Notice number: 6.003

COMMISSION OF INQUIRY INTO DNA PROJECT 13

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

STATEMENT OF GENEROSA LUNDIE

I, **Generosa Lundie**, care of Queensland Health Forensic and Scientific Service, Forensic Scientist, do solemnly and sincerely declare that:

1. On 24 October 2023, I provided a written statement to this Commission responding to Notice 6.001 "Requirement to Given Information in a Written Statement".

2. On 25 October 2023, I was requested to provide a statement responding to Notice 6.003 "Requirement to Give Information in a Written Statement".

3. This statement provides my written response to Notice 6.003.

Identification

Question 1(a) - State your full name:

4. My full name is Generosa Lundie.

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

5. Please see my statement dated 24 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.

6. Please see my statement dated 24 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.

[redacted] [redacted]

Generosa Lundie Witness

7. **Annexed and marked Exhibit GL-01** is a table of my responses to Question 2.

8. My solicitors have provided me with 11 versions of the standard operating procedure (**SOP**) relevant to the DNA IQ protocol.

9. **Annexed and marked Exhibit GL-02** is a copy of version 1 of the SOP relevant to the DNA IQ protocol dated 24 October 2007.

10. **Annexed and marked Exhibit GL-03** is a copy of version 2 of the SOP relevant to the DNA IQ protocol dated 11 January 2008.

11. **Annexed and marked Exhibit GL-04** is a copy of version 3 of the SOP relevant to the DNA IQ protocol dated 27 March 2008.

12. **Annexed and marked Exhibit GL-05** is a copy of version 4 of the SOP relevant to the DNA IQ protocol dated 21 May 2008.

13. **Annexed and marked Exhibit GL-06** is a copy of version 5 of the SOP relevant to the DNA IQ protocol which is undated.

14. **Annexed and marked Exhibit GL-07** is a copy of version 6 of the SOP relevant to the DNA IQ protocol dated 13 August 2009.

15. **Annexed and marked Exhibit GL-08** is a copy of version 7 of the SOP relevant to the DNA IQ protocol dated 9 November 2010.

16. **Annexed and marked Exhibit GL-09** is a copy of version 8 of the SOP relevant to the DNA IQ protocol dated 27 June 2012.

17. **Annexed and marked Exhibit GL-10** is a copy of version 9 of the SOP relevant to the DNA IQ protocol dated 3 January 2014.

18. **Annexed and marked Exhibit GL-11** is a copy of version 10 of the SOP relevant to the DNA IQ protocol dated 12 June 2015.

19. **Annexed and marked Exhibit GL-12** is a copy of version 11 of the SOP relevant to the DNA IQ protocol dated 30 January 2017.

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

20. I do not know.

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.

21. I do not know.

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.,

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

Generosa Lundie Witness

Lundie, G., Iannuzzi, C., Ientile, V. Automation/LIMS Implementation Project, DNA Analysis FSS (August 2008)?

22. No.

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 26 October 2023.

[redacted]

Generosa Lundie Witness

EXHIBITS INDEX

Exhibits Index – Generosa Lundie Statement

Question	Exhibit	Document Title
2	GL-01	Table of Generosa Lundie's responses to question 2 in Notice 6.003
2	GL-02	Version 1 of the SOP relevant to the DNA IQ protocol dated 24 October 2007.
2	GL-03	Version 2 of the SOP relevant to the DNA IQ protocol dated 11 January 2008.
2	GL-04	Version 3 of the SOP relevant to the DNA IQ protocol dated 27 March 2008.
2	GL-05	Version 4 of the SOP relevant to the DNA IQ protocol dated 21 May 2008.
2	GL-06	Version 5 of the SOP relevant to the DNA IQ protocol undated.
2	GL-07	Version 6 of the SOP relevant to the DNA IQ protocol dated 13 August 2009.
2	GL-08	Version 7 of the SOP relevant to the DNA IQ protocol dated 9 November 2010.
2	GL-09	Version 8 of the SOP relevant to the DNA IQ protocol dated 27 June 2012.
2	GL-10	Version 9 of the SOP relevant to the DNA IQ protocol dated 3 January 2014.
2	GL-11	Version 10 of the SOP relevant to the DNA IQ protocol dated 12 June 2015.
2	GL-12	Version 11 of the SOP relevant to the DNA IQ protocol dated 30 January 2017.

[redacted] .....

Generosa Lundie

[redacted] .....

Witness

GL-01

Table of Generosa Lundie's responses to Question 2 in Notice 6.003

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	(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
Version 1 of the SOP relevant to the DNA IQ protocol dated 24 October 2007	Please see attached Exhibit GL-02.	Please see section 7 for automated extraction.	Please see section 7 (item 82) for automated extraction.	Please see section 7 for automated extraction.	Please see section 7 for automated extraction.
Version 2 of the SOP relevant to the DNA IQ protocol dated 11 January 2008.	Please see attached Exhibit GL-03.	Please see section 7 for automated extraction. Please see subsection 16.2.2 for manual extraction.	Please see subsection 7(7) for automated extraction. Please see subsection 16.2.2 for manual extraction.	Please see subsection 7(1) for automated extraction. Please see subsection 16.2.2(2) for manual extraction.	Please see subsection 7(5) for automated extraction. Please see subsections 16.2.2 (16) & (17) for manual extraction.
Version 3 of the SOP relevant to the DNA IQ protocol dated 27 March 2008.	Please see attached Exhibit GL-04.	Please see section 9 for automated exaction. Please see subsection 18.4.2 for manual extraction.	Please see subsections 18.3.2 (27) & (30) for automated extraction. Please see subsection 18.4.2(11) for manual extraction.	Please see subsection 18.3.2(24) for automated extraction. Please see subsection 18.4.2(2) for manual extraction.	Please see subsection 9(3) for automated extraction. Please see subsections 18.4.2(16) & (17) for manual extraction.
Version 4 of the SOP relevant to the DNA IQ protocol dated 21 May 2008.	Please see attached Exhibit GL-05.	Please see section 9 for automated extraction. Please see subsection 18.4.2 for manual extraction.	Please see subsections 9 (29) & (32) for automated extraction. Please see subsubsections 18.4.2 (19) & (24) for manual extraction.	Please see subsection 8(12) for automated extraction. Please see subsection 18.4.2(2) for manual extraction.	Please see subsection 9(3) for automated extraction. Please see subsections 18.4.2 (16) & (17) for manual extraction.
Version 5 of the SOP relevant to the DNA IQ protocol which is undated.	Please see attached Exhibit GL-06.	Please see subsection 8.2 for automated extraction. Please see subsection 17.1.4 for manual extraction.	Please see subsubsection 9.5 (7) for automated extraction.	Please see subsection 8.1(7) for automated extraction.	Please see subsection 9.5(5) for automated extraction.

			Please see subsection 17.1.4 (20) & (24) for manual extraction.	Please see subsection 17.1.4(1) for manual extraction.	Please see subsections 17.1.4 (17) & (18) for manual extraction.
Version 6 of the SOP relevant to the DNA IQ protocol dated 13 August 2008.	Please see attached Exhibit GL-07.	Please see subsection 9.5 for automated extraction. Please see subsection 17.1 for manual extraction.	Please see subsection 9.5(7) for automated extraction. Please see subsections 17.1 (21) & (25) for manual extraction.	Please see subsection 8.1(7) for automated extraction. Please see subsection 17.1.4(1) for manual extraction.	Please see subsections 9.5(5) for automated extraction. Please see subparagraphs 17.1 (18) & (19) for manual extraction.
Version 7 of the SOP relevant to the DNA IQ protocol dated 9 November 2010.	Please see attached Exhibit GL-08.	Please see subsections 7.1 & 7.2 for automated extraction. Please see subsections 9.4 & 9.5 for manual extraction.	Please see subsection 8.6.7 for automated extraction. Please see subsection 9.5 (27) & (31) for manual extraction.	Please see subsection 7.1(7) for automated extraction. Please see subsection 9.5(3) for manual extraction.	Please see subsection 8.6.5 for automated extraction. Please see subsections 9.5 (24) & (25) for manual extraction.
Version 8 of the SOP relevant to the DNA IQ protocol dated 27 June 2012.	Please see attached Exhibit GL-09.	Please see subsections 7.1 & 7.2 for manual extraction. Please see section 8 for automated extraction.	Please see subsection 9.3.7 for automated extraction. Please see subsections 7.1 (21) & (25) for manual extraction.	Please see subsection 8(7) for automated extraction. Please see subsection 7.1(2) for manual extraction.	Please see subsection 9.3.5 for automated extraction. Please see subsections 7.1(18) & (19) for manual extraction.
Version 9 of the SOP relevant to the DNA IQ protocol dated 3 January 2014.	Please see attached Exhibit GL-10.	Please see subsections 7.1 & 7.2 for manual extraction. Please see section 8 for automated extraction.	Please see subsection 9.3.7 for automated extraction. Please see subsection 7.1 (21) & (25) for manual extraction.	Please see section 8(7) for automated extraction. Please see subsection 7.1(2) for manual extraction.	Please see subsection 9.3.5 for automated extraction. Please see subsections 7.1(22) & (23) for manual method.
Version 10 of the SOP relevant to the DNA IQ protocol dated 12 June 2015.	Please see attached Exhibit GL-11.	Please see subsections 7.1 & 7.2 for manual extraction. Please see section 8 for automated extraction.	Please see subsection 9.3.7 for automated extraction. Please see subsections 7.1 (21) & (25) for manual extraction.	Please see subsubsection 8(6) for automated extraction. Please see subsubsection 7.1(6) for manual extraction.	Please see subsection 9.3.5 for automated extraction. Please see subsections 7.1(18) & (19) for manual extraction.
Version 11 of the SOP relevant to the DNA IQ protocol dated 20 January 2017.	Please see attached Exhibit GL-12.	Please see subsections 7.1 & 7.2 for manual extraction.	Please see subsections 7.1 (21) & (25) for manual extraction.	Please see subsection 7.1(6) for manual extraction.	Please see subsections 7.1(18) & (19) for manual extraction.

GL-02

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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.

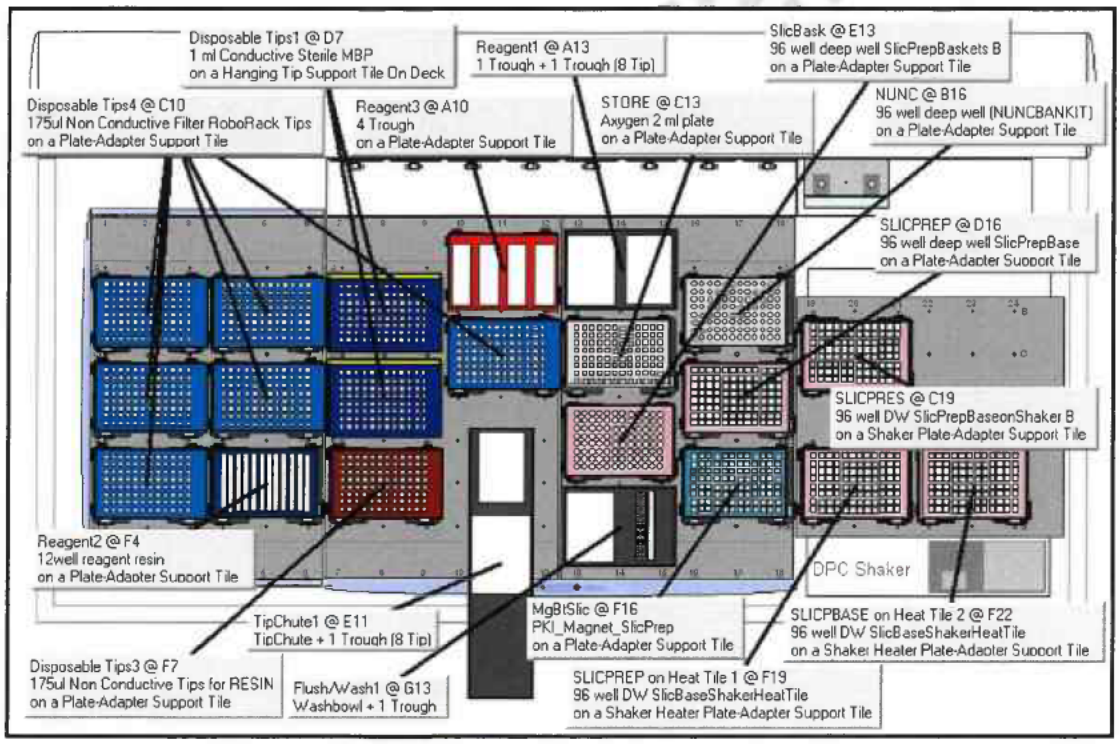


Figure 3. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

9. Ensure that the DPC Shaker and Heater Controller Box are switched on.
   For EP-A: Tile 3 should be at F19 (50°C), Tile 1 at F22 (85°C).
   For EP-B: Tile 1 should be at F19 (50°C), Tile 2 at F22 (85°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°C and moved to the PKI Magnet, a User message (step 79) will appear requesting:
"**Push down the Slicprep on the PKI Magnet then press OK**."
Allow to the plate to cool slightly before pushing down on all four corners of the plate to lock it into place on the PKI Magnet. Once it is firmly in place, click OK to continue. After the second elute, the prompt will appear again. Repeat the steps.

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

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

Recording Reagent Details in AUSLAB

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

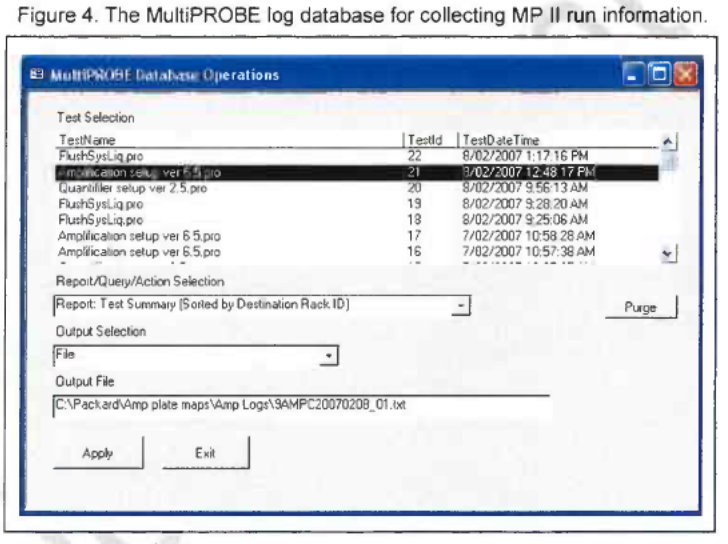
Finalising the MP II run

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

Importing the MP II log file into AUSLAB

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

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



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

Importing Extraction "Results" into AUSLAB

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

8 SAMPLE STORAGE

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

9 VALIDATION

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

10 QUALITY ASSURANCE/ACCEPTANCE CRITERIA

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

11 REFERENCES

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13. Promega, Protocols & Applications Guide. Chapter 9. rev. 7/06.
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15. Promega, DNA IQ™ System-Database Protocol. Promega Technical Bulletin #TB297, 2006. Rev 4/06: p. 1-14.
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18. Vandenberg, N., van Oorchot., R. A. H., & Mitchell, R. J., An evaluation of selected DNA extraction strategies for short tandem repeat typing. Electrophoresis, 1997. 18: p. 1624-1626.

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.

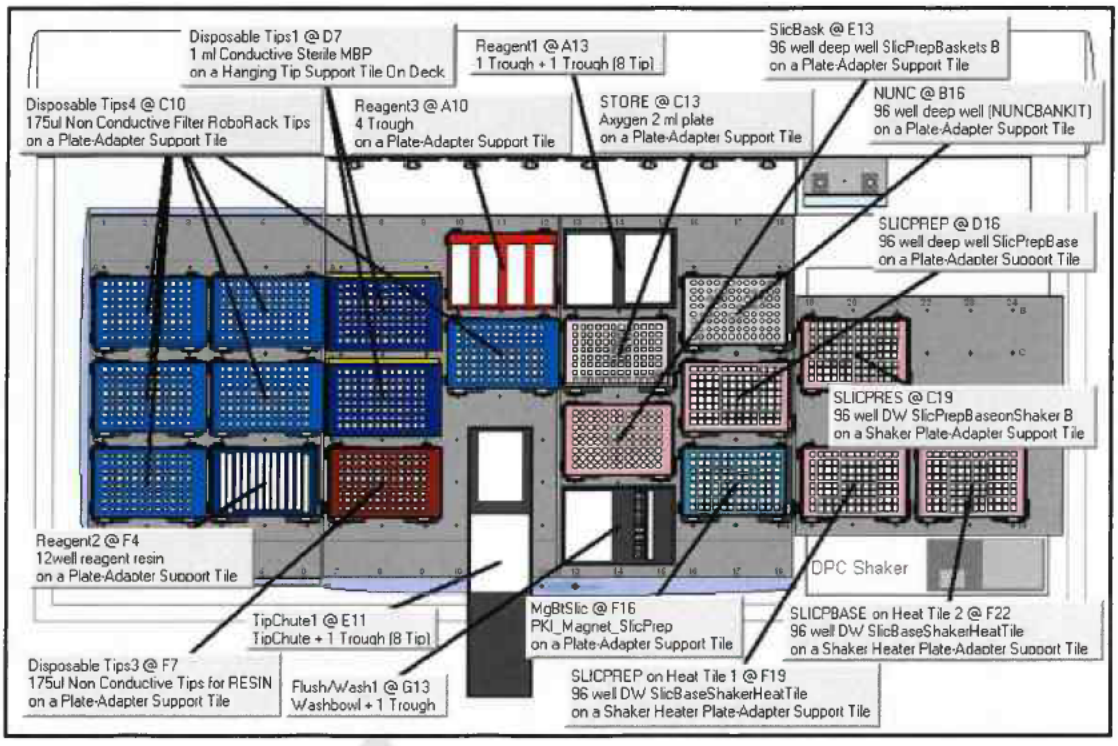


Figure **2**. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

13. Ensure that the DPC Shaker and Heater Controller Box are switched on.
    For EP-A: Tile 3 should be at F19 (50°C), Tile 1 at F22 (85°C).
    For EP-B: Tile 1 should be at F19 (50°C), Tile 2 at F22 (85°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°C (real temp 37°C). When current temperature reaches 50°C click "**Continue**".

