 minutes at room temperature at maximum speed (15800g). The centrifuged lysate was combined with the lysate in the original 1.5mL tube. The samples were incubated at 65°C on a Thermomixer Comfort (Eppendorf) at 1100 rpm for 10 minutes. Samples were then either stored in the fridge or freezer (Table 1) until automated DNA IQ™ extraction was performed up to 4 days later.

Table 1. Storage conditions and storage time for all 5 sets of samples.

Day	Set Number	$T_{storage}$ (°C)	Days of storage prior to extraction
Day 1	Set 1	4	4
	Set 2	-20	4
Day 2	Set 3	4	3
Day 3	Set 4	4	2
Day 4	Set 5	4	1

4.2 Off-deck lysis: assessing differences between hot blocks and Thermomixer

4.2.1. Sample creation for hot block testing

A total of 15 samples each of blood on cotton swab and blood on rayon swab, and 10 of cells on rayon swab were created. Swab heads were cut

off the swab shafts and placed in sterile 1.5mL tubes. For blood samples, 10µL of donor whole blood was spotted on to small cotton swabs and 30µL was spotted on to rayon swabs. For cell samples, 10µL of donor cell suspension was spotted on to rayon swabs. Samples were dried on a hot block (Ratek) at 56°C for 2 hours.

4.2.2. Off-deck lysis protocol

Samples that were created for testing were divided equally into 3 sets, and processed using a similar off-deck lysis protocol, except for minor differences where a hot block (Ratek) or Thermomixer Comfort (Eppendorf) was used, and which vortexing routine was performed (see Table 2). 500µL of Extraction Buffer was added to each tube. Each tube was vortexed, before incubating at 37°C for 45 minutes either on a hot block (Ratek) or Thermomixer Comfort (Eppendorf) set at 1000 rpm. The sample substrate material was transferred to a DNA IQ™ Spin Basket and centrifuged for 2 minutes at room temperature at maximum speed (15800g). The centrifuged lysate was combined with the lysate in the original 1.5mL tube. The samples were then incubated at 65°C for 10 minutes either on a hot block (Ratek) or Thermomixer Comfort (Eppendorf) at 1100 rpm. Samples were stored in the freezer until automated DNA IQ™ extraction was performed.

Table 2. Incubation protocols for the 3 sets of samples.

Sample Set Number	Incubation protocol
Set 1	On hot block; vortex 5 sec before and 5 sec after incubation.
Set 2	On hot block; vortex 5 sec before and after incubation, and 5 sec during incubation.
Set 3	On Thermomixer, shaking at 1100 rpm.

4.3 Automated DNA IQ™ protocol

Sample lysates from the fridge or freezer were allowed to thaw or come to room temperature, then added into a Slicprep™ 96 Device (without basket) using the STORstar instrument. Automated DNA IQ™ was then performed (without the automated addition of Extraction Buffer).

4.4 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.

4.5 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.

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4.6 Capillary electrophoresis and fragment analysis

PCR product was prepared for capillary electrophoresis using the manual 9+1 protocol as per QIS 19978. Capillary electrophoresis was performed on an ABI Prism® 3130 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® 3130 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%.

5. Results and Discussion

5.1 Testing the effect of storage condition and storage time of samples that have undergone off-deck lysis prior to automated DNA IQ™

Off-deck lysis was performed on five sets of samples across 5 days, with 2 sets being processed on Day 1, and 1 set processed every day for Day 2, 3 and 4 (see Table 1). One of the sample sets processed on Day 1 was stored in the freezer (-20°C) after off-deck lysis was completed, while all other sets were stored in the fridge (4°C). This resulted in a series of replicate samples that were processed using the same off-deck lysis protocol, but stored in the fridge or freezer for varying lengths of time, up to 4 days. Sample lysates were thawed or allowed to come to room temperature on Day 5, and the automated DNA IQ™ extraction protocol was performed (with the Extraction Buffer addition step removed).

DNA quantitation results for all samples, as determined by the Quantifiler™ system, are outlined in Table 3 below.

Table 3. DNA quantitation results (ng/µL) for samples stored for varying lengths of time in Extraction Buffer, prior to automated DNA IQ™ extraction.