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

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

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

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

30. The next User prompt will appear with the following directions:
"**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°C (real temp 65°C). **DO NOT PRESS CONTINUE** it will continue automatically when temperature has reached 85°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 4. below)

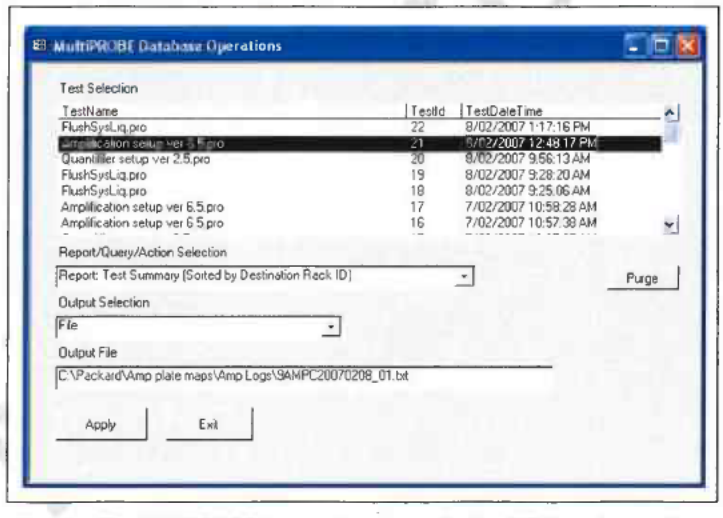


Figure 4. The MultiPROBE log database for collecting MP II run information

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

61. Press **[Esc]**.

Importing Extraction "Results" into AUSLAB

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

8 SAMPLE STORAGE

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

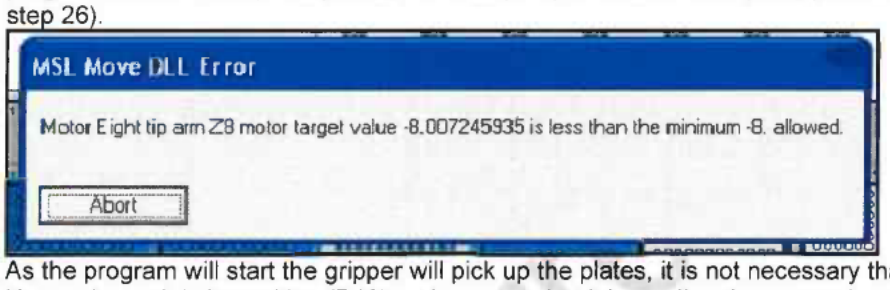
9 TROUBLESHOOTING

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

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

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

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



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

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

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

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

9. If the message:

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

10 VALIDATION

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

11 QUALITY ASSURANCE/ACCEPTANCE CRITERIA

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

12 REFERENCES

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

13 STORAGE OF DOCUMENTS

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

14 ASSOCIATED DOCUMENTS

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

15 AMENDMENT HISTORY

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

16 APPENDIX

16.1 Reagents Calculation Tables

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

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

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

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

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

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

16.2 Manual method for extraction using DNA IQ™

16.2.1 Sampling and Sample Preparation

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

Table 3. Sample storage locations.

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

QC samples

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

Table 4. Extraction Quality Controls

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

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

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

Create the Extraction Batch

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

Locating Samples

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

When all samples have been located:

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

Sequence Check the tubes

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

16.2.2 Procedure

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

2. Turn on the Eppendorf Thermo mixer and set the temperature to 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 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 4°C 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.

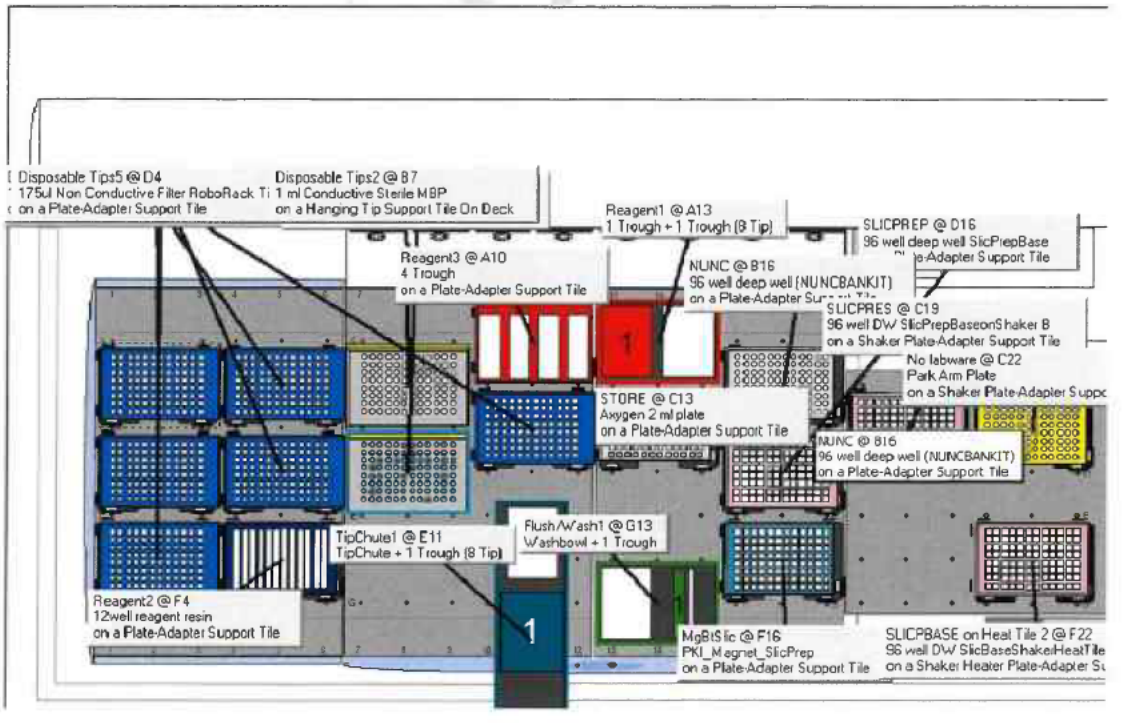

Figure 2. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

13. Ensure that the DPC Shaker and Heater Controller Box are switched on.
    For EP-A: Tile 1 at F22 (85°C).
    For EP-B: Tile 2 at F22 (85°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. **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):

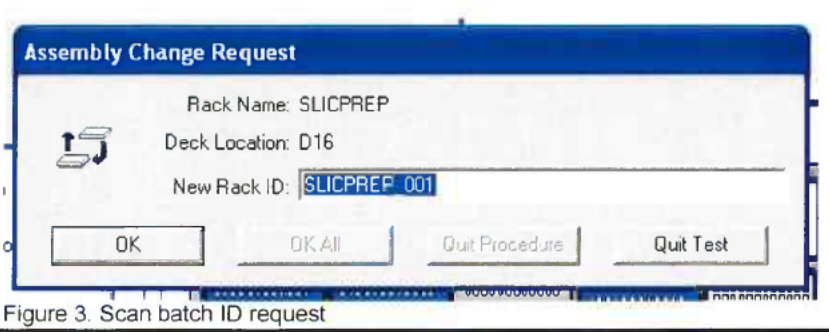

Figure 3. Scan batch ID request

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

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)

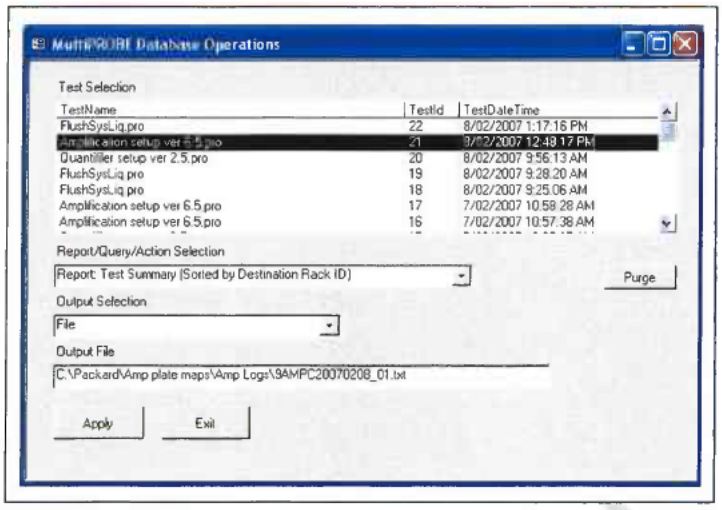

Figure 4. The MultiPROBE log database for collecting MP II run information

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).

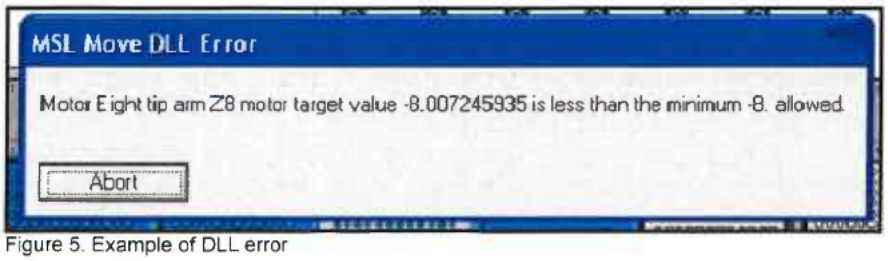

Figure 5. Example of DLL error

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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5. Greenspoon, S.B., J., Robotic Extraction of mock sexual assult samples using the Biomek® 2000 and the DNA IQ™ System. Profiles in DNA, 2002. 5: p. 3-5.
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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).
7. Log onto the WinPrep® software by entering your username and password, then press **"Enter"**.

Figure 7 The WinPrep® icon.

8. Ensure the **System Liquid 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.

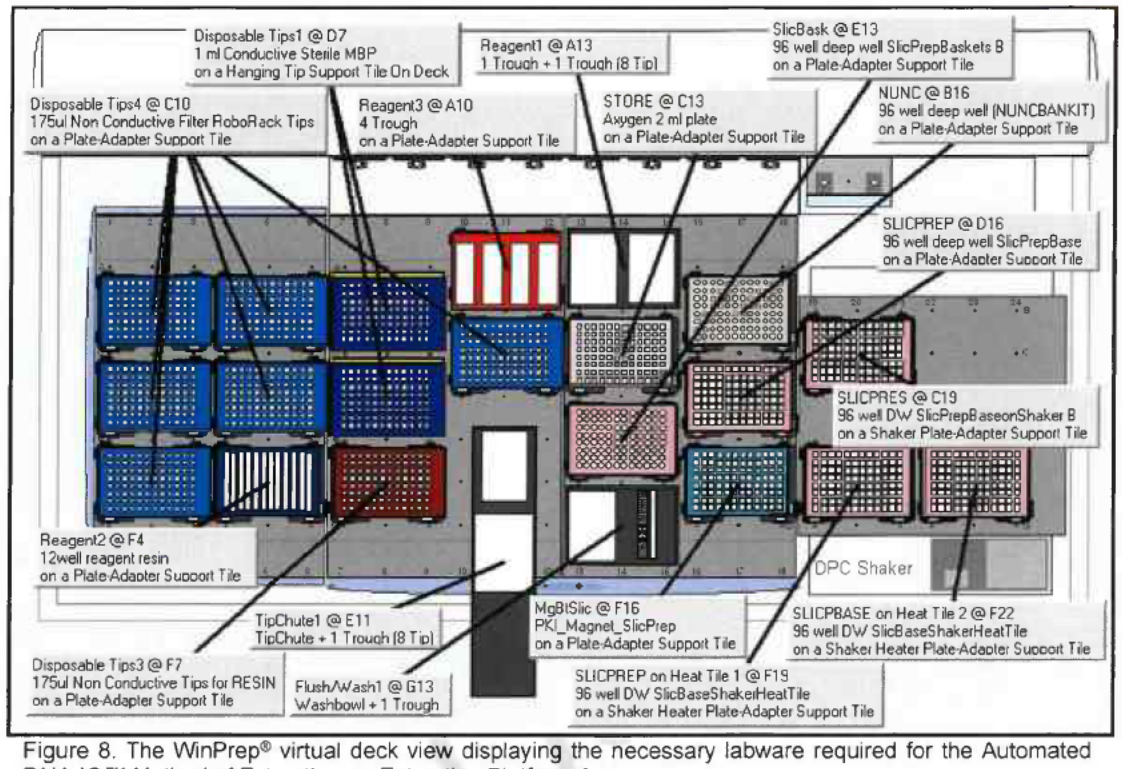

Figure 8. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

13. Ensure that the DPC Shaker and Heater Controller Box are switched on.
    For EP-A: Tile 3 should be at F19 (50°C), Tile 1 at F22 (85°C).
    For EP-B: Tile 1 should be at F19 (50°C), Tile 2 at F22 (85°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°C (real temp 37°C). When current temperature reaches 50°C click "**Continue**".

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

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

27. Add the Resin to the 12 Channel plate. Pipette mix thoroughly. Then dispense 50uL of resin into each well of the Slicprep™ 96 device.

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

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

30. The next User prompt will appear with the following directions:
"**Ensure Wash Buffer has been added to trough 4 at A10.**
Manually add 50uL resin to each well of the SlicPrep plate
Place the plate in position D16.
Add the Elution Buffer to the 12 channel plate.
THEN
Press OK when ready." Press **"OK"** when steps 27-29 have been performed.

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

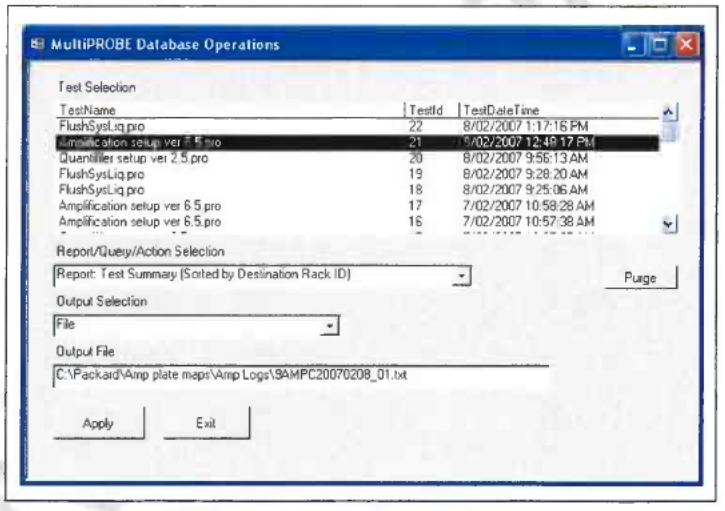

Figure 9. The MultiPROBE log database for collecting MP II run information

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 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 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

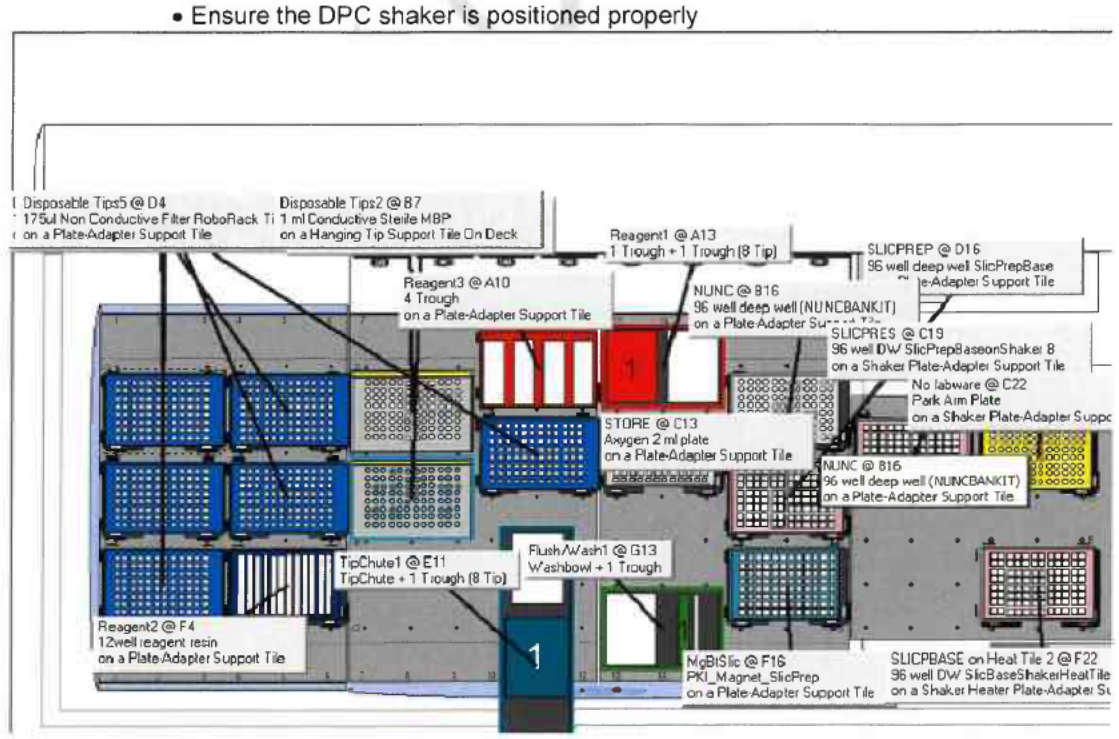

Figure 2. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

14. Ensure that the DPC shaker and Heater Controller Box are switched on.
    For EP-A: Tile 1 at F22 (85°C).
    For EP-B: Tile 2 at F22 (85°C).
    Note: Press the start/stop button twice at the front of the DPC shaker to ensure that it displays zero on the screen.

15. Ensure the heat transfer tile is clicked into the plate adapter tile properly. This is critical to ensure correct incubation temperatures.

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

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

18. **Nunc™ Bank-It™ tube rack**: Check that is the same AUSLAB batch number as the platemap to be run. Ensure that the barcode of the first (A1) and last tube (H12) are the respective ones. Check that the batch label and batch barcode labels are attached to front side of rack. Add B1-Lite generated "**NUNC**" barcode to the right side of the Nunc™ Bank-It™ tube rack. Then place the rack into position **B16**.

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

20. **ABgene 96-deep well plate containing lysates**: Centrifuge plate for 2 minutes at 3021rpm before gently removing adhesive seal and place into position **D16** ensuring the plate is oriented such that the long side of the plate with the words "Front" written on at time of STORstar processing is visible from the front. (This should correspond with the cut corner at H1 being visible to the front of the operator)

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

22. Message will appear (Figure 3 below):

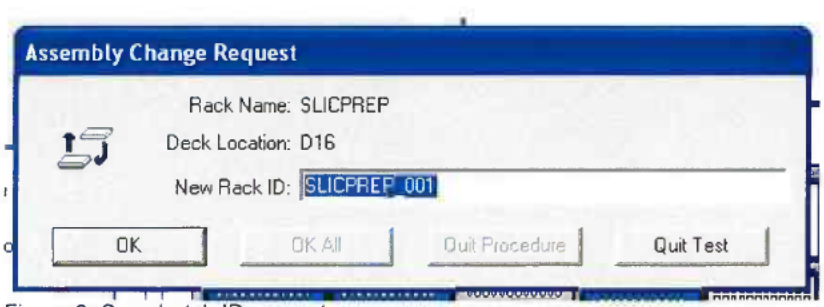

Figure 3. Scan batch ID request

Into "New Rack ID:" Scan barcode of ABgene 96-deep well plate (matches batch ID).

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

24. Select the correct platemap by browsing to **C:\PACKARD\EXT PLATE MAPS**. Ensure that the platemap selected matches the batch ID affixed to the ABgene 96-deep well plate containing Lysates located in position **D16.** Once this has been done, click "**Start**", to continue.

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

26. Add the Resin to the 12 Channel plate. Pipette mix thoroughly. Then dispense 50uL of resin into each well of the ABgene 96-deep well plate containing lysates and return plate to position **D16**.

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

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

29. The next User prompt will appear with the following directions:
"**Ensure Wash Buffer has been added. Manually add 50uL of Resin and place the ABgene plate in position D16. Ensure Elution Buffer has been added.**" Press **"OK"** when steps 24-26 have been performed.

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

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)

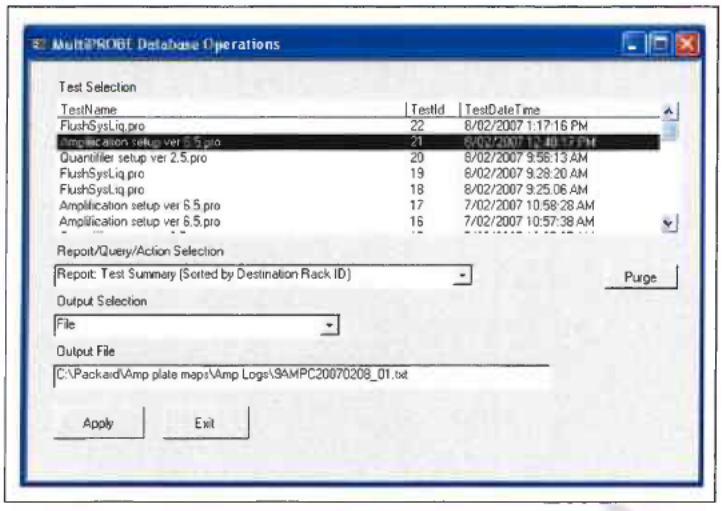

Figure 4. The MultiPROBE log database for collecting MP II run information

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).

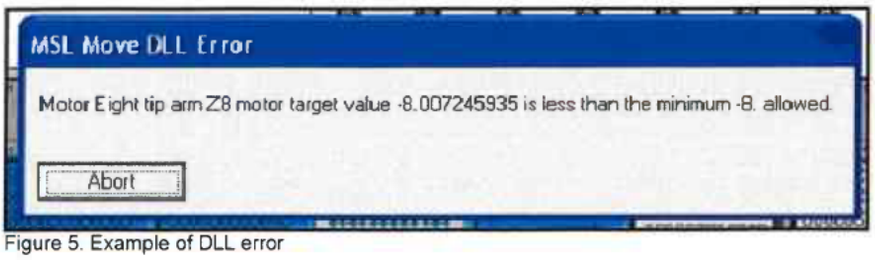

Figure 5. Example of DLL error

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:

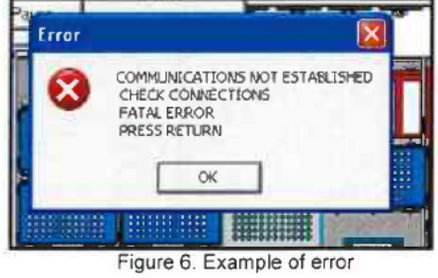

Figure 6. Example of error

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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15 STORAGE OF DOCUMENTS

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

16 ASSOCIATED DOCUMENTS

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

17 AMENDMENT HISTORY

Revision	Date	Author/s	Amendments
0	23 Oct 2007	B. Gallagher, T. Nurthen, C. Iannuzzi, V. Hlinka, G. Lundie, I Muharam.	First Issue
1	12 Dec 2007	M Harvey, C Iannuzzi, A McNevin	Reviewed and updated after initial training
2	19 March 2008	M Harvey, B Andersen, C Iannuzzi, A McNevin	Addition of Off-deck Lysis procedure, Retention of fully automated method as Appendix, addition of reagent record tables into Appendix
3	April 2008	QIS2 Migration Project	Headers and Footers changed to new CaSS format. Amended Business references from QHSS to FSS, QHPSS to CaSS and QHPS to Pathology Queensland

18 APPENDIX

18.1 Reagents Calculation Tables

Table 7. **Less than 48 samples** (note difference is in DNA IQ RESIN Solution)

Lysis-DTT Buffer		Volume (in mL)
Lysis Buffer	(Nx1.35)+0.75	
DTT (1M)	Lysis Buffer/100	
Extraction Buffer		
TNE Buffer	Nx0.56	
Prot K (20 mg/L)	Nx0.03	
Sarcosyl (40 %)	Nx0.015	
DNA IQ RESIN Solution		
LYSIS Buffer	0.054x(N+8)	
DNA IQ RESIN	0.009x(N+8)	
DNA IQ 1X Wash Buffer	Nx0.36	
DNA IQ Elution Buffer	Nx0.144	

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

Table 8. **Greater than 48 samples** (note difference is in DNA IQ RESIN Solution)

Lysis-DTT Buffer		Volume (in mL)
Lysis Buffer	(Nx1.35)+0.75	
DTT (1M)	Lysis Buffer/100	
Extraction Buffer		
TNE Buffer	Nx0.56	
Prot K (20 mg/L)	Nx0.03	
Sarcosyl (40 %)	Nx0.015	
DNA IQ RESIN Solution		
LYSIS Buffer	0.054x(N+16)	
DNA IQ RESIN	0.009x(N+16)	
DNA IQ 1X Wash Buffer	Nx0.36	
DNA IQ Elution Buffer	Nx0.144	

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

18.2 Reagent & Batch details recording tables (DNA IQ™ Lysis Batch & Extraction Batch)

Off Deck Lysis Batch ID:	DNA IQ Extraction Batch ID:

Lysis batch:

Samples located by:	
Sample set 1	**Sample set 2**
Operator:	Operator:
Sequence check performed by:	Sequence check performed by:
Transfer tubes sequence checked:	Transfer tubes sequence checked:
37°C Incubation temp:	37°C Incubation temp:
65°C Incubation temp:	65°C Incubation temp:

Extraction Buffer made by:	TNE Buffer Lot#:
40% Sarcosyl Lot#:	Proteinase K Lot#:
Comments:	

Extraction batch:

Plate Lot#:	Lysate/STORstar Operators:
Nunc tube/STORstar Operators:	Lysate Logfile uploaded:
Nunc Logfile uploaded:	
Comments:	

MultiPROBE Platform:	Operator:
Date and Start time:	

Kit Lot#:	1xWash Buffer Lot#:
Lysis Buffer Lot#:	DTT Lot#:
Resin Lot#:	Elution Buffer Lot#:
MP II Logfile uploaded:	Results file uploaded:
Comments:	

18.3 Fully automated method for extraction using DNA IQ™

18.3.1 Sampling and Sample Preparation

FTA® Samples waiting for extraction will have been punched into a Slicprep™ 96 device according to "*FTA® Processing*" SOP (QIS document 24823) and stored in the Fridge located in room 6127.

18.3.2 Procedure

Preparation of Reagents prior to extraction

1. Defrost Prot K and DTT
2. Refer to table 2 for reagent volumes to make up the required amount of Extraction Buffer, Lysis Buffer (with DTT) and Resin solution. Also measure the required amount of 1X Wash Buffer.
3. Record the Lot numbers of all the reagents used onto the AUSLAB worksheet.

Setting up the EP-A or EP-B MPIIs

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

4. Turn on the instrument PC.
5. Log onto the network using the **Robotics** login.
6. Double click the WinPrep® icon on the computer desktop (Figure 7).
7. Log onto the WinPrep® software by entering your username and password, then press **"Enter"**.

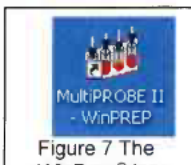

Figure 7 The WinPrep® icon.

8. Ensure the **System Liquid reservoir is FULL** and fully submerged in the system liquid before every run and perform a Flush/Wash.
9. Ensure that the daily/weekly start-up has been performed before running any program. If WinPrep® has been closed or been idle for a long period of time initialise the MP II platform as described in QIS 23939.
10. Open the Extraction setup MP II test file in WinPrep® by selecting:
    - **File**
    - **Open**, navigate to **C:\PACKARD\MULTIPROBE\BIN\QHSS PROTOCOLS**
    - Select "**DNA IQ Extraction_Ver1.3.mpt.**"
    - Click the "**Open**" button
11. Check the whole program there are no bold fonts (e.g. plates may lose gripability and the program will not run). See the line manager.
12. Decontaminate the required labware by wiping with 5% TriGene followed by 70% ethanol before placing the labware on to the designated grid positions as outlined in the virtual deck view within WinPrep® (Figure 2).

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

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

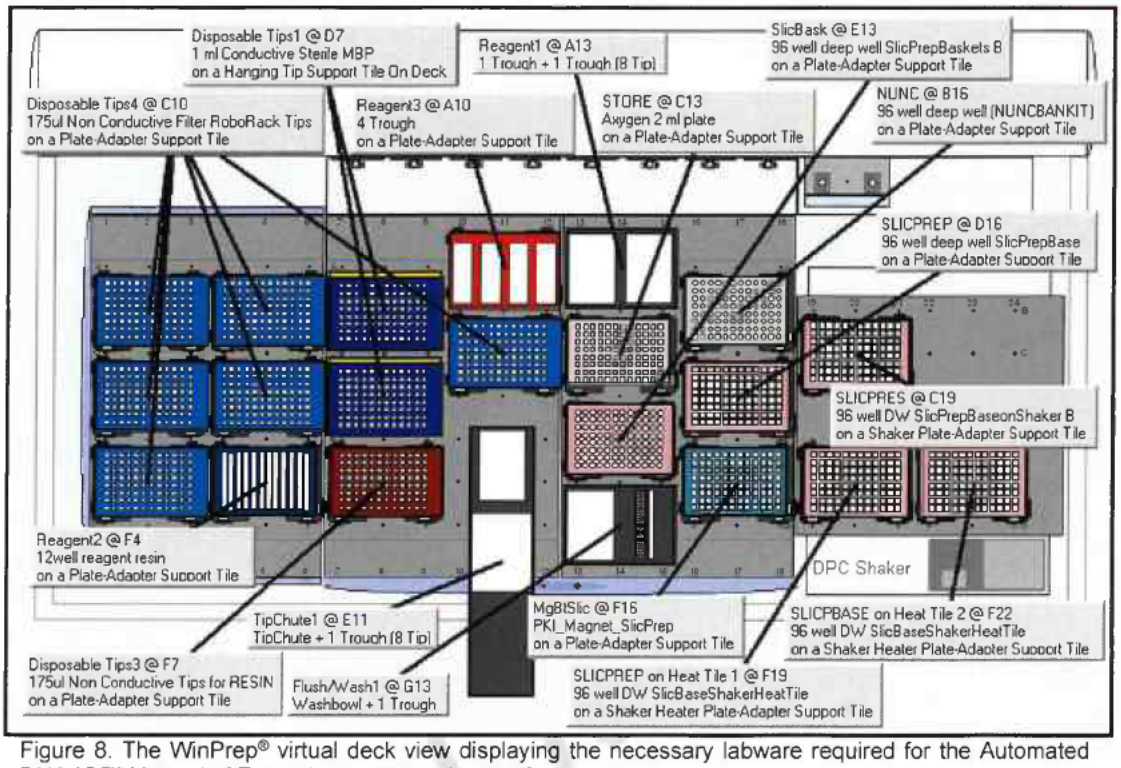

Figure 8. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

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

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

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

16. **Nunc™ Bank-It™ tube rack**: Check that is the same AUSLAB batch number as the platemap to be run. Ensure that the barcode of the first (A1) and last tube (H12) are the respective ones. Attach batch label and batch barcode label to front side of rack. Add B1-Lite generated "**NUNC**" barcode to the right side of the Nunc™ Bank-It™ tube rack. Then place the rack into position **B16**

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

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

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

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

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

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

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

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

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

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

27. Add the Resin to the 12 Channel plate. Pipette mix thoroughly. Then dispense 50uL of resin into each well of the Slicprep™ 96 device.

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

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

30. The next User prompt will appear with the following directions:
"**Ensure Wash Buffer has been added to trough 4 at A10.**
Manually add 50uL resin to each well of the SlicPrep plate
Place the plate in position D16.
Add the Elution Buffer to the 12 channel plate.
THEN
Press OK when ready." Press **"OK"** when steps 27-29 have been performed.

31. Once the lysis and wash steps have been completed, a message will appear waiting for the heating tile to reach 85^{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)

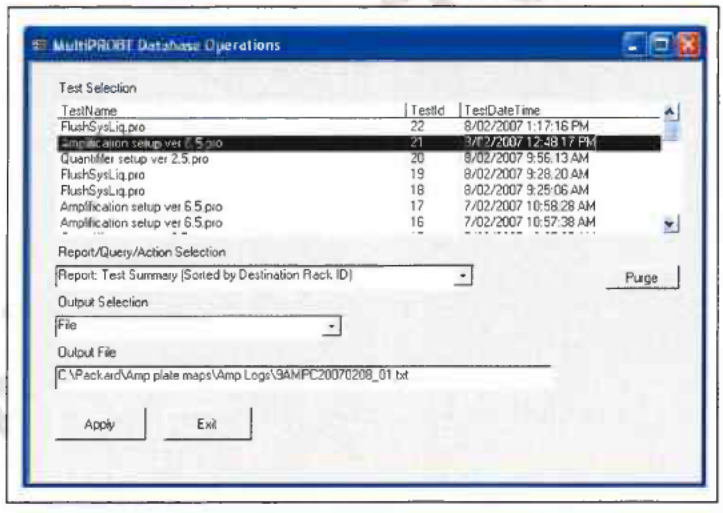

Figure 9. The MultiPROBE log database for collecting MP II run information

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"**.

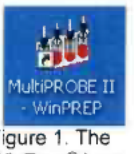

Figure 1. The WinPrep® icon.

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 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 6.4_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°C).
    For EP-B: Tile 2 at F22 (85°C).
    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):

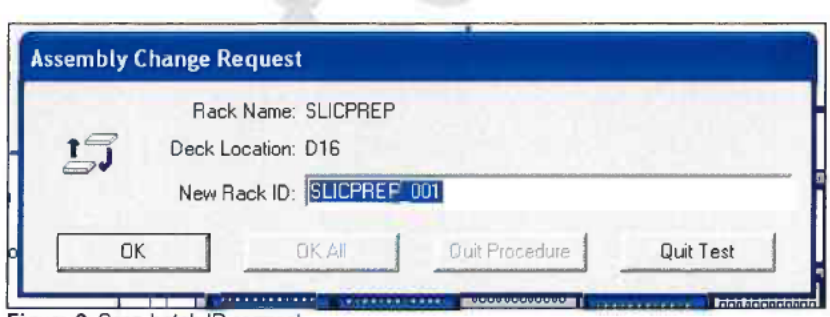

Figure 2. Scan batch ID request

    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 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; Nunc™ Bank-it™ storage tube.

Note: Spin baskets are not required for the Negative Extraction control, tape lifts, nails and other non absorbent substrates. For these samples, excluding the Negative Extraction control, label a 2mL tube instead of a spin basket.

4. Have a second operator perform a sequence check of all tubes. This person must enter the sequence check in AUSLAB.

5. Using Table 7, prepare Extraction Buffer, Lysis Buffer & Resin solution. Ensure that the DNA IQ™ Resin solution is thoroughly vortexed prior to use.
   Note: Reagents need to be prepared fresh before each run and Lysis Buffer-DTT solution and Resin solution need to be prepared in the fume hood.