Samples	Number of days in storage				
	1 (Fridge)	2 (Fridge)	3 (Fridge)	4 (Fridge)	4 (Frozen)
NegCtl	*0.0000*	*0.0005*	*0.0006*	*0.0008*	*0.0007*
Blood QC	1.1100	1.7400	1.7200	1.7500	1.2500
Blood QC	0.8140	0.9930	1.4500	1.5700	1.3900
Blood QC	0.2390	0.9240	1.1000	1.5500	1.2200
Blood QC	1.0500	0.9280	0.9490	1.6200	1.0400
Blood QC	1.0400	1.4300	1.4300	1.2600	1.0500
Mean Blood QC	*0.8506*	*1.2030*	*1.3298*	*1.5500*	*1.1900*
Std Dev	*0.36*	*0.37*	*0.31*	*0.18*	*0.15*
Blood Urea	1.0600	1.5900	1.2000	1.0400	1.6000
Blood Urea	1.2700	0.9580	1.5900	0.9770	1.2000
Blood Urea	1.7600	1.7300	1.3400	0.8540	1.0600
Blood Urea	1.1400	1.2900	1.5000	1.4200	1.4300
Blood Urea	1.0900	1.1200	0.8840	1.0700	1.1400
Mean Blood Urea	*1.2640*	*1.3376*	*1.3028*	*1.0722*	*1.2860*
Std Dev	*0.29*	*0.32*	*0.28*	*0.21*	*0.22*
Cells	0.4930	0.1780	0.1060	0.3510	0.1330
Cells	0.3750	0.1140	0.2690	0.3520	0.2070
Cells	0.0999	0.1710	0.1040	0.3380	0.3530
Cells	0.1770	0.2060	0.1960	0.4530	0.2020
Cells	0.1010	0.1930	0.3030	0.3220	0.3360
Mean Cells	*0.2492*	*0.1724*	*0.1956*	*0.3632*	*0.2462*
Std Dev	*0.18*	*0.04*	*0.09*	*0.05*	*0.09*

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The combined mean quantitation results for blood QC samples, blood samples spiked with urea and cell samples that were stored in Extraction Buffer across four days of storage (in fridge *or* freezer) were 1.2247, 1.2525 and 0.2453ng/µL respectively. On average, the absolute difference in individual mean results for each set was only 0.0978ng/µL (between 5.7-19.9%) away from the combined mean quantitation result. This indicates little variation in quantitation results between samples that had remained in storage for 1, 2, 3 or 4 days, either in the fridge or freezer.

DNA profiling results (Table 4) also do not indicate any variation between results for samples that had remained in storage over varying lengths of time. All samples generated full profile (18/18) results. Samples spiked with urea to mimic inhibition generated similar results to un-spiked samples, indicating no change in the ability of DNA IQ™ to remove inhibitors, but also no damage to the lysate material while it remained in storage.

Table 4. Number of reportable alleles (out of 18) for samples that were stored for varying lengths of time in Extraction Buffer, prior to automated DNA IQ™ extraction.

Samples	Number of days in storage				
	1 (Fridge)	2 (Fridge)	3 (Fridge)	4 (Fridge)	4 (Frozen)
NegCtl	0	0	0	0	0
Blood QC	18	18	18	18	18†
Blood QC	18	18	18	18	18
Blood QC	18†	18‡	18	18	18
Blood QC	18	18¥	18	18	18
Blood QC	18	18	18	18†	18
Blood Urea	18	18	18	18	18
Blood Urea	18	18	18	18	18
Blood Urea	18	18	18†	18†	18
Blood Urea	18	18	18	18	18
Blood Urea	18†	18	18	18	18
Cells	18	18	18	18	18
Cells	18	18	18¥	18	18
Cells	18	N*	18	18	18
Cells	18	18	18	18†	18
Cells	18	18†	18	18	18

†Allelic imbalance greater than 65% at one locus
‡ Allelic imbalance less than 64.9% but greater than 60% at one locus
¥Allelic imbalance less than 59.9% at one or more loci
*PP and AI with ULP due to bad injection

When compared to results for extraction positive controls (QC blood swabs) that were extracted since January 2008 as part of routine laboratory processes, the positive controls that were included in these series of experiments generated higher quantitation values but similar DNA profile results (Table 5). The off-deck positive controls produced an average DNA concentration of 1.22ng/µL (SD 0.35), compared to 0.27ng/µL (SD 0.12) for routine QC blood swabs, i.e. the concentration of off-deck controls was over 4-fold greater than controls extracted using the current protocol. Positive controls that were extracted using the off-deck method displayed more allelic imbalance compared to routine positive controls, i.e. 20% (5/25) compared to 9% (3/34). Four out of the five occurrences of allelic imbalance in off-deck controls were one locus events with a peak height ratio greater than 60%, and therefore pass the in-house acceptance criteria for extraction positive controls.

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Table 5. Comparison of results for extraction positive controls (QC blood swabs) extracted using the off-deck lysis protocol or the current automated method.

	n	Mean $_{[DNA]}$ (ng/µL)	*SD*	% OK Profile	% AI Profile	% Pass QC
Off-Deck Lysis	25	1.22	0.35	80	20	96
Routine Work	34	0.27	0.12	88*	9	97

* One sample was involved in an OQI and excluded from this data pool.

Based on the results in this study, it appears that storage of extracts in extraction buffer for a few days rather than a single day does not affect the quality of the DNA for subsequent use in quantitation and DNA profiling, and the results are comparable to the current method.

5.2 Comparison between hot block and Thermomixer for performing off-deck lysis

For the comparison between hot blocks and Thermomixer, off-deck lysis was performed on a hot block for two sample sets, and a third set was incubated on the Thermomixer. For sets 1 and 2 where the hot block was used, the minor difference between the two sets is a 5 second vortex *during* incubation for Set 2 samples (see Table 2).

Table 6. Quantitation results (ng/µL) for a comparison between off-deck lysis performed on a hot block or a Thermomixer.

Sample type	Set 1 Hot block (vortex before and after)	Set 2 Hot block (vortex before, after, during)	Set 3 Thermomixer (1100 rpm)
10µL blood, cotton swab	0.1060	0.0279	0.1260
	0.0750	0.0871	0.3210
	0.0568	0.0209	0.1240
	0.1020	0.0788	0.3110
	0.0641	0.0267	0.1710
Mean	*0.0808*	*0.0483*	*0.2106*
30µL blood, rayon swab	0.8630	0.6760	0.6850
	0.8440	0.0851	0.7900
	0.7920	0.5060	1.5400
	0.5340	0.4580	0.4530
	0.6430	0.6240	0.3990
Mean	*0.7352*	*0.4698*	*0.7734*
10µL buccal cells, rayon swab	0.0135	0.0102	0.0036
	0.0127	0.0006	0.0016
	0.0023	0.0126	0.0016
	0.0022	0.0027	0.0000
	0.0042	0.0031	0.0007
	0.0083	0.0040	0.0021
	0.0042	0.0022	0.0000
	0.0000	0.0000	0.0000
	0.0079	0.0037	0.0000
	0.0038	0.0007	0.0000
Mean	*0.0059*	*0.0040*	*0.0010*

Quantitation results indicate that all three sets generated similar results, except Set 2 where the mean results were generally lower (Table 6). The vortexing of samples during incubation may have resulted in temperature variations that affected lysis efficiency. Vortexing before and after incubation on a hot block produced similar results to performing lysis incubation on the Thermomixer. For buccal cell samples, however, the Thermomixer samples (Set 3) produced more undetermined results (0ng/µL) compared to the hot block samples (Set 1), but most results were generally low and close to the validated LOD of 0.00426ng/µL and therefore indistinguishable from the background (Hlinka *et al.*, 2006).

For blood samples, either on cotton or rayon swabs, all sample sets produced full (18/18) DNA profiles (data not shown). The results for cell samples were slightly varied and were mostly non-reportable or NSD, as suggested by the low DNA quantitation results (Table 6). Out of 180 possible alleles (excluding Amelogenin) for buccal cell samples, Set 1 samples yielded 12 allele calls and 52 non-reportable alleles, compared to Set 3 that produced 0 allele calls and 14 non-reportable alleles (Table 7). In comparison, Set 2 samples generated only 1 allele call and 39 reportable alleles (Table 7).

Table 7. Number of reportable and non-reportable alleles generated for samples incubated on a hot block and Thermomixer.

	Alleles	NR's
Set 1 (Hot block, vortex before and after)	12	52
Set 2 (Hot block, vortex before, after and during)	1	39
Set 3 (Thermomixer, 1100 rpm)	0	14

Results indicate that performing off-deck lysis on a hot block does not generate results that are worse than samples that were incubated on the Thermomixer. Performing a 5 second vortex before and after incubation appears to be the best method for mixing samples that are incubated on the hot block.

6. Summary and Recommendations

Samples that were lysed off-deck were able to be stored either in the fridge or freezer for up to 4 days without affecting the possibility of obtaining DNA profiles, suggesting stability of the lysate in Extraction Buffer containing inactivated Proteinase K. Furthermore, off-deck lysis was able to be performed using either the Ratek hot blocks or the Eppendorf Thermomixer Comfort without producing significant variation in results.

We recommend:

1. Off-deck lysis using Extraction Buffer can be performed on either a hot block or a Thermomixer, prior to automated DNA IQ™ extraction (without the automated addition of Extraction Buffer). If performed on a hot block, vortexing the sample before and after incubation for 5 seconds each is an adequate substitute for continuous shaking.
2. Samples that have had off-deck lysis performed can be stored either in the fridge or freezer for up to 4 days.