Table 7. Table of reagent volumes for DNA IQ Manual Extraction

Reagent (volume per sample)	Constituent	Volume per sample (µL)	Volume for 12 Samples (mL)
Extraction Buffer (300µL/sample)	**TNE Buffer**	277.5	4.0
	Prot K (20mg/mL)	15.0	0.216
	Sarcosyl (40% w/v)	7.5	0.108
Lysis Buffer – DTT (726µL/sample)	**Lysis Buffer**	660	10.0
	DTT	6.6	0.1
Resin-Lysis Solution (50µL/sample)	**Lysis Buffer with DTT** (from above)	43	0.645
	DNA IQ RESIN	7	0.105
DNA IQ 1X Wash Buffer (300µL/sample)	See Reagent preparation		4.0
DNA IQ Elution Buffer (100µL/sample)	Use directly from Kit		1.4

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

6. Add 300µL of Extraction Buffer. Ensure that large substrates including tape lifts are fully submerged. Vortex, then incubate at 37°C on the Thermomixer at 1000 rpm for 45 minutes. If no thermomixer available, incubate samples within a hotblock, vortex mix at the start and end of the incubation and at least one during the incubation.

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

8. Transfer the substrate to spin basket. Centrifuge spin basket for 2 minutes at maximum speed. Retain the spin basket in the original 5ml tube and transfer the flow through back into sample tube.

Note: If original sample tube is not a 2mL tube, transfer flow through from spin basket and the supernatant from the original tube into a 2mL tube.

9. For samples that do not require a spin basket, pulse spin and transfer supernatant to the labelled 2mL tube.

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

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

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

13. Remove from the Multitube shaker, vortex and immediately place into the magnetic stand. Separation will occur instantly.
    Note: If resin does not form a distinct pellet on the side of the tube, or if the magnetic beads have re-suspended while in the stand, vortex the tube and quickly place back in the stand.

14. Carefully transfer the solution (supernatant) into the 1.5mL supernatant tube, ensuring that the resin is not disturbed. Remove from the magnetic stand.
    Note: If some resin is drawn up in the pipette tip, gently expel resin back into tube to allow re-separation.

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

16. Once separation has occurred, transfer the Lysis-DTT Buffer to the supernatant 1.5mL tube. Remove from the magnetic stand.

17. Add 100µL of 1X Wash Buffer, vortex and place into the magnetic stand. Once separation has occurred remove and discard the Wash Buffer. Remove from the magnetic stand.

18. Repeat the Wash Buffer step (step 17) two times for a total of three washes. Ensure that all of the solution has been removed after the last wash.

19. In a Biohazard hood, uncap the tubes and place the lids down onto a clean rediwipe. Air-dry the resin in the hood for 15 minutes and then recap tubes and remove from the Biohazard hood. Remove from the magnetic stand.

Note: Do not dry for more than 20 minutes, as this may inhibit the elution of DNA.

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

21. Incubate in the Thermomixer at 65°C for 3 minutes and then continue to incubate for a further 3 minutes shaking at 1100 rpm. If no thermomixer available, incubate samples within a hotblock, vortex mix at the start and end of the incubation. Remove samples.

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

23. Carefully transfer the DNA extract to the corresponding labelled Nunc™ Bank-It™ tube.

24. Remove from the magnetic stand and repeat the Elution Buffer steps (step 20-23). The final volume after the double elution is approximately 95µL of DNA extract.

25. DNA extracts are stored in temporary storage in freezer 6117-2 (-20°C) located in the workflow area. Tubes containing magnetic beads and supernatants are stored in the allocated boxes in freezer 6117-2 (-20°C) located in the workflow area.

26. 5mL tubes containing the origingal substrate are to be stored in spin basket boxes in freezer 6117-5 located in the workflow area.

17.1.5. Procedure (Retain Supernatant)

1. Turn on the Eppendorf Thermomixer and set the temperature to 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; Nunc™ Bank-It™ storage tube.
4. Note: Spin baskets are not required for the Negative Extraction control, tape lifts, nails and other non absorbent substrates. For these samples, excluding the Negative Extraction control, label a 2mL tube instead of a spin basket.
5. Have a second operator perform a sequence check of all tubes. This person must enter the sequence check in AUSLAB.
6. Using Table 8, prepare Lysis Buffer & Resin solution. Ensure that the DNA IQ™ Resin solution is thoroughly vortexed prior to use.
   Note: Reagents need to be prepared fresh before each run and Lysis Buffer-DTT solution and Resin solution need to be prepared in the fume hood.

Table 7. Table of reagent volumes for DNA IQ Manual Extraction

Reagent (volume per sample)	Constituent	**Volume per sample (µL)**	**Volume for 12 Samples (mL)**
Lysis Buffer – DTT (726µL/sample)	**Lysis Buffer**	660	10.0
	DTT	6.6	0.1
Resin-Lysis Solution (50µL/sample)	**Lysis Buffer with DTT** (from above)	43	0.645
	DNA IQ RESIN	7	0.105
DNA IQ 1X Wash Buffer (300µL/sample)	See Reagent preparation		4.0
DNA IQ Elution Buffer (100µL/sample)	Use directly from Kit		1.4

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

7. Add 450µL of TNE buffer and vortex.
8. Incubate at room temperature for 30 minutes.
9. Vortex, then centrifuge at maximum speed (14,000rpm) for 3 minutes.
10. Remove 150µL of supernatant and place into the respective 1.5mL tube labelled with "sup" (for further testing).
11. Add 14µL of 20mg/mL Proteinase K and 7µL 40% (w/v) Sarcosyl to the original sample tube containing substrate and TNE buffer, then vortex.Add 300µL of Extraction Buffer. Ensure that large substrates including tape lifts are fully submerged.
12. Vortex, then incubate at 37°C on the Thermomixer at 1000 rpm for 45 minutes. If no thermomixer available, incubate samples within a hotblock, vortex mix at the start and end of the incubation and at least one during the incubation.
13. Remove from the Thermomixer and increase the temperature on the Thermomixer to 65°C (for use in the Elution steps).
14. Transfer the substrate to spin basket. Centrifuge spin basket for 2 minutes at maximum speed. Retain the spin basket in the original 5ml tube and transfer the flow through back into sample tube.
    Note: If original sample tube is not a 2mL tube, transfer flow through from spin basket and the supernatant from the original tube into a 2mL tube.
15. For samples that do not require a spin basket, pulse spin and transfer supernatant to the labelled 2mL tube.

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

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

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

19. Remove from the Multitube shaker, vortex and immediately place into the magnetic stand. Separation will occur instantly.
    Note: If resin does not form a distinct pellet on the side of the tube, or if the magnetic beads have re-suspended while in the stand, vortex the tube and quickly place back in the stand.

20. Carefully transfer the solution (supernatant) into the 1.5mL supernatant tube, ensuring that the resin is not disturbed. Remove from the magnetic stand.
    Note: If some resin is drawn up in the pipette tip, gently expel resin back into tube to allow re-separation.

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

22. Once separation has occurred, transfer the Lysis-DTT Buffer to the supernatant 1.5mL tube. Remove from the magnetic stand.

23. Add 100µL of 1X Wash Buffer, vortex and place into the magnetic stand. Once separation has occurred remove and discard the Wash Buffer. Remove from the magnetic stand.

24. Repeat the Wash Buffer step (step 17) two times for a total of three washes. Ensure that all of the solution has been removed after the last wash.

25. In a Biohazard hood, uncap the tubes and place the lids down onto a clean rediwipe. Air-dry the resin in the hood for 15 minutes and then recap tubes and remove from the Biohazard hood. Remove from the magnetic stand.

Note: Do not dry for more than 20 minutes, as this may inhibit the elution of DNA.

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

27. Incubate in the Thermomixer at 65°C for 3 minutes and then continue to incubate for a further 3 minutes shaking at 1100 rpm. If no thermomixer available, incubate samples within a hotblock, vortex mix at the start and end of the incubation. Remove samples.

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

29. Carefully transfer the DNA extract to the corresponding labelled Nunc™ Bank-It™ tube.

30. Remove from the magnetic stand and repeat the Elution Buffer steps (step 20-23). The final volume after the double elution is approximately 95µL of DNA extract.

31. DNA extracts are stored in temporary storage in freezer 6117-2 (-20°C) located in the workflow area. Tubes containing magnetic beads and supernatants are stored in the allocated boxes in freezer 6117-2 (-20°C) located in the workflow area.

32. 5mL tubes containing the origingal substrate are to be stored in spin basket boxes in freezer 6117-5 located in the workflow area.

17.1.6. Sample storage

Refer to "DNA Analysis Workflow Procedure" (QIS 24919).

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

1. PURPOSE AND SCOPE

This method describes the routine method for the extraction of DNA using the DNA IQ™ kit (Promega Corp., Madison, WI, USA). The automated method is the preferred procedure, utilising the MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms (PerkinElmer BioDiscovery, Downers Grove, IL, USA). The manual method has also been included. This method applies to all DNA Analysis staff members that are required to extract DNA from samples.

Reference samples and casework samples must be extracted separately. If casework and reference samples are to be extracted on the same instrument, the instrument (including all required labware) must be decontaminated between operations.

2. DEFINITIONS

DNA IQ™ Resin	Magnetic resin beads used to bind DNA
DTT	1,4 Dithiothreitol
EDTA	Ethylenediaminetetraacetatic acid
EP-A	Extraction Platform A
EP-B	Extraction Platform B
Extracts	Samples that have had a DNA extraction processes performed
Lysates	Samples that have had the off-deck lysis step performed, but have not yet completed the entire extraction process
MPII	MultiPROBE® II PLUS HT EX Platform
Paramagnetic	To become magnetic with the application of a magnetic force
Pro K	Proteinase K
Samples	Sample substrates (in tubes) awaiting DNA extraction
Sarcosyl	N-Lauroylsarcosine sodium
TNE	Tris, NaCl and EDTA buffer (10mM Tris, 100mM NaCl, 1mM EDTA, pH 8.0)

3. PRINCIPLE

Sample Pre-lysis

The Extraction Buffer used in the pre-lysis of cellular material contains Proteinase K (Pro K), TNE buffer (10mM Tris, 100mM NaCl, 1mM EDTA, pH 8.0) and Sarcosyl. TNE acts as a basic buffer with EDTA chelating ions in solution. Sarcosyl is a detergent that lyses open cell membranes. Proteinase K is added to digest cellular material, helping the DNA to be released. It also digests proteins that interfere with the DNA binding capacity of the resin. In addition, Proteinase K rapidly inactivates enzymatic activity that could potentially degrade DNA (e.g. nucleases).

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

DNA IQ™ Kit

The DNA IQ™ kit (Promega Corp., Madison, WI, USA) is designed to purify DNA from a number of different substrates and has been optimised for trace DNA samples. An in-house validation was performed using a modified version of the PerkinElmer automated protocol.

The in-house protocol includes:

- Off-deck lysis steps with the option to retain a portion of the supernatant for further testing;
- The use of 300µL Extraction Buffer containing TNE (10mM Tris, 100mM NaCl, 1mM EDTA, pH 8.0) and Proteinase K to lyse cellular material prior to performing the DNA IQ process;
- The use of tubes and spin baskets for off-deck lysis of samples prior to extraction on the MPII platform. At the conclusion of off-deck lysis, lysates are transferred to individual Nunc Bank-It™ tubes;
- Nunc Bank-It™ tubes (arranged in sequence using STORstar) containing lysates are presented to the MPII platform for automated transfer of lysates into a 96-deep well plate;
- DNA IQ™ Resin is added using the MPII platform, followed by addition of two volumes of DNA IQ™ Lysis Buffer;
- The 96-deep well plate containing DNA IQ™ Resin and Lysis Buffer is sealed using an adhesive aluminium film and is placed on a MixMate to mix the contents of each well. The plate is centrifuged and the aluminium film is then pierced using a 96 well half skirt PCR microplate and the plate is returned to the MPII platform;
- A double elution step is performed using two dispenses of DNA IQ™ Elution Buffer at 60µL, resulting in a final DNA extract volume of 100µL;
- DNA extracts are automatically transferred into Nunc Bank-It™ tubes for storage.

Cell lysis is performed using DNA IQ™ Lysis Buffer containing Dithiothreitol (DTT). 1,4 Dithiothreitol is a reducing agent used in extractions to break disulfide bonds of proteins. The Lysis Buffer used is a proprietary buffer containing chaotropic salts required for binding of DNA to the magnetic beads. According to the MSDS (Promega Corporation 2006), the Lysis Buffer contains 50-75% guanidinium thiocyanate, < 2.5% EDTA, < 2% 3-[(3-Choalamidopropryl)dimethylammonio] propanesulfonic and < 2% polyethylene glycol tert-octylphenyl ether.

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

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

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

The DNA IQ™ Elution Buffer removes the DNA from the magnetic bead resin. The Elution Buffer changes the salt content. Heating the complex to 65°C allows the DNA to be released from the magnetic beads. The Elution Buffer is a low ionic strength buffer that

reduces the affinity of the DNA for the silica that covers the magnetic beads, by re-hydration of the phosphate backbone.

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

MultiPROBE® II HT EX PLUS with Gripper™ Integration Platform
Within DNA Analysis, routine DNA extractions are performed on casework or reference samples using two MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms (EP-A or EP-B) located in Room 6127.

Each platform uses a computer-controlled Cartesian XYZ liquid handling system designed for the efficient automation of sample preparation. Liquid transfer is performed by an 8-tip system with VersaTip® and VariSpan™ options. The VersaTip® option allows the use of both fixed and disposable tips (conductive and non-conductive). The VariSpan™ option permits variable probe spacing between each of the individual probes so that a wide range of labware such as micro plates, tubes, vials and reagent troughs can be accessed. Each sample probe is capable of independent motion in the Z direction due to independent Z drives.

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

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

For automated DNA extraction using the DNA IQ™ kit, a platemap is utilised to provide the necessary information for correct volumes and locations for pipetting. It also contains information regarding batch and plate identification, sample positions, lab numbers of samples, and lists all the samples in the batch, providing the total number of samples. The program will fail to work without a platemap.

4. REAGENTS AND EQUIPMENT

4.1. Reagents

- DNA IQ™ System Kit (400 sample kit)
  - DNA IQ™ Resin
  - Lysis Buffer (LB)
  - 2x Wash Buffer (2xWB)
  - Elution Buffer (EB)
- TNE (10mM Tris, 100mM NaCl, 1mM EDTA, pH 8.0)
- Proteinase K (Pro K) 20mg/mL
- Dithiothreitol (DTT) 1M
- 5% TriGene
- 70% Ethanol
- 10% Bleach 7x Solution
- 1% Amphyl
- 0.2% Amphyl
- Isopropyl Alcohol
- AnalR 100% Ethanol

- 40% Sarcosyl
- Nanopure Water

These reagents are stored in locations as per Table 1.

Table 1. Reagent storage locations.

Reagent	Device	Storage Location
Pro K	Freezer	Room 6120
DTT	Freezer	Room 6120
40% Sarcosyl	Shelf	Room 6122
Isopropyl Alcohol	Shelf	Room 6122
AnalR 100 %Ethanol	Shelf	Room 6122
TNE pH 8 Buffer	Shelf	Room 6122
DNA IQ™ Kit	Shelf	Room 6122
Amphyl (1% and 0.2%)	Shelf	Room 6127
Nanopure Water	Shelf	Room 6127
5% TriGene	Shelf	Room 6127
10% Bleach 7x Solution	Shelf	Room 6127

Table 2 shows the volume of reagents for a full plate or half plate. All reagents can be made on the bench, with the exception of the Lysis Buffer-DTT which needs to be made in a fume hood. DNA IQ™ reagents are prepared by staff performing the method. Refer to "*Receipt, Storage and Preparation of Chemicals, Reagents and Kits*" (QIS 17165) for preparation of TNE Buffer.

Table 2. Table of reagent volumes.

Reagent	Volume for 96 samples (mL)	Volume for 48 samples (mL)	Volume for 24 samples (mL)
Extraction Buffer			
TNE buffer	33.3	20	10
Proteinase K (20mg/mL)	1.8	1.08	0.54
Sarcosyl (40%)	0.9	0.54	0.27
Lysis-DTT Buffer			
DNA IQ™ Lysis Buffer	90.0	50	N/A
DTT (1M)	0.9	0.5	N/A
DNA IQ™ Resin solution			
Lysis-DTT Buffer	6.0	3	N/A
DNA IQ™ Resin	1.0	0.5	N/A
DNA IQ™ 1x Wash Buffer	35.0	18	N/A
DNA IQ™ Elution Buffer	14.0	8	N/A

Note: Do not directly pipette from reagent stock bottles. Stock reagents should be decanted into working containers where appropriate. Volume for 24 samples is for off-deck lysis samples only.

4.2. Extraction Buffer

Note: Prepare Extraction Buffer just prior to commencing the off-deck lysis or extraction procedure.

1. Determine the required volumes of reagents by using Table 2.
2. Remove the required amount of 20mg/mL Proteinase K from the freezer and thaw. Vortex and centrifuge before use.
3. Ensure that the 40% (w/v) Sarcosyl is completely dissolved and appears clear in the stock solution. If not dissolved, invert the container a few times and leave at room temperature.
4. Retrieve an aliquot of TNE buffer of the appropriate volume size from the falcon tube storage container in Room 6122.
5. Add the appropriate volumes of 20mg/mL Proteinase K and 40% (w/v) Sarcosyl to the falcon tube containing TNE buffer, and invert gently to mix.
6. Label the tube with "Extraction Buffer", your initials and the date.

4.3. Lysis Buffer with DTT Solution

Note: Lysis Buffer is supplied with the DNA IQ™ kit. The Lysis Buffer with DTT solution is prepared just prior to commencing the extraction procedure.

Warning: Lysis Buffer and DTT are toxic, use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling Lysis Buffer.