3. Off-deck lysis can be used to processes a greater variety of samples types (e.g. tapelifts) using the DNA IQ™ system.

7. Acknowledgements

Experiments were performed by the Automation/LIMS Implementation Project team, with the assistance of the Analytical Section.

8. References

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Managing Scientist
DNA Analysis
Forensic and Scientific Services
PO Box 594, Archerfield QLD Australia 4108

Or by telephone (07) 3274 9169

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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

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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.

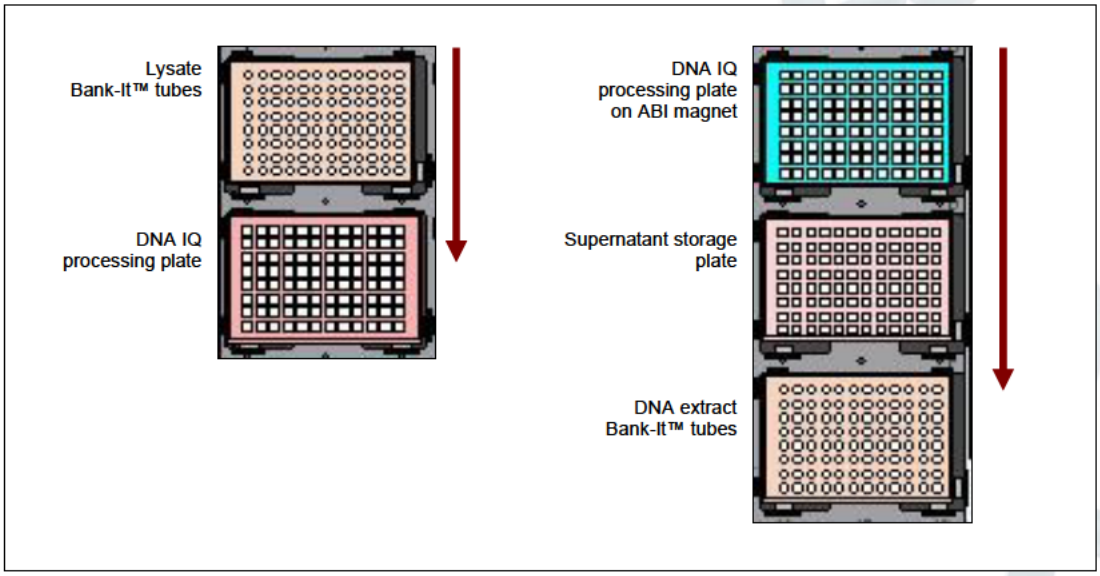

Figure 1 Movement of samples between different racks and plates occur from the back to the front of the instrument, therefore only passing over one column at a time. No diagonal movements occur when samples are present in tips, therefore minimising the risk of contamination across the plate.

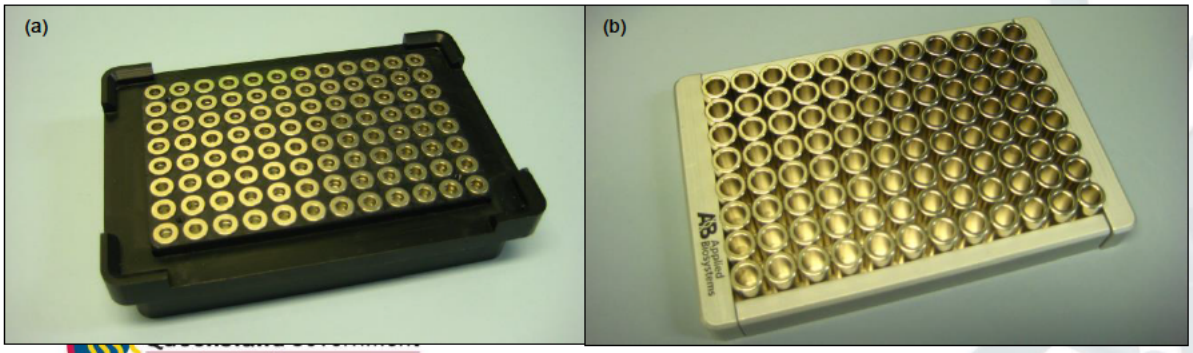

Figure 2 The PKI magnet (a) has corners that often required manual intervention to allow full contact between the plate surface and the magnet. The ABI magnet (b) has no corner holders and the magnets are slightly raised, therefore allowing full contact with the plate.

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*xl* 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*xl* 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.

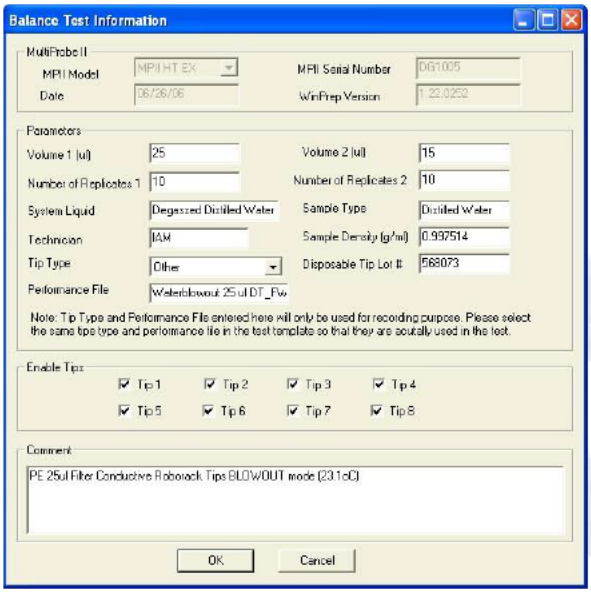

Figure 3 The Balance Test Information Window as present within the Balance Test DT program. All pipetting parameters are entered here and are subsequently transferred to the result output file.

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.

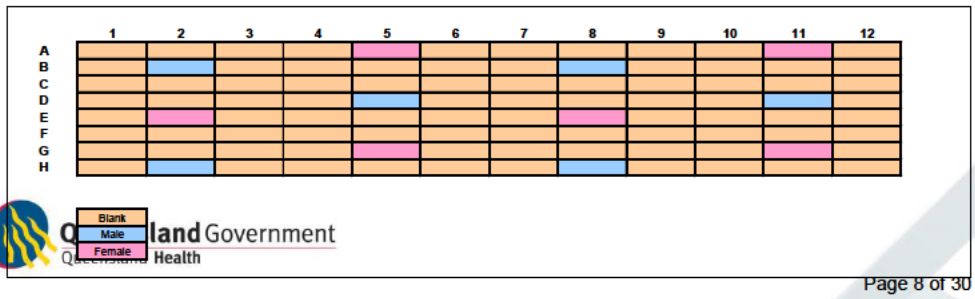

Figure 4 Anti-contamination plate in soccerball format. Each plate contained 6 DNA samples consisting of male DNA and 6 DNA samples consisting of female DNA, and the remainder were negative (blank) samples consisting of water and no template DNA.

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.

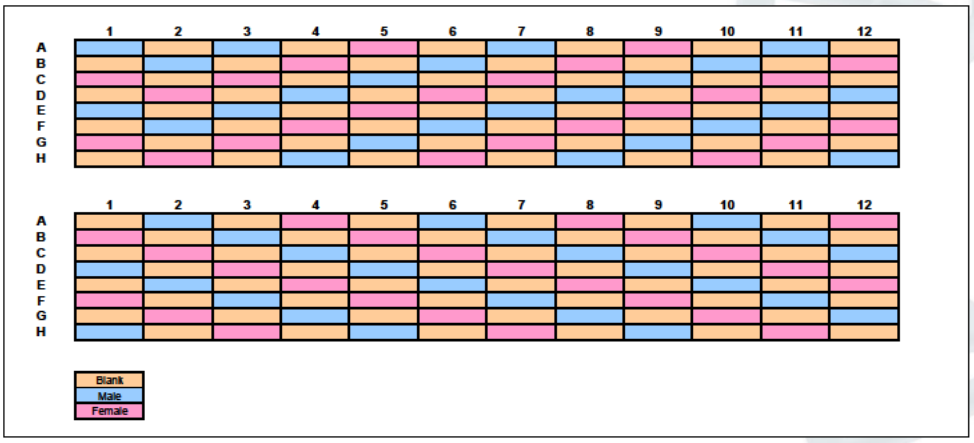

Figure 5 Anti-contamination plates in checkerboard format. Each plate consisted of 48 DNA samples (24 male and 24 female) and 48 negative (blank) samples consisting of water and no template DNA.