1. Determine whether a half- or full-plate of reagents are required (Table 2).
2. Remove the required amount of DTT from the freezer and thaw. Vortex and centrifuge before use.
3. In the fume hood add the required volume of Lysis Buffer to a sterilised glass Schott bottle and then add the required volume of DTT.
4. Label the glass Schott bottle with "Lysis Buffer + DTT", your initials and the date.

4.4. DNA IQ™ Resin

Note: DNA IQ™ Resin is supplied with the DNA IQ™ kit. The resin is prepared at the start of each run. Ensure the resin is properly mixed by *vortexing* prior to use.

1. Determine whether a half- or full-plate of reagents are required (Table 2).
2. Into a 10mL (or 5mL) sterile tube, add the required volume of Lysis Buffer with DTT solution (from 4.1.2) followed by the required volume of DNA IQ™ Resin.
3. Mix by gentle inversion.
4. Label the tube with "Resin", your initials and the date.

4.5. 1x Wash Buffer

Note: 2x Wash Buffer is supplied with the DNA IQ™ kit. To prepare 1x Wash Buffer, add 35mL of *AnalR* Ethanol and 35mL of Isopropyl Alcohol to the 2x Wash Buffer bottle. Then label the lid and side of the bottle with "1x Wash Buffer," your initials and the date. Also fill out the Reagent Log (DNA IQ Reagents).

1. Determine whether a half- or full-plate of reagents are required (Table 2).
2. Into a Falcon tube, add the required volume of 1x Wash Buffer.
3. Label the falcon tube with "Wash Buffer", your initials and the date.

4.6. Elution Buffer

Note: Elution Buffer is supplied with the DNA IQ™ kit. The Elution Buffer can be used directly from the kit. The Elution Buffer is removed from the kit and stored in the automated extraction room (6127).

5. EQUIPMENT

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

Table 3. Equipment used and location.

Equipment	Asset No.	Location
STORstar (B system)	10238493	6122
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (EP-A)	10076438	6127
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (EP-B)	10076437	6127
DPC shaker (EP-A)	N/A	6127
DPC shaker (EP-B)	N/A	6127
Automated Temperature Controller, Heat Block tiles and heat block adapters (EP-A)	N/A	6127
Automated Temperature Controller, Heat Block tiles and heat block adapters (EP-B)	N/A	6127
Eppendorf 5804 centrifuge	10238416	6127
Vortex	30087015	6127
Fridge	30433424	6127
Micro centrifuge	None	6127
MixMate		6127
Decapper	None	6127
4titude 4seal Sealer	30512847	6127

Table 4. Consumables used for extraction.

Consumables	Location
175µL Clear Non-Conductive Filter RoboRack tips – Pre-Sterilised	6127
MβP Pure 1000uL Tips – Pre-Sterilised	6127
SlicPrep™ 96 device plate	6122
ABgene 96-deep well plate	6120
Axygen 2mL deep well storage plate	6127
96 well Half Skirt PCR Microplate	6127
1.5mL or 2mL Eppendorf tubes with spin baskets	6120
12 Channel plate	6127
Nunc Bank-it™ tubes	6120
Nunc Bank-it™ caps	6127
Sterile 50mL Falcon tubes	6122
Sterile 10mL or 5mL tubes	6122
Autoclaved 100mL glass bottles	6122
Autoclaved 250mL glass bottles	6122
Aluminium sealing film	6127
300µL ART tips	6120
1000µL ART tips	6120

6. SAFETY

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

The automated platforms, labware and associated equipment should be wiped with 5% TriGene™ followed by 70% Ethanol before and after use. In addition, clean the work area around the MPII instrument with 10% (v/v) Bleach 7x and 70% Ethanol.

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

Warning: Tris base, EDTA, Sarcosyl and Lysis Buffer are irritants. DTT is a reducing agent that destroys disulfide bonds. Handle carefully and wear appropriate PPE.

Lysis Buffer contains guanidinium thiocyanate (GuSCN) which is toxic and can be harmful if inhaled, swallowed or comes in contact with skin. Left over Lysis Buffer-DTT is disposed of

in a brown Winchester bottle in the fume hood. Never dispose down the sink. If spillage occurs, absorb with liquid-binding material (such as sand or kitty litter) and dispose in a biohazard bin. Do not combine bleach with Lysis Buffer and do not wipe areas of Lysis Buffer spillage with bleach, as the two reagents produce cyanide gas when combined. Handle carefully and wear appropriate PPE, including safety glasses, when handling Lysis Buffer. If Lysis Buffer is spilt onto PPE (eg. gloves, gowns), discard the PPE and obtain new PPE.

7. SAMPLING AND SAMPLE PREPARATION

7.1. Sample Locations

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

Table 5. Sample storage locations.

Sample type	Storage Device	Storage Location
Urgent/High/Medium Priority Samples	Freezer	6117-2
Lysates in 1.0mL Nunc Bank-it™ tubes	Freezer	6117
Extracts in 1.0mL Nunc Bank-It™ tubes	Freezer	6117

Note: Some Medium and Low Priority storage boxes may be located in the Exhibit Room (6106).

7.2. QC Samples

For all off-deck lysis batches (with 48 samples or less) and extraction batches; one negative control and one positive control is required to be registered. For all off-deck lysis batches with > 48 samples; two negative and two positive controls is required to be registered.

Table 6. Extraction Quality Controls

QC Name	Batch Type	Description
Negative Control	Off-Deck Lysis	Negative Extraction control – empty well
Positive Control	Off-Deck Lysis	Positive Extraction control – dried blood swab from a known donor
Negative Control	IQ Extraction	Negative Extraction control – empty well
Positive Control	IQ Extraction	Internal IQ Efficiency Control

7.2.1. Registration of QC Samples

The registration of control samples is covered in the DNA Analysis workflow procedure (QIS 24919)

7.3. Create the DNA IQ™ Lysis or Retain Supernatant batch

Creation of Lysis and retain supernatant batches is covered in the DNA Analysis Workflow Procedure (QIS 24919).

7.4. Locating Samples

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

8. OFF-DECK LYSIS PROCEDURE

8.1. Off-Deck Lysis (No Retained Supernatant)

1. For batches of 48 samples or less, one set of controls and one operator is required. For larger batches, separate the batch into two smaller batches of 48 samples, including one set of controls in each. If a single operator is performing the whole procedure, the second batch can be started during an appropriate incubation step.

   Note: For full batches positions 1-4 will be the two sets of controls, positions 5-50 will be the first 46 samples and 51-96 will be the second set of 46 samples.

2. For each sample label:
   - Original sample tube
   - Spin basket or 1.5mL tube as required
   - 1.0mL Nunc Bank-It™ tube

   Note 1: Samples that require a spin basket are: swabs, fabric, paper and cigarette butts. Samples that require a 1.5mL tube are: tapelifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

   Note 2: If samples are in a 2mL QPS tube and require a spin basket, label a new tube for the substrate to be retained in.

3. If substrate is in a 0.5mL tube, transfer to a labelled 1.5mL tube.

4. Have a second operator sequence check the tubes and enter the sequence check in AUSLAB.

5. Prepare Extraction Buffer as per Section 4.1.1.

6. Add 300µL of Extraction Buffer and vortex briefly. Ensure that substrates are immersed in the Extraction Buffer.

7. Incubate on a Thermomixer at 37°C for 45 minutes at 1000rpm or on the hotblock (if using hotblock vortex samples at 22-23 minutes and again at the end of the incubation). Record temperature on worksheet.

8. Remove from the Thermomixer/hotblock. Transfer substrate to spin basket if required or transfer the lysate to an appropriately labelled 1.5mL tube. Retain original tube containing the substrate in if no spin basket used.

9. Centrifuge spin basket at maximum speed (14,000rpm) for 2 minutes.

10. Retain spin basket containing the substrate and transfer flow through back to original lysis tube.

11. Vortex lysate, then incubate in hotblock/Thermomixer at 65°C for 10 minutes. Record temperature on worksheet.

12. Centrifuge at maximum speed (14,000rpm) for 1 minute.

13. Transfer 300uL of lysate to the corresponding Nunc Bank-It™ tube.

Note: If more than 300uL of lysate is present, retain the remaining lysate in the 1.5mL tube. In AUSLAB, write a specimen note for that sample (eg. "extra lysate retained from sample XXXXXXXXX."). Store the retained 1.5mL lysate tube in appropriate box in freezer.

14. In AUSLAB, enter reagent and hotblock/Thermomixer temperature details and complete the batch.

15. Transfer substrates from spin baskets to an appropratiely labelled tube (may use original sample tube if no remaining lysate)

16. Store lysates in temporary storage boxes in freezer 6117-2 (-20°C). Store tubes containing substrates in "Spin Basket boxes" in freezer 6117-5 (-20°C).

8.2. Off-Deck Lysis (Retained Supernatant)

1. For batches of 48 samples or less, one set of controls and one operator is required. For larger batches, separate the batch into two smaller batches of 48 samples, including one set of controls in each. If a single operator is performing the whole procedure, the second batch can be started during step 7.

   Note: For full batches positions 1-4 will be the two sets of controls, positions 5-50 will be the first 46 samples and 51-96 will be the second set of 46 samples.

2. For each sample label:
   - Original sample tube
   - Spin basket or 1.5mL tube as required
   - 1.5mL tube (also labelled with "**sup**" to indicate supernatant)
   - 1.0mL Nunc Bank-It™ tube

   Note 1: Samples that require a spin basket are: swabs, fabric, paper and cigarette butts. Samples that require a 1.5mL tube are: tapelifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

   Note 2: If samples are in a 2mL QPS tube and require a spin basket, label a new 5mL tube for the substrate to be retained in.

3. If substrate is in a 0.5mL tube, transfer to a labelled 1.5mL tube.

4. Have a second operator sequence check the tubes and enter the sequence check in AUSLAB.

5. Add 450µL of TNE buffer and vortex.

6. Incubate at room temperature for 30 minutes.

7. Vortex, then centrifuge at maximum speed (14,000rpm) for 3 minutes.

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

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

10. Incubate in Thermomixer at 37°C for 45 minutes at 1000rpm or on the hotblock (if using hotblock vortex samples at 22-23 minutes and again at the end of the incubation). Record temperature on worksheet.

11. Remove from the Thermomixer/hotblock. Transfer substrate to spin basket if required or transfer the lysate to an appropriately labelled 1.5mL tube. Retain original tube containing the substrate in if no spin basket used.

12. Centrifuge spin basket at maximum speed (14,000rpm) for 2 minutes.

13. Retain spin basket containing the substrate and transfer flow through back to original lysis tube.

14. Vortex Lysate, then incubate in hotblock/Thermomixer at 65°C for 10 minutes. Record temperature on worksheet.

15. Centrifuge at maximum speed (14,000rpm) for 1 minute.

16. Transfer 300uL of lysate to the corresponding Nunc Bank-It™ tube.

Note: If more than 300uL of lysate is present, retain the remaining lysate in the 1.5mL tube. In AUSLAB, write a specimen note for that sample (eg. "extra lysate retained from sample XXXXXXXXX."). Store the retained 1.5mL lysate tube in appropriate box in freezer.

17. In AUSLAB, enter reagent and hotblock/Thermomixer temperature details and complete the batch.

18. Transfer substrates from spin baskets to an appropratiely labelled tube (may use original sample tube if no remaining lysate)

19. Store supernatants in the "S/N Retention" boxes in Freezer 6117-2 (-20°C). Store lysates in temporary storage boxes in freezer 6117-2 (-20°C). Store tubes containing substrates in "Spin Basket boxes" in freezer 6117-5 (-20°C).

9. AUTOMATED EXTRACTION OF LYSED SAMPLES

9.1. Create the DNA IQ Extraction batch

Creation of extraction batch is covered in the DNA Analysis Workflow Procedure (QIS 24919).

9.2. Locating samples

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

9.3. Sequence checking the Nunc Bank-It™ tubes

The procedure for the automated checking of sample tubes is covered in the Procedure for the use of the STORstar unit for automated sequence checking (QIS 24256)

9.4. MPII Extraction Procedure

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

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

9.5. Summary of DNA IQ™ Extraction Version 6.5_ODL (following off-deck lysis)

1. ***Transfer of lysates from Nunc Bank-It™ tubes into the ABgene 96-deep well plate***
   Lysates from the off-deck lysis protocol, contained in individual Nunc Bank-It™ tubes, are transferred automatically into an ABgene 96-deep well plate prior to commencing automated sample processing. Instead of a 96-deep well plate, the use of individual Bank-It™ tubes for storage of lysates removes the need for an adhesive film and therefore reduces the risk of well-to-well contamination from the action of peeling back an adhesive plate cover.

2. ***Automated addition of DNA IQ™ Resin and Lysis Buffer***
   DNA IQ™ Resin is added automatically into the ABgene 96-deep well plate on the platform. The program uses a waste mode dispensing step to add 53µL DNA IQ™ Resin-Lysis Buffer solution to each well in either half the plate or the whole plate. Because of this, batch sizes are restricted to either 48 or 96 samples in order to

maintain efficiency and economy. Two volumes of Lysis Buffer are then added to promote binding of DNA to the paramagnetic resin.

3. ***Mixing using a MixMate to bind DNA to resin***
   Manual intervention is required to seal the ABgene 96-deep well plate with a 4titude Pierce Seal and sealing plate using 4titude sealer pre-heated at 175 °C. The plate is then transferred onto a MixMate instrument for mixing at 1100rpm for 5 minutes, followed by centrifugation on an Eppendorf 5804 centrifuge at 3000rpm for 2 minutes. The seal is then carefully pierced with a 96 well PCR microplate and the ABgene plate is returned to the Applied Biosystems magnet on the MPII platform.

4. ***Removing lysis reagents for storage***
   At this point, most of the DNA is bound to the paramagnetic resin. With the positioning of the ABgene plate on the ABI magnet, DNA IQ™ Resin becomes immobile at the bottom of the plate. The lysis reagents from each well are transferred automatically to a storage plate on the MPII platform without disturbing the DNA IQ™ Resin. The purpose of the storage plate is for retaining supernatant that may potentially still contain DNA material. The storage plate may also become useful in quality investigations.

5. ***Washing of the resin-DNA complex***
   Washing steps are performed to remove any inhibitors in solution. The first wash uses 125µL Lysis Buffer with shaking at room temperature for 1 minute on the DPC shaker to ensure that the DNA is bound to the paramagnetic resin. The plate is moved to the ABI magnet and the supernatant is transferred into the storage plate. The next three washes incorporate 100µL of 1x Wash Buffer with shaking at room temperature for 1 minute on the DPC shaker. During each wash cycle, the ABgene 96-deep well plate is moved to the ABI magnet and the supernatant is discarded into the tip chute.

6. ***Removing any excess of 1x Wash Buffer***
   The samples are allowed to air dry at room temperature for 5 minutes in order to remove all traces of Ethanol from the Wash Buffer. The presence of Ethanol may potentially inhibit both the elution process and also downstream PCR.

7. ***Elution of DNA from the resin-DNA complex***
   A double elution method is employed in this procedure. At each elution step, 60µL of Elution Buffer is added to each sample, followed by incubation at 65°C for 6 minutes (3 minutes without shaking followed by 3 minutes shaking on the DPC shaker). The ABgene plate is moved to the ABI magnet and the eluted solution (supernatant containing eluted DNA) is transferred to fresh Nunc™ Bank-It™ tubes.

8. ***Flushing of capillaries***
   As a decontamination measure, the MPII capillaries and liquid pathway are washed with Amphyl and Nanopure water at the conclusion of the automated extraction process.

9.6. Preparation of reagents for the automated extraction process

Note: Reagents are prepared during the setting up of the MPII platforms (Section 4.3).

9.7. Setting up the MPII platforms for automated DNA IQ™ processing

The following steps are carried out in the automated extraction room (Room 6127).

1. Remove the Nunc Bank-It™ tubes containing lysates from the fridge to allow to come to room temperature before commencing the extraction procedure.

   Note: If the lysates are frozen, remove them from the freezer and thaw in Room 6127. Also remove the required amount of DTT to thaw.

2. Restart or turn on the instrument PC.

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

4. Open WinPrep® by double clicking icon on the computer desktop (Figure 1).

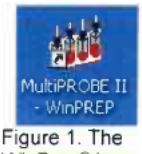

Figure 1. The WinPrep® icon.

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

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

7. Ensure the **System Liquid reservoir is FULL** and tubing is fully submerged in the system liquid before every run and perform a Flush/Wash. If visible air bubbles have appeared in tubing or in syringes between setting up the deck and executing the test, another flush wash will need to be performed before starting the MPII extraction run.

8. Open the Extraction setup MP II test file in WinPrep® by selecting:
   - **File**
   - **Open**, navigate to **C:\PACKARD\MULTIPROBE\BIN\QHSS PROTOCOLS**
   - Select "**DNA IQ Extraction_Ver 6.5_ODL.mpt**"
   - Click the "**Open**" button

9. Check the tree pane of the whole program for any bold fonts. See the Analytical Senior Scientist if bold fonts are present.

10. Copy the required plate map from the network folder **I:\EXTRACTION** into the local folder **C:\PACKARD\EXT PLATE MAPS.** Open the plate map using Excel and check that the plate map is complete, and make changes if necessary. If there are blanks (samples that have been removed), make all volumes to 0. If it is not a full batch, delete the rows of blank samples at the end of the platemap. Save all changes made to the platemap (as a .txt file).

11. Decontaminate the required labware with 5% TriGene followed by 70% Ethanol and place onto the instrument deck in the designated grid positions (as shown in the WinPrep® software). Additionally, ensure the DPC shaker is positioned properly.