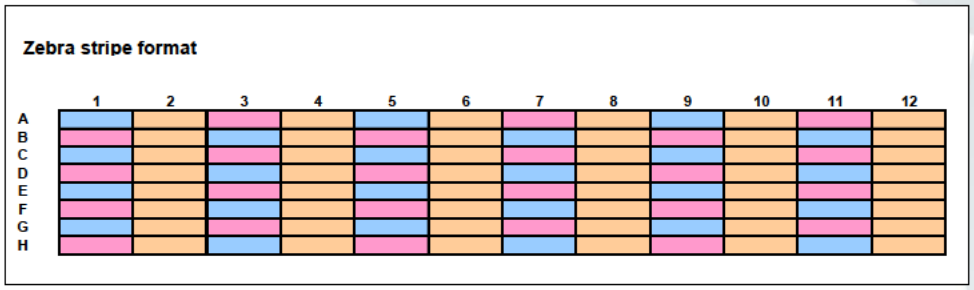

Figure 6 Anti-contamination plates in zebra stripe format. Each plate consisted of 48 DNA samples (24 male and female) and 48 negative (blank) samples consisting of water and no template DNA.

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 AmpFℓSTR® 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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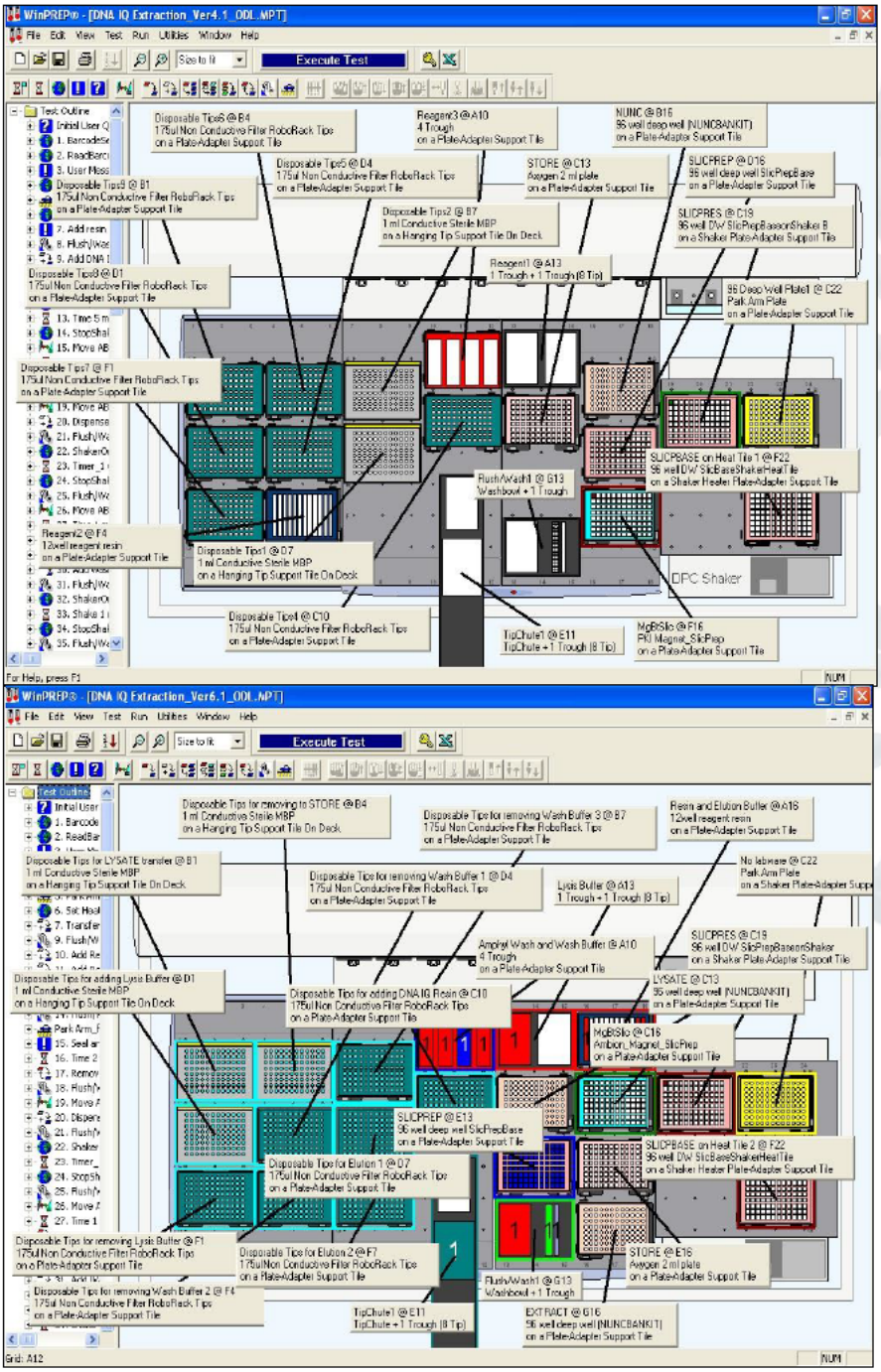

Figure 7 Virtual deck layouts for version 4.1 (top) and version 6.5 (bottom), showing changes in labware and positioning of labware on the MPII deck.