12. Ensure that the DPC shaker and Heater Controller Box are switched on.
    For EP-A: Tile 1 at F22 (85°C).
    For EP-B: Tile 2 at F22 (85°C).
    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):

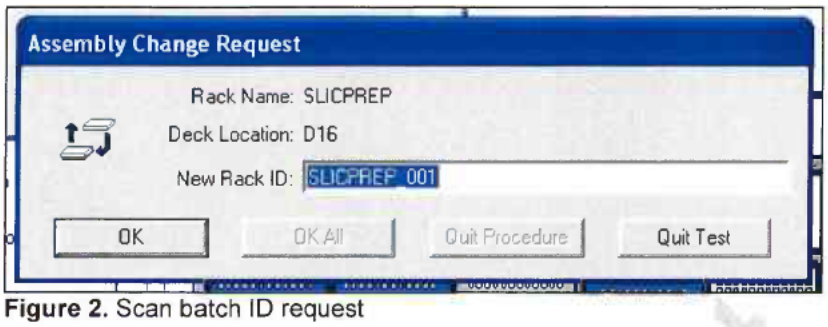

Figure 2. Scan batch ID request

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\EXT A MPII\Logs\CWIQEXT20071115_01.csv) and press **[Enter]**. For more details on importing files into AUSLAB refer to Batch functionality in AUSLAB SOP (QIS 24469).

9.10. Importing Extraction "results" into AUSLAB

1. Import the results file, entering the filename and extension. For more details on importing files into AUSLAB refer to Batch functionality in AUSLAB SOP (QIS 24469).
2. The file will be imported into AUSLAB and appear in the DNA file table.
3. Highlight entry and press **[Enter]**, for access to the DNA results table.
4. Page down through the table and check that all sample results have been imported.
5. Press **[SF8]** ***Table Sort Order***, this sorts the table, sorting samples that have failed Autovalidation to the top. Samples that have failed are those that have processing comments present.
6. For samples that have failed, check the **Processing Comments,** by entering into the sample.
7. If processing comments state sample is to be sent to another batch type **other** than quant. Proceed with the following steps:
   a. Request the appropriate rework test code via the **[SF7]** results history table and the **[SF8]** request rework functions (e.g. samples requiring Microcon, NucleoSpin and pooling).
   b. Press **[Esc]** to exit back to the DNA results table. Do not toggle accept.
   c. Add the extraction batch ID into the 9PLEX or 9FTAR completed date fields in AUSLAB.
8. If processing comments do not state next step the sample will be processed as normal:
   a. Press **[Esc]** to exit back to the DNA results table.
   b. Highlight any entries to be changed and press **[SF7]** ***Toggle Accept.***
9. Press **[F7]** ***Complete Batch***, all of the samples with a ***Yes*** in the Accept column will be transferred to the outstanding Quant Casework or Quant Reference lists.
10. File the extraction worksheet into the relevant folder in Room 6117.

9.11. Sample Storage

Refer to "Analytical Sample Storage" (QIS 24255) for how to store the DNA extract Nunc™ Bank-It™ tubes, ABgene 96-deep well and Axygen store plates.

10. TROUBLESHOOTING

1. If the resin is not pipette mixing correctly (eg. resin is not being drawn up into the pipette tip), pause the Winprep program and check that the support tile is clicked onto the MPII deck correctly. Alternatively, pipette and mix resin manually one more time from the corresponding column in the 12 channel plate.

2. If the Gripper is not placing the rack/plate properly on the deck, pause the program and manually adjust the rack/plate properly on the adapter support tile.

3. If the Gripper appears to be not gripping a plate correctly, or the pipetting alignment of a labware appears to be incorrect, the deck and / or labware can be re-calibrated. This is covered in the Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform SOP (QIS 23939)

11. VALIDATION

- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 9. Automation: Report on the Evaluation of DNA Extraction Chemistries." June 2007.

- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 11: Report on the Validation of the Automated Extraction Chemistry Kit using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Casework Platform." 2007.
- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 13: Report on the Verification of the Automated Extraction Chemistry Kit using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Casework Platform." 2007.
- Muharam I., Hlinka V., Gallagher, B., Lundie, G., Iannuzzi, C., Nurthen T., McNevin A., Ientile V. "Project 21: A Modified DNA IQ™ Method Consisting of Off-Deck Lysis to Allow Supernatant Retention for Presumptive Identification of α-Amylase" 2008
- Muharam I., Hlinka V., Gallagher, B., Lundie, G., Iannuzzi, C., Nurthen T., McNevin A., Ientile V. "Project 22: A Modified DNA IQ™ Method for Off-Deck Lysis Prior to Performing Automated DNA Extraction" 2008

12. QUALITY ASSURANCE/ACCEPTANCE CRITERIA

- Positive and negative (reagent blank) controls are included in each extraction batch as per Table 6. These controls are processed as normal samples through to completion.
- If any results are obtained from the reagent blank, either at quantification or during the CE QC check, then the possible source of the contamination is investigated. The samples extracted with this control are thoroughly checked and repeated if possible.

13. REFERENCES

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7. Mandrekar, P., V., Flanagan, L., & Tereba, A., Forensic Extraction and Isolation of DNA Form Hair, Tissue and Bone. Profiles in DNA, 2002: p. 11.
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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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°C).
    For EP-B: Tile 2 at F22 (85°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. 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):

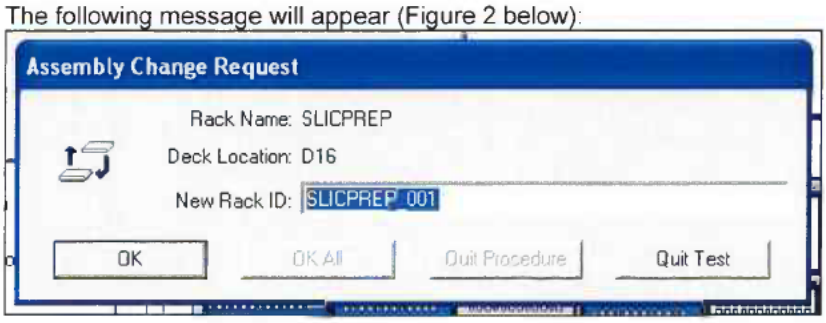

Figure 2. Scan batch ID request

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 37°C. 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 °C, allows the DNA to be released from the magnetic beads. The Elution Buffer is a low ionic strength buffer that reduces the affinity of the DNA for the silica that covers the magnetic beads, by re-hydration of the phosphate backbone. To prevent PCR inhibition caused by the presence of very small DNA fragments within extracts the DNA IQ™ System selectively isolates DNA fragments greater than 80bp.

3.2 OFF-DECK LYSIS PROCEDURE

Samples must undergo a pre-extraction Off-Deck Lysis step before processing with the DNA IQ™ reagents on the MPII instrument.

The Extraction Buffer used for the Off-Deck Lysis is designed to digest cellular material and release DNA. It contains TNE buffer (Tris, NaCl, EDTA, pH 8.0), Sarcosyl and Pro K.

TNE acts as a basic buffer with EDTA chelating ions in solution.

Sarcosyl is a detergent that releases cellular material from the substrate.

Pro K is an enzyme from the fungus *Engyodontium album* (formerly *Tritirachium album*). As a highly active endopeptidase, it cleaves peptide bonds adjacent to the carboxyl group of N-substituted (blocked alpha amino) hydrophobic, aliphatic and aromatic amino acids. Pro K is added to the extraction buffer to digest cellular material, helping the DNA to be released. It also digests proteins that interfere with the DNA binding capacity of the resin and rapidly inactivates enzymatic activity that could potentially degrade DNA (e.g. nucleases).

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°C).
    For EP-B: Tile 2 at F22 (85°C).

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):

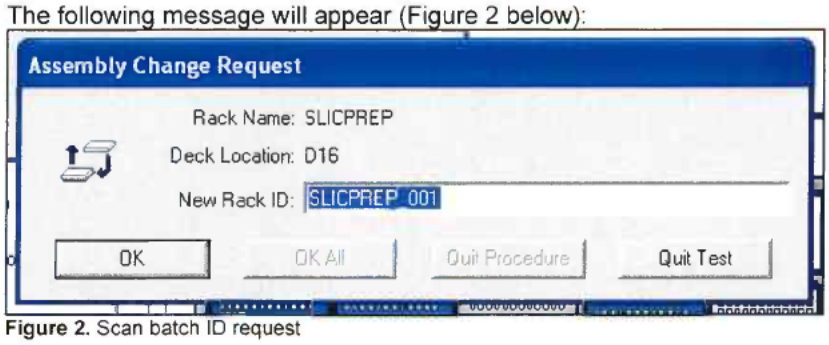

Figure 2. Scan batch ID request

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

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- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 11: Report on the Validation of the Automated Extraction Chemistry Kit using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Casework Platform." 2007.
- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 13: Report on the Verification of the Automated Extraction Chemistry Kit using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Casework Platform." 2007.
- Muharam I., Hlinka V., Gallagher, B., Lundie, G., Iannuzzi, C., Nurthen T., McNevin A., Ientile V. "Project 21: A Modified DNA IQ™ Method Consisting of Off-Deck Lysis to Allow Supernatant Retention for Presumptive Identification of α-Amylase" 2008
- Muharam I., Hlinka V., Gallagher, B., Lundie, G., Iannuzzi, C., Nurthen T., McNevin A., Ientile V. "Project 22: A Modified DNA IQ™ Method for Off-Deck Lysis Prior to Performing Automated DNA Extraction" 2008

11.3 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 °C, allows the DNA to be released from the magnetic beads. The Elution Buffer is a low ionic strength buffer that reduces the affinity of the DNA for the silica that covers the magnetic beads by re-hydration of the phosphate backbone. To prevent PCR inhibition caused by the presence of very small DNA fragments within extracts the DNA IQ™ System selectively isolates DNA fragments greater than 80bp.

3.2 OFF-DECK LYSIS PROCEDURE

Samples must undergo a pre-extraction Off-Deck Lysis step before processing with the DNA IQ™ reagents on the MPII instrument.

The Extraction Buffer used for the Off-Deck Lysis is designed to digest cellular material and release DNA. It contains TNE buffer (Tris, NaCl, EDTA, pH 8.0), Sarcosyl and Pro K.

TNE acts as a basic buffer with EDTA chelating ions in solution.

Sarcosyl is a detergent that releases cellular material from the substrate.

Pro K is an enzyme from the fungus *Engyodontium album* (formerly *Tritirachium album*). As a highly active endopeptidase, it cleaves peptide bonds adjacent to the carboxyl group of N-substituted (blocked alpha amino) hydrophobic, aliphatic and aromatic amino acids. Pro K is added to the extraction buffer to digest cellular material, helping the DNA to be released. It also digests proteins that interfere with the DNA binding capacity of the resin and rapidly inactivates enzymatic activity that could potentially degrade DNA (e.g. nucleases).

3.3 MULTIPROBE® II HT EX PLUS WITH GRIPPER™ INTEGRATION PLATFORM

Within Forensic DNA Analysis, DNA extractions are performed on casework or reference samples using two MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms (MPII-A or MPII-B) located in Room 3191.

Each platform uses a computer-controlled Cartesian XYZ liquid handling system designed for the efficient automation of sample preparation. Liquid transfer is performed by an 8-tip system with VersaTip® and VariSpan™ options. The VersaTip® option allows the use of both fixed and disposable tips (conductive and non-conductive). The VariSpan™ option permits variable probe spacing between each of the individual probes so that a wide range of lab-ware such as micro plates, tubes, vials and reagent troughs can be accessed. Each sample probe is capable of independent motion in the Z direction due to independent Z drives.

The 8-tip system is also capable of multichannel liquid-level sensing by utilising Accusense™ technology. This technology works by each probe detecting a change in capacitance when in contact with the liquid. This capacitive mode of detection is also

possible when using conductive disposable tips. Accusense™ also permits the detection of low or non-ionic polar solutions and solvents. Pipetting on the platforms is driven by 8 individually controlled syringes via positive displacement of the system liquid (nanopure water) when transferring liquid.

The Gripper™ Integration allows for automated identification of lab-ware via the scanning of barcodes and also allows for automated transfer of plates from one position on the deck to another. To increase deck space and capacity, the platforms are equipped with a left deck extension.

For automated DNA extraction using the DNA IQ™ kit, a plate map is utilised to provide the necessary information for correct volumes and locations for pipetting. It also contains information regarding batch and plate identification, sample positions, lab numbers of samples, and lists all the samples in the batch, providing the total number of samples. The program will fail to work without a plate map.

4 REAGENTS AND EQUIPMENT

4.1 REAGENTS

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

Table 1 Reagents with storage room and location

Reagent	Room	Location
Amphyl (0.2 and 1%)	3191	Sink
DNA IQ™ Elution Buffer	3188	In-use tray or shelf
DNA IQ™ Lysis Buffer	3188	In-use tray or shelf
DNA IQ™ Resin	3188	In-use tray or shelf
DNA IQ™ 2x Wash Buffer	3188	In-use tray or shelf
DTT (1M)	3188	Freezer
70 % v/v ethanol	3188, 3189, 3191	Sink or bench
100 % v/v ethanol	3188	Shelf
5 % v/v Hypo 10 bleach	3188, 3189, 3191	Sink or bench
Isopropyl alcohol	3188	Shelf
Nanopure water	3188, 3194	Sink
40 % w/v Sarcosyl	3188	In-use tray or shelf
Pro K (20 mg/mL)	3188	Freezer
TNE	3188	In-use tray or shelf
5 % v/v Trigene	3191	Sink

4.1.1 Extraction Buffer

Prepare Extraction Buffer just prior to commencing the Manual DNA IQ™ or Off-Deck Lysis procedures.

1. Use the correct table in the appendices (Section 18) for the required procedure to determine the reagent volumes.
2. Remove the appropriate aliquot/s of 20 mg/mL Pro K from the freezer and thaw. Vortex mix and briefly centrifuge before use.

3. Ensure that the 40 % w/v Sarcosyl is completely dissolved and appears clear in the stock solution. If not dissolved, invert the container a few times and leave at room temperature.
4. In the biohazard cabinet, aliquot the required amount of TNE buffer into a 50 mL tube. Do not use directly from the stock bottle, use a smaller working aliquot instead.
5. Add the appropriate volume of 20 mg/mL Pro K and 40% w/v Sarcosyl to the TNE buffer and invert gently to mix.
6. Label the tube with **"Extraction Buffer"**, your initials and the date.
7. Note down all reagent lot numbers.

4.1.2 Lysis Buffer with DTT Solution

Lysis Buffer is supplied with the DNA IQ™ kit. The Lysis Buffer with DTT solution is prepared just prior to commencing the Manual or Automated DNA IQ™ procedures.

Warning: Lysis Buffer and DTT are toxic, use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling Lysis Buffer.

1. Use the correct table in the appendices (Section 18) for the required procedure to determine the reagent volumes.
2. Remove the appropriate aliquot/s of DTT from the freezer and thaw. Vortex mix and centrifuge briefly before use.
3. In the biohazard cabinet, add the required volume of Lysis Buffer to a sterilised glass Schott bottle or 50mL tube.
4. Add the required volume of DTT to the Lysis Buffer and gently swirl to mix.
5. Label the container with **"Lysis Buffer + DTT"**, your initials and the date.
6. Note down all reagent lot numbers.

4.1.3 DNA IQ™ Resin

DNA IQ™ Resin is supplied with the DNA IQ™ kit. The resin solution is prepared using the Lysis Buffer with DTT solution (from section 4.1.2) just prior to commencing the Manual or Automated DNA IQ™ procedures.

Warning: Lysis Buffer and DTT are toxic, use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling Lysis Buffer.

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

1. Use the correct table in the appendices (Section 18) for the required procedure to determine the reagent volumes.
2. In the biohazard cabinet, aliquot the required amount of Lysis Buffer with DTT solution (from 4.1.2) into a 10, 5 or 2 mL tube.
3. Thoroughly vortex mix the DNA IQ™ resin and immediately add the required volume to the Lysis Buffer with DTT solution.
4. Mix by gentle inversion.
5. Label the tube with **"Resin"**, your initials and the date.
6. Note down all reagent lot numbers.

4.1.4 1x Wash Buffer

Note: 2x Wash Buffer is supplied with the DNA IQ™ kit.

To prepare 1x Wash Buffer:

1. In a biohazard cabinet, add 35 mL of *AnalR* 100 % Ethanol to the 2x Wash Buffer bottle.
2. Add 35 mL of Isopropyl Alcohol to the 2x Wash Buffer bottle.
3. Update the 1x Wash Buffer reagent audit trail in AUSLAB:

- From the main page press <2> "Sample Processing"
- Press <8> "Materials Processing"
- Press <2> "Consumable Inventory"
- Highlight "DNA IQ Wash Buffer" in the list and press <enter>
- Highlight the correct lot number and press <SF8> "Consumable Lot Audit"
- Add audit entry that specifies the 1x Wash Buffer aliquot (e.g. A1) and the lot numbers of the ethanol and isopropyl alcohol used.

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

1. Use the correct table in the appendices (Section 16) for the required procedure to determine the reagent volume.
2. In the biohazard cabinet, aliquot the required amount of 1x Wash Buffer into a 50 or 10 mL tube.
3. Label the tube with **"Wash Buffer"**, your initials and the date.
4. Note down the reagent lot number.

4.1.5 Elution Buffer

Elution Buffer is supplied with the DNA IQ™ kit.

1. Use the correct table in the appendices (Section 18) for the required procedure to determine the reagent volume.
2. In the biohazard cabinet, aliquot the required amount of 1x Wash Buffer into a 50 or 10 mL tube.
3. Label the tube with **"Wash Buffer"**, your initials and the date.
4. Note down the reagent lot number.

4.2 EQUIPMENT

4.2.1 Equipment and consumables required for Manual DNA IQ™ and Off-Deck Lysis

Table 2 outlines the equipment and location required for Manual DNA IQ™ or Off-Deck Lysis procedures.

Table 2 Equipment with asset number and location for each procedure

Equipment	Asset No.	Location	Procedure
Fridge	30433424	3189	Manual DNA IQ™; Off-deck Lysis
Freezers		3189 / 3190	Manual DNA IQ™; Off-deck Lysis
Biological safety cabinet class II x 5		3188 x 1 3189 x 3	Manual DNA IQ™; Off-deck Lysis
PCR cabinet		3189	Manual DNA IQ™; Off-deck Lysis
96 well tube racks	N/A	3189	Manual DNA IQ™; Off-deck Lysis
Vortex x 4	30435255 30435256 002123941 806021325	3189	Manual DNA IQ™; Off-deck Lysis
Hot block x 4	30435115 30435113 30435114 30435112	3189 / 3191	Manual DNA IQ™; Off-deck Lysis
Centrifuge x 4	30433323 30433324 10233209 30433322	3189	Manual DNA IQ™; Off-deck Lysis

Mini centrifuges x 4	30434993 30087075 30087057 041129	3189	Manual DNA IQ™; Off-deck Lysis
Finnpipettes 100 – 1000 µL	N/A	3189	Manual DNA IQ™; Off-deck Lysis
Finnpipette 20 – 200 µL	N/A	3189	Manual DNA IQ™
Thermo mixer x 2		3189	Manual DNA IQ™
Magnetic rack	N/A	3189	Manual DNA IQ™
Shaker		3191	Manual DNA IQ™

Table 3 outlines the consumables and location required for Manual DNA IQ™ or Off-Deck Lysis procedures.

Table 3 Consumables and location for each procedure

Consumables	Location	Procedure
5 mL, 10 mL and 50mL sterile tubes	3188 / 3189	Manual DNA IQ™; Off-deck Lysis
1000 µL ART or One-touch tips	3188 / 3189	Manual DNA IQ™; Off-deck Lysis
1.5mL or 2mL tubes	3188 / 3189	Manual DNA IQ™; Off-deck Lysis
Spin baskets and 2 mL collection tubes	3189	Manual DNA IQ™; Off-deck Lysis
Inoculation sticks	3189	Manual DNA IQ™; Off-deck Lysis
300 µL ART or 200 µL One-touch tips	3188 / 3189	Manual DNA IQ™
Nunc Bank-it™ tubes	3189	Manual DNA IQ™; Off-deck Lysis
Nunc Bank-it™ caps	3189	Off-deck lysis
Sharps bin	3188 / 3189	Manual DNA IQ™; Off-deck Lysis

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

4.2.2 Equipment and consumables required for Automated DNA IQ™

Table 4 outlines the equipment with asset number and location required for Automated DNA IQ™.

Table 4 Equipment with asset number and location for Automated DNA IQ™

Equipment	Asset No.	Location
STORstar (B)	10238493	3190
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (MPII-A)	10076438	3191
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (MPII-B)	10076437	3191
DPC shaker x 2	N/A	3191
Automated temperature controller, heat block tiles and heat block adapters x 2	N/A	3191
Milli-Q Integral 3 (A10) water purification system		3194
Eppendorf 5804 centrifuge	10238416	3191
Vortex	30087015	3191
Fridge	30433424	3191
MixMate	30512822	3191
Capit-All automated decapper	None	3191
4titude 4seal sealer	30512847	3191

Table 5 outlines the consumables and location required for Automated DNA IQ™.

Table 5 Consumables and location for Automated DNA IQ™

Consumables	Location

175µL Clear Non-Conductive Filter RoboRack tips – Pre-Sterilised	3191
MβP Pure 1000µL Tips – Pre-Sterilised	3191
Abgene 96-deep well plate	3191
Axygen 2mL deep well storage plate	3191
96 well Half Skirt PCR Microplate	3191
4titude Piercing Film	3191
12 Channel plate	3191
Nunc Bank-it™ tubes	3191
Nunc Bank-it™ caps	3191
Sterile 50mL Falcon tubes	3188
Sterile 10mL or 5mL tubes	3188
Autoclaved 100mL glass bottles	3191
Autoclaved 250mL glass bottles	3191
Aluminium sealing film	3191
300uL ART tips	3188
1000µL ART tips	3191

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

5 SAFETY

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

The MPII instruments, labware, biohazard cabinets and centrifuges should be wiped with **5% v/v Trigene only**, followed by 70 % v/v ethanol.
Work benches and non-metallic equipment should be wiped with 5 % v/v Hypo 10 bleach, followed by 70 % v/v ethanol.

Warning: Do not place any part of the body inside the cabinet while the MPII is running a procedure.

If it is necessary to access the deck, pause the program using the computer software or the emergency STOP button located on the front of the instrument.

Warning: Tris base, EDTA, Sarcosyl and Lysis Buffer are irritants. DTT is a reducing agent that destroys disulfide bonds. Handle carefully and wear appropriate PPE.

Lysis Buffer contains guanidinium thiocyanate (GuSCN) which is toxic and can be harmful if inhaled, swallowed or comes in contact with skin. Left over Lysis Buffer-DTT is to be disposed of in a brown Winchester bottle in the ventilated cupboard in Room 3191. Never dispose down the sink. If spillage occurs, absorb with liquid-binding material (such as sand or kitty litter) and dispose in a biohazard bin.

Do not combine bleach with Lysis Buffer and do not wipe areas of Lysis Buffer spillage with bleach, as the two reagents produce cyanide gas when combined. Handle carefully and wear appropriate PPE, including safety glasses, when handling Lysis Buffer. If Lysis Buffer is spilt onto PPE (eg. Gloves, gowns), remove and discard the contaminated item/s in a biohazard bin.

6 SAMPLING AND SAMPLE PREPARATION

6.1 SAMPLE LOCATIONS

Samples waiting to be extracted are stored in freezers as detailed in Table 6.

Table 6 Sample storage locations

Sample type	Storage Device	Storage Location
Urgent/High/Medium Priority Samples	Freezer	3190 or 6106*
Lysates in 1.0mL Nunc Bank-it™ tubes	Freezer	3190
Extracts in 1.0mL Nunc Bank-It™ tubes	Freezer	3194 A

* Some Medium and Low Priority storage boxes are located in Block 6, within the Exhibit Room (6106).

6.2 QC SAMPLES

One negative reagent control and one positive blood control (from a known donor) must be registered for all extraction batches. Additionally, off-deck lysis batches require five blank controls as detailed in Table 7.

Table 7 Extraction Quality Controls

Batch Type	Control
Off-deck lysis	Positive control (x1)
	Negative control (x1)
	Blank control (x5)
Manual DNA IQ™	Positive control (x1)
	Negative control (x1)
Retain supernatant DNA IQ™	Positive control (x1)
	Negative control (x1)

6.3 REGISTRATION OF QC SAMPLES

Register control samples according to the standard laboratory procedure QIS 24919 Forensic DNA Analysis Workflow Procedure. CQ samples for off-deck lysis and Auto extractions are to be registered with a 9PLEX test code. QC samples for a DNA IQ Maxwell extraction are to be registered using the XPLEX test code.

6.4 CREATION OF EXTRACTION BATCHES

Create extraction batches according to the standard laboratory procedure QIS 24919 Forensic DNA Analysis Workflow Procedure. All samples that are registered using a 9PLEX test code will be extracted using the automated DNA IQ method with the exception of tapelifts that will be processed using the DNA IQ Maxwell procedure.

6.5 LOCATING SAMPLES

Locate samples according to the standard laboratory procedure QIS 23959 Storage Guidelines for Forensic DNA Analysis.

6.6 SEQUENCE CHECKING AUTOMATED EXTRACTION BATCHES

Sequence check lysates in Nunc Bank-It tubes for automated extraction batches according to the standard laboratory procedure QIS 24256 Procedure for the use of the STORstar unit for automated sequence checking.

6.7 ELECTRONIC WORKFLOW DIARY

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

7 MANUAL METHODS FOR EXTRACTION USING DNA IQ™

7.1 MANUAL DNA IQ™ (NO RETAIN SUPERNTANT)

Note: All manual extractions are to be performed within a biohazard cabinet in room 3189.

1. Prepare fresh reagents within the biohazard hood of the clean room (3188) prior to commencing the procedure. Refer to Section 4.1 for preparation of reagents. Use Table 8 in Appendix 12.1 for reagent volumes.

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

3. Label for each sample:
   - Original sample tube;
   - Spin basket (if required);
   - 2mL tube; and
   - Nunc™ Bank-It™ tube.

Note: A spin basket is required for swabs, fabric, paper and cigarette butts. Samples that do not require a spin basket are tape-lifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

4. Have a second operator perform a sequence check of all tubes. Record the sequence check in AUSLAB.

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

6. Vortex and incubate at 37 °C on the Thermomixer at 1400 rpm for 45 minutes.

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

8. Transfer the substrate to spin basket (if required) or transfer the lysate to an appropriately labelled 2.0 mL tube. Retain the original tube containing the substrate if no spin basket is used.

9. Centrifuge the spin basket at 15800 xg for 2 minutes.

10. Transfer the lysate from the original sample tube and the flow-through from the collection tube of the spin basket to an appropriately labelled 2 mL tube. Transfer the substrate from the spin basket back into the original sample tube.

    Note: If original sample tube is an appropriate 2 mL tube, transfer the substrate into the new 2 mL tube and add the flow-through from the spin basket to the lysate still in the original sample tube.

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

12. Add 50 µL of DNA IQ™ Resin-Lysis Buffer-DTT solution to the lysate. Pipette mix or vortex the solution frequently to keep a homogenous suspension of resin within the solution.

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

14. Remove from the Multitube shaker, vortex and immediately place into the magnetic stand. Separation will occur instantly.

    Note: If resin does not form a distinct pellet on the side of the tube, or if the magnetic beads have re-suspended while in the stand, vortex the tube and quickly place back in the stand.

15. Carefully remove and discard the liquid, ensuring that the resin is not disturbed. Remove from the magnetic stand.

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

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

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

18. Add 100 µL of 1X Wash Buffer, vortex and place into the magnetic stand. Once separation has occurred remove and discard the liquid. Remove the tube from the magnetic stand.

19. Repeat step 18 twice for a total of three Wash Buffer washes. Ensure that all of the liquid has been removed after the last wash. Remove the tube from the magnetic stand.

20. Uncap the tubes and place the lids down onto a clean rediwipe within the biohazard cabinet. Allow the resin to air-dry in the hood for 15 minutes and then recap the tubes.

    Note: Do not allow the resin to dry for more than 20 minutes as this may inhibit DNA elution.

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

22. Incubate in the Thermomixer at 65°C for 3 minutes and then continue to incubate for a further 3 minutes shaking at 1100 rpm. If no thermomixer is available, incubate samples within a hot block and vortex mix at the start and end of the second 3 minute incubation. Remove samples.

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

24. Carefully transfer the DNA extract to the corresponding labelled Nunc™ Bank-It™ tube.

25. Remove from the magnetic stand and repeat steps 21-23 once. The final volume after the second elution is approximately 95 µL of DNA extract.

26. Store the DNA extracts in temporary storage located within the Pre-PCR sorting room 3194-A in the upright freezer with equipment number 30512883.

27. Store tubes containing substrates in Spin Basket boxes located within the Pre-PCR sorting room 3194-A in the upright freezer with equipment number 30512883.

7.2 PROCEDURE FOR MANUAL DNA IQ™ (RETAIN SUPERNTANT)

Note: All manual extractions are to be performed within a biohazard cabinet in room 3189.

1. Prepare fresh reagents within the biohazard hood of the clean room (3188) prior to commencing the procedure. Refer to Section 4.1 for preparation of reagents. Use Table 9 in the Appendix for reagent volumes.

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

3. Label for each sample:
   - Original sample tube;
   - 1.5 or 2 mL tube labelled 'SUP' for supernatant;
   - Spin basket (if required);
   - 2 mL tube; and
   - Nunc™ Bank-It™ tube.

   Note: A spin basket is required for swabs, fabric, paper and cigarette butts. Samples that do not require a spin basket are tape-lifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

4. Have a second operator perform a sequence check of all tubes. This person must enter the sequence check in AUSLAB.

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

6. Incubate at room temperature for 30 minutes.

7. Vortex, then centrifuge at 15800 xg for 3 minutes.

8. Remove 150 µL of supernatant and place into the respective 1.5 mL tube labelled with 'SUP' (for further testing).

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

10. Vortex, then incubate at 37°C on the Thermomixer at 1400 rpm for 45 minutes. If no thermomixer available, incubate samples within a hot block, vortex mix at the start and end of the incubation and at least one during the incubation.

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

12. Transfer substrate to spin basket if required or transfer the lysate to an appropriately labelled 2.0mL tube. Retain original tube containing the substrate in if no spin basket used.

13. Centrifuge spin basket at 15800 g for 2 minutes.

14. Transfer the substrate in the spin basket into a new and labelled 2mL tube to be retained. Transfer the flow through (lysate) back into the original sample tube.

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

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

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

18. Remove from the Multitube shaker, vortex and immediately place into the magnetic stand. Separation will occur instantly.

    Note: If resin does not form a distinct pellet on the side of the tube, or if the magnetic beads have re-suspended while in the stand, vortex the tube and quickly place back in the stand.

19. Carefully remove and discard the solution, ensuring that the resin is not disturbed. Remove from the magnetic stand.

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

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

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

22. Add 100µL of 1X Wash Buffer, vortex and place into the magnetic stand. Once separation has occurred remove and discard the Wash Buffer. Remove from the magnetic stand.

23. Repeat the Wash Buffer step (step 23) two times for a total of three washes. Ensure that all of the solution has been removed after the last wash. Remove from the magnetic stand.

24. Uncap the tubes and place the lids down onto a clean rediwipe. Air-dry the resin in the hood for 15 minutes and then recap tubes

    Note: Do not dry for more than 20 minutes, as this may inhibit the elution of DNA.

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

26. Incubate in the Thermomixer at 65°C for 3 minutes and then continue to incubate for a further 3 minutes shaking at 1100 rpm. If no thermomixer available, incubate samples within a hot block, vortex mix at the start and end of 2nd 3 minute incubation. Remove samples.

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

28. Carefully transfer the DNA extract to the corresponding labelled Nunc™ Bank-It™ tube.

29. Remove from the magnetic stand and repeat the Elution Buffer steps (step 26-29). The final volume after the double elution is approximately 95µL of DNA extract.

30. Store the DNA extracts in temporary storage located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

31. Store tubes containing substrates in Spin Basket boxes located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

32. Store supernatants in the S/N Retention boxes located within the Extraction sorting room in the Orford Freezer 30512883 (Room 3190).

33. Ensure that the negative extraction control from the batch is also inserted onto the **'Saliva List'**:

    - Log into Auslab
    - 3 for Patient Enquiry
    - Type/Scan the lab number for the negative extraction control
    - From the 9Plex page or the XPLEX Page press Shift F12
    - A prompt will appear 'Enter List Name'
    - Type Saliva or use the F1 lookup list.
    - Press Enter

8 OFF-DECK LYSIS PROCEDURE

1. All reagents are to be made fresh within the clean room (3188) prior to commencing each process. Use Table 10 in Appendix 12.3 for reagent volumes.

2. All manual extractions are to be performed within a biohazard cabinet in Room 3189

3. For each sample label:
    - Original sample tube
    - Spin basket as required
    - 1.0mL Nunc Bank-It™ tube
    - 1.5mL or 2.0mL tube

    Note: Samples that require a spin basket are: swabs, fabric, paper and cigarette butts. Samples that do not require a spin basket are: tapelifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

4. If substrate is in a 0.5mL tube, transfer to a labelled 1.5mL or 2.0mL tube.

5. Have a second operator sequence check the tubes and enter the sequence check in AUSLAB.

6. Add 300µL of Extraction Buffer and vortex briefly. Ensure that substrates are immersed in the Extraction Buffer.

7. Incubate for 45 minutes at 37 degrees on a hotblock. It is best practice to vortex the samples intermittently throughout the duration of the incubation period. Record temperature on worksheet.

8. Remove from the hotblock. Transfer substrate to spin basket if required or transfer the lysate to an appropriately labelled 1.5mL or 2mL tube. Retain original tube containing the substrate if no spin basket is used.

9. Centrifuge spin baskets at 15800 g for 2 minutes.

10. Transfer the substrate in the spin basket to a new and appropriately labelled 1.5mL or 2mL tube and retain.Transfer the flow through back to original lysis tube.

11. Vortex lysate, then incubate on hotblock at 65°C for 10 minutes. Record temperature on worksheet.

12. Centrifuge at 15800 g for 1 minute.

13. Transfer 300µL of lysate to the corresponding Nunc Bank-It™ tube.

Note: If more than 300µL of lysate is present, retain the remaining lysate in the 1.5mL tube. In AUSLAB, write a specimen note for that sample (eg. Extra lysate retained from sample XXXXXXXXX.). Store the retained 1.5mL lysate tube in appropriate box in freezer.

14. In AUSLAB, enter reagent and hotblock temperature details and complete the batch.

15. Store lysates in temporary storage boxes located within the extraction sorting room in the orford Freezer with equipment number 102384430 (Room 3190).

16. Store tubes containing substrates or extra lysate in Spin Basket boxes located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

9 AUTOMATED EXTRACTION OF LYSED SAMPLES

9.1 MPII Extraction PROCEDURE

Automated setup of DNA IQ extractions in 96-deep well format is performed using the MultiPROBE® II PLUS HT EX EP-A and EP-B platforms located in Room 3191.

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

9.2 PREPARATION OF REAGENTS FOR THE AUTOMATED EXTRACTION PROCESS

All reagents are to be made fresh within the clean room (3188) prior to commencing each process. Use Table 11 in the Appendix for reagent volumes.

9.3 SUMMARY OF DNA IQ™ EXTRACTION VERSION 6.6_ODL

9.3.1 Transfer of lysates from Nunc Bank-It™ tubes into the Abgene 96-deep well plate

Lysates from the off-deck lysis protocol, contained in individual Nunc Bank-It™ tubes, are transferred automatically into an Abgene 96-deep well plate prior to commencing automated sample processing. Instead of a 96-deep well plate, the use of individual Nunc Bank-It™ tubes for storage of lysates removes the need for an adhesive film and therefore reduces the risk of well-to-well contamination from the action of peeling back an adhesive plate cover.