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.

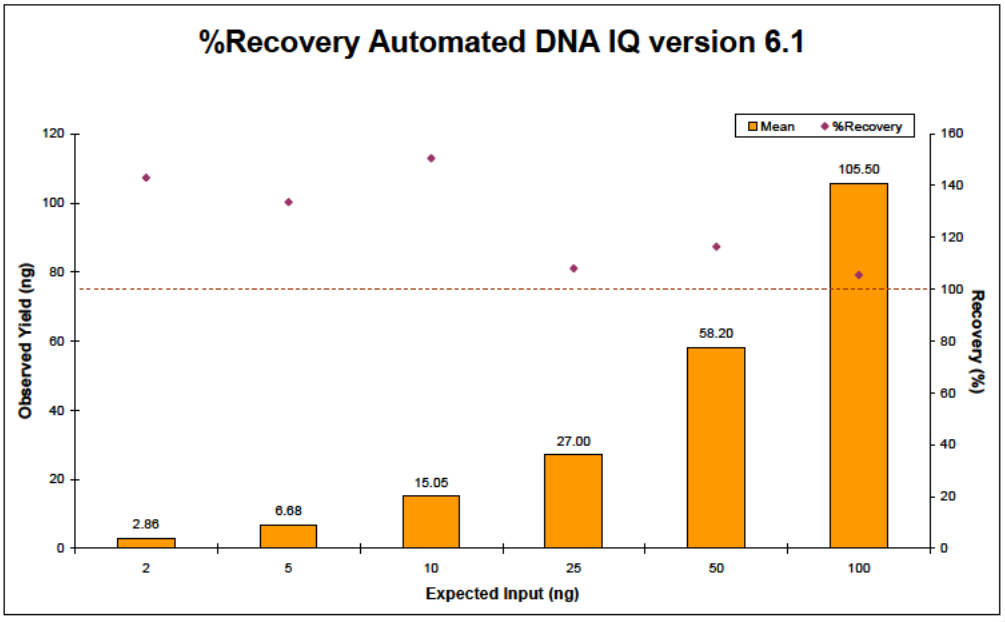

Figure 8 The recovery rate of the modified automated DNA IQ™ procedure (version 6.1).

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.

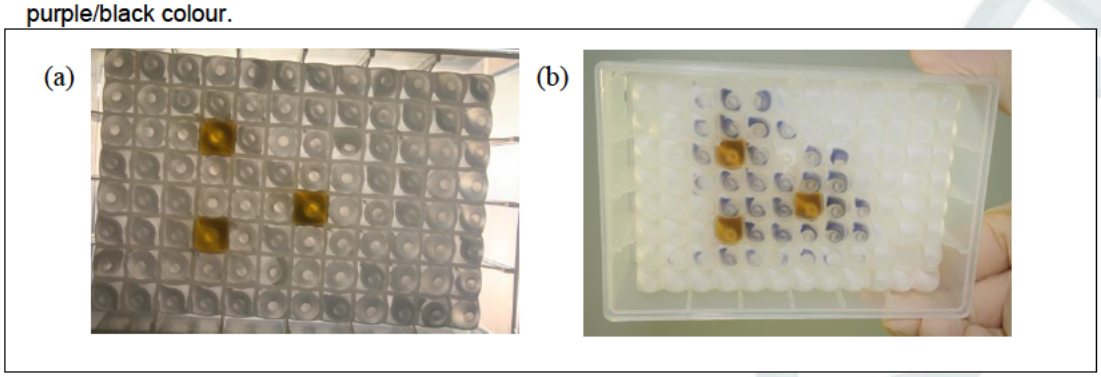

Figure 9 (a) ABgene 2.2ml DWP sealed with septa mat after mixing. Yellow wells contain NaI, surrounding wells contain bleach and cardboard. **(b)** Wells after mixing and centrifuging of 2.2ml DWP. Wells with dark staining contain NaI transferred from the yellow coloured wells.

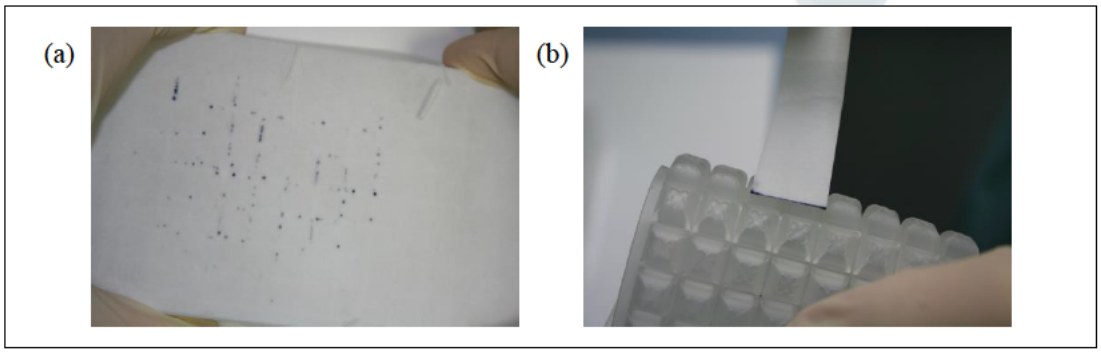

Figure 10 (a) Cardboard swab of DWP after removal of the septa mat. **(b)** Cardboard swab between the grooves of the septa mat.

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.

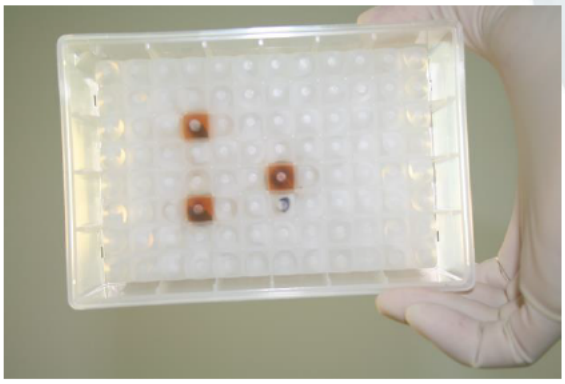

Figure 11 Contamination present after inverting the plate and centrifuging. Contamination is identified by the dark colour in a previously blank well.

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.

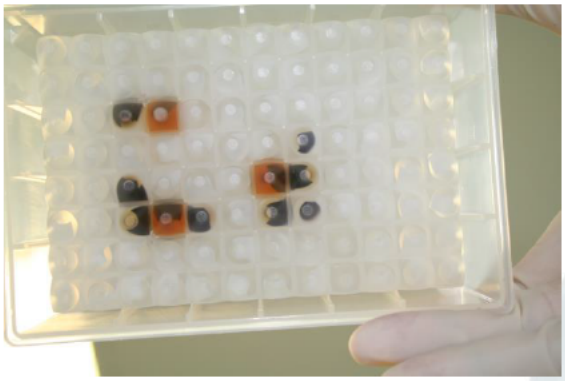

Figure 12 Plate left sitting upside on bench for 5 minutes and then centrifuged.

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.

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.

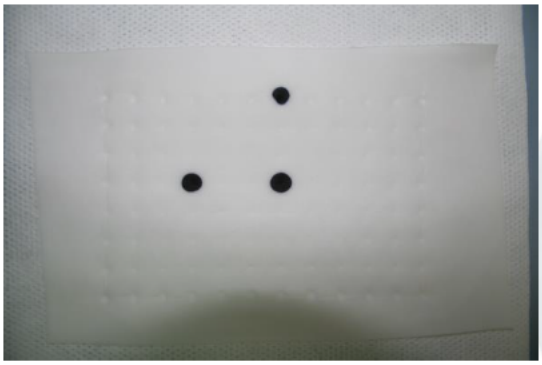

Figure 13 NaI reactions with bleach on the underside of a PCR plate after it has been used to pierce a heat seal.

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.

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.

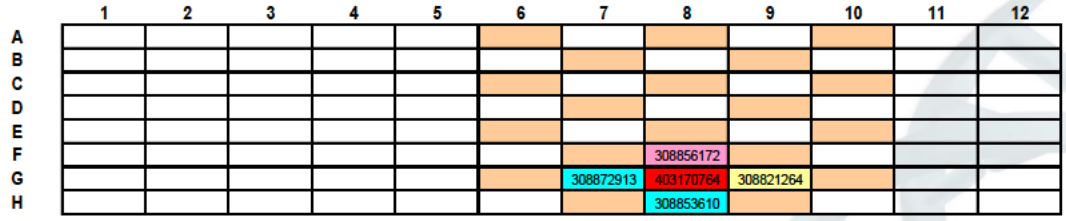

Figure 14 Plate map indicating position of sample 403170764 on the extraction batch. All alleles obtained for the contaminated negative control are represented in the profile of sample 308821264.

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.

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.

10. References

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