9.3.2 Automated addition of DNA IQ™ Resin and Lysis Buffer

DNA IQ™ Resin is added automatically into the Abgene 96-deep well plate on the platform. The program uses a waste mode dispensing step to add 53µL DNA IQ™ Resin-Lysis Buffer solution to each well in either half the plate or the whole plate. Because of this, batch sizes are restricted to either 48 or 96 samples in order to maintain efficiency and economy. Two volumes of Lysis Buffer are then added to promote binding of DNA to the paramagnetic resin.

9.3.3 Mixing using a MixMate to bind DNA to resin

Manual intervention is required to seal the Abgene 96-deep well plate with a 4titude Pierce Seal film using the 4titude sealer pre-heated at 175 °C. The plate is then transferred onto a MixMate instrument for mixing at 1100rpm for 5 minutes, followed by centrifugation on an Eppendorf 5804 centrifuge at 3000rpm for 2 minutes. The seal is then carefully pierced with a 96 well PCR microplate and the Abgene plate is returned to the Applied Biosystems magnet on the MPII platform.

9.3.4 Removing lysis reagents for storage

At this point, most of the DNA is bound to the paramagnetic resin. With the positioning of the Abgene plate on the ABI magnet, DNA IQ™ Resin becomes immobile at the bottom of the plate. The lysis reagents from each well are transferred automatically to a storage plate on the MPII platform without disturbing the DNA IQ™ Resin.

9.3.5 Washing of the resin-DNA complex

Washing steps are performed to remove any inhibitors in solution. The first wash uses 125µL Lysis Buffer with shaking at room temperature for 1 minute on the DPC shaker to ensure that the DNA is bound to the paramagnetic resin. The plate is moved to the ABI magnet and the supernatant is transferred into the storage plate. The next three washes incorporate 100µL of 1x Wash Buffer with shaking at room temperature for 1 minute on the DPC shaker. During each wash cycle, the Abgene 96-deep well plate is moved to the ABI magnet and the supernatant is discarded into the tip chute.

9.3.6 Removing any excess of 1x Wash Buffer

The samples are allowed to air dry at room temperature for 5 minutes in order to remove all traces of Ethanol from the Wash Buffer. The presence of Ethanol may potentially inhibit both the elution process and also downstream PCR.

9.3.7 Elution of DNA from the resin-DNA complex

A double elution method is employed in this procedure. At each elution step, 60µL of Elution Buffer is added to each sample, followed by incubation at 65°C for 6 minutes (3 minutes without shaking followed by 3 minutes shaking on the DPC shaker). The Abgene plate is moved to the ABI magnet and the eluted solution (supernatant containing eluted DNA) is transferred to fresh Nunc™ Bank-It™ tubes.

9.3.8 Flushing of liquid pathway

As a decontamination measure, the liquid pathway is washed with Amphyl and Nanopure water at the conclusion of the automated extraction process.

9.4 SETTING UP THE MPII PLATFORMS FOR AUTOMATED DNA IQ™ PROCESSING

The following steps are carried out in the automated extraction room (Room 3191).

1. Remove the Nunc Bank-It™ tubes containing lysates from the fridge to allow to come to room temperature before commencing the extraction procedure.

   Note: If the lysates are frozen, remove them from the freezer and thaw in Room 3191.

2. Restart or turn on the instrument PC.
3. Log onto the network using the **Robotics** login.
4. Open WinPrep® by double clicking icon on the computer desktop (Figure 1).

Figure 1.
The WinPrep® icon.

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

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

7. Ensure the **System Liquid reservoir is FULL** and tubing is fully submerged in the system liquid before every run and perform a Flush/Wash. If visible air bubbles have appeared in tubing or in syringes between setting up the deck and executing the test, another flush wash will need to be performed before starting the MPII extraction run.

8. Open the Extraction setup MP II test file in WinPrep® by selecting:

   - **File**
   - **Open**, navigate to **C:\PACKARD\MULTIPROBE\BIN\QHSS PROTOCOLS**
   - Select "**DNA IQ Extraction_Ver 6.6_ODL.mpt**"
   - Click the "**Open**" button

9. Check the tree pane of the whole program for any bold fonts. See the Analytical Senior Scientist if bold fonts are present.

10. Copy the required plate map from the network folder **I:\EXTRACTION** into the local folder **C:\PACKARD\EXT PLATE MAPS.** Open the plate map using Excel and check that the plate map is complete, and make changes if necessary. If there are blanks (samples that have been removed), make all volumes to 0. If it is not a full batch, delete the rows of blank samples at the end of the platemap. Save all changes made to the platemap (as a .txt file).

11. Decontaminate the required labware with 5% TriGene followed by 70% Ethanol and place onto the instrument deck in the designated grid positions (as shown in the WinPrep® software). Additionally, ensure the DPC shaker is positioned properly.

12. Ensure that the DPC shaker and Heater Controller Box are switched on.
    For EP-A: Tile 1 at F22 (850C).
    For EP-B: Tile 2 at F22 (850C).

    Note: Press the start/stop button twice at the front of the DPC shaker to ensure that it displays zero on the screen.

13. Ensure the heat transfer tile is clicked into the plate adapter tile properly.

    Note: This is critical to ensure correct incubation temperatures.

14. To the Amphyl wash station in position **A10**, add 1% Amphyl to the far left hand side, 0.2% diluted Amphyl to the middle left and place an empty (waste) reagent trough in the middle right position of the station.

15. Add Wash Buffer to the far right hand side trough of the Amphyl wash station in position **A10**.

16. Add Lysis Buffer to the labelled 150mL reagent trough on the left hand side of the 2 trough holder in position **A13**.

17. Place the 12 channel plate into position **A16**. Add Elution Buffer to the plate by dividing the Elution Buffer between channels 11 and 12.

18. Add Resin to channel 1 of the 12 channel plate in position **A16**. It is important to add the resin in a uniform fashion to ensure equal distribution of resin along the channel.

19. **Nunc Bank-It™ lysate tubes:** The lysates should now be at room temperature. Ensure that the rack is labelled with the correct **AUSLAB batch ID** and that the label matches the batch ID on the worksheet and platemap. Additionally, randomly check some visible barcodes on the side of lysate tubes against the positions on the worksheet to ensure the correct batch of samples is to be processed.

20. Place the rack onto the MixMate to shake for 1 minute at 1000rpm.

21. Centrifuge the rack at 3000rpm for 2 minutes in the Eppendorf 5804 centrifuge and then place into position **C13**.

22. **Abgene 96-deep well plate:** With the plate in its correct orientation label the **left hand side** of the plate with both the correct **AUSLAB batch ID** and **batch ID barcode**. With a marker, print the word "**FRONT-lysate**" on the front of the plate. Place the plate in its correct orientation in position **E13**.

23. **2mL 96-deep well storage plate:** With the plate in its correct orientation label the **left hand side** of the plate with both the correct **AUSLAB batch ID** and **batch ID barcode**. With a marker, print the word "**FRONT-store**" on the front of the plate. Place the plate in its correct orientation in position **E16**.

24. **Nunc Bank-It™ extract tubes:** Ensure that the rack is labelled with the correct **AUSLAB batch ID** on the **front** of the Nunc Bank-It™ tube rack. Additionally, randomly check some visible barcodes on the side of lysate tubes against the positions on the worksheet to ensure the correct batch of samples is to be processed. Place the rack in its correct orientation in position **G16**.

25. Add Nanopure water to the 160mL trough in the Flush/Wash station in position **G13**.

26. Ensure that all necessary labware have been positioned correctly as displayed within WinPrep®, then click "**EXECUTE TEST**". Record run information in the Run Log book.

27. The following message will appear (Figure 2 below):

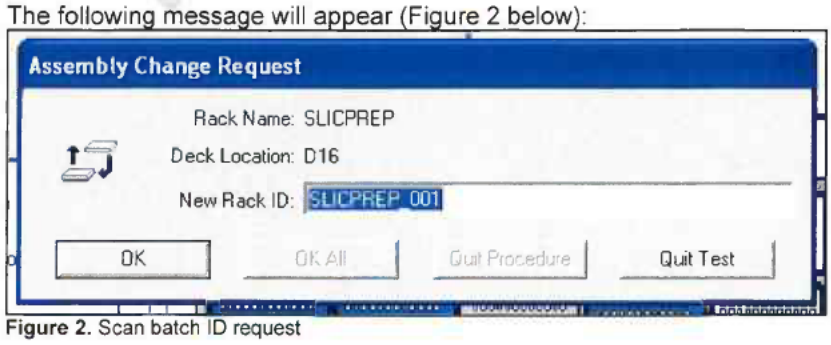

Figure 2. Scan batch ID request

Into "**New Rack ID:**" scan barcode off the worksheet. It is important this corresponds to the labelling of labware on the deck and the plate maps used.

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

29. Select the correct platemap by browsing to **C:\PACKARD\EXT PLATE MAPS**. Ensure that the platemap selected corresponds to the labelling of labware on the deck, the paperwork used and the **"New Rack ID"** entered.

30. For a full batch of 76 samples, ensure that all nodes are checked. Click "**Next**" to check all other nodes.

31. For a batch of 48 samples or less, uncheck the node: **"Add resin to second half of plate"**. Click "**Next**" to check all other nodes.

32. Click "**Start**" to continue.
    - The MPII instrument will begin and user prompts will appear as a reminder to confirm the deck setup. **Always decap tubes from positions H1 to A1, H2 to A2 etc.**
    - Ensure all steps on the first prompt have been complete, Click **OK** to continue.
    - Ensure all steps on the second prompt have been complete, Click **OK** to continue.

33. The program will progress to transfer the lysates followed by automated addition of the Lysis-DTT buffer and the DNA IQ™ Resin solution. The next user prompt will appear. Follow the steps as outlined in the user prompt. Then click **OK** when ready.

    Note: Ensure that the plate is heated and sealed properly with the Pierce Seal on the 4titude Heat Sealer. Mix the plate on the mixmate for 5min at 1100rpm and centrifuge. Pierce the plate with a PCR Microplate and then discard. (A new plate is to be used each time). Ensure the pierced plate is returned to the magnet.

34. Once lysis steps have been completed, a user prompt will appear. Follow the directions as outlined in the user prompt. Then click **OK** when ready.

    Note 1: The Lysate Nunc tubes (including the rack) should be placed into a clipseal bag, sealed and discarded into a biohazard waste bin. The Store plate can also be removed, heat sealed and discarded into a biohazard waste bin.

    Note 2: When the supernatant storage plate is sealed, check the plate for the transfer of beads. To do this, place the plate onto the ABI magnet (if beads are present they will settle to the bottom of the plate) and then inspect the bottom of the plate. If no beads are present, discard the store plate. If, however, beads are present, refer to the Section 15, Troubleshooting.

35. Once the wash steps have been completed, a user prompt will appear. Follow the directions as outlined in the user prompt. Then click **OK** when ready.

    Note: The Nunc extract tubes can be decapped on the bench in the MPII cabinet while the samples are drying and the heating tile is stabilising. At this point, the operator has approximately **12 minutes** to decap the tubes. If the operator has been interrupted and requires more time to decap the tubes, the program may be paused at this step.

36. A message will appear waiting for the heating tile to reach 85°C (for incubation at 65°C). **DO NOT PRESS CONTINUE** as the program will continue automatically when the temperature has been reached with sufficient stability.

37. A user prompt will appear. Follow the directions as outlined in the user prompt. Then click **OK** when ready.

38. Once the elution is completed, a user prompt appears. Follow the directions as outlined in the user prompt. Then click **OK** when ready.

39. The Lysate Plate is heat sealed and kept in temporary storage for one month.

40. Once all plates are removed from the deck and sealed. Click "**OK**" to proceed to the Amphyl wash step.

41. A final message will advise that the run has completed. Click "**OK**".

9.5 FINALISING THE MPII RUN

1. Transfer left over Resin solution from the 12 channel plate and the Lysis Buffer-DTT (wearing safety glasses) into the glass Lysis-DTT bottle previously used. Discard the 12 channel plate in the biohazard waste bin. Transfer the contents of glass bottle into the brown Winchester bottle located in the safety cupboard in room 3191.

2. Discard the contents of the Amphyl wash station into the sink and rinse the troughs with Nanopure water.

3. Remove all labware from the deck and clean with 5% TriGene™ followed by 70% Ethanol, and setup for the next run if necessary. In addition, clean the work area around the MPII instrument with 10% (v/v) Bleach 7x and 70% Ethanol.

4. Remove the tip chute and funnel, rinse with warm tap water to remove any residue inside the chute before cleaning with 5% TriGene and 70% Ethanol.

5. Import the platemap into Auslab and then delete it.

9.6 IMPORTING MPII LOG FILE INTO AUSLAB

1. Click on the Microsoft Access icon in the WinPrep® main menu to open the MultiPROBE log database.

2. Click **"Open"** when prompted.

3. Click on the relevant run Test ID in the Test Selection box. In the Report/Query/Action Selection dropdown menu, select **"Report: Test Summary (Sorted by Destination Rack ID)"**

4. In the Output Selection dropdown menu, select **"File"**. Save the output file as *.csv format to **C:\PACKARD\EXT PLATE MAPS\EXT LOGS** with the same name as the AUSLAB batch ID and click "**Apply**".

5. Open the log file and check for any errors that may have arisen during the extraction process. Compare the listed errors to any that were encountered during the run. Report any critical errors to the line manager.

6. Import the log file, entering the path, filename and extension (e.g. C:\Packard\ext plate maps\ext logs....) and press **[Enter]**. Delete the log file after importing.

7. For more details on importing files into AUSLAB refer to Batch functionality in AUSLAB SOP (QIS 24469).

9.7 IMPORTING EXTRACTION "RESULTS" INTO AUSLAB

1. Import the results file, entering the filename and .txt extension (e.g. CWIQEXT20100917_06.txt). For more details on importing files into AUSLAB refer to Batch functionality in AUSLAB SOP (QIS 24469).

2. The file will be imported into AUSLAB and appear in the Results Import Table.

3. Highlight entry and press **[Enter]**, for access to the Results Table.

4. Page down through the table and check that all sample results have been imported.

5. Press **[SF8]** ***Table Sort Order***, this sorts the table, sorting samples that have failed Autovalidation to the top. Samples that have failed are those that have processing comments present.

6. For samples that have failed, check the **Processing Comments,** by entering into the sample.

7. If processing comments state sample is to be sent to another batch type **other** than quant. Proceed with the following steps:
   a. Request the appropriate rework test code via the **[SF7]** results history table and the **[SF8]** request rework functions (e.g. samples requiring Microcon, NucleoSpin and pooling).
   b. Press **[Esc]** to exit back to the DNA results table. Do not toggle accept.
   c. Add the extraction batch ID ONLY into the 9PLEX or 9FTAR completed date fields in AUSLAB.

8. If processing comments do not state next step the sample will be processed as normal:
   a. Press **[Esc]** to exit back to the DNA results table.
   b. Highlight any entries to be changed and press **[SF7]** ***Toggle Accept.***

9. Press **[F7]** ***Complete Batch***, all of the samples with a ***Yes*** in the Accept column will be transferred to the outstanding Quant Casework or Quant Reference lists.

10. File the extraction worksheet into the relevant intray for scanning in room 3194A (Pre PCR-Sorting).

10 SAMPLE STORAGE

Refer to QIS 23959 Storage Guidelines for Forensic DNA Analysis for how to store the DNA extract Nunc™ Bank-It™ tubes and Abgene 96-deep well. Refer to QIS 24919 Forensic DNA Analysis Workflow Procedure for storage of extracts.

11 TROUBLESHOOTING WITH THE MPII

1. If the resin is not pipette mixing correctly (eg. Resin is not being drawn up into the pipette tip), pause the Winprep program and check that the support tile is clicked onto

the MPII deck correctly. Alternatively, pipette and mix resin manually one more time from the corresponding column in the 12 channel plate.

2. If the Gripper is not placing the rack/plate properly on the deck, pause the program and manually adjust the rack/plate properly on the adapter support tile.

3. If the Gripper appears to be not gripping a plate correctly, or the pipetting alignment of a labware appears to be incorrect, the deck and / or labware can be re-calibrated. This is covered in QIS 23939 Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform

12 QUALITY ASSURANCE/ACCEPTANCE CRITERIA

- Positive and negative (reagent blank) controls are included in each extraction batch. However, for off deck-lysis batches, an additional five negative (reagent blank) controls are included. These controls are processed as normal samples through to completion.
- If any results are obtained from the reagent blank, either at quantification or during the CEQ check, then the possible source of the contamination is investigated. The samples extracted with this control are thoroughly checked and repeated if possible

13 VALIDATION

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- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 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.
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- Muharam I., Hlinka V., Gallagher, B., Lundie, G., Iannuzzi, C., Nurthen T., McNevin A., Ientile V. "Project 22: A Modified DNA IQ™ Method for Off-Deck Lysis Prior to Performing Automated DNA Extraction" 2008

14 STORAGE OF DOCUMENTS

The off-deck lysis worksheets are to be stored within the extraction sorting room (3190) in the nominated intray. It is essential that ALL off-deck lysis worksheets go within this intray so the operational officers are aware that batches are ready and waiting to be combined for extraction on the automated robots.

All worksheets, after auto extraction has been completed, are stored within the Pre-PCR sorting room (3194-A) in an allocated in-tray for scanning by the operational officers.

15 REFERENCES

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16 ASSOCIATED DOCUMENTS

QIS 17165 Receipt, Storage and Preparation of Chemicals, Reagents and Test Kits
QIS 17171 Method for Chelex Extraction
QIS 22857 Anti-Contamination procedure
QIS 23939 Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform
QIS 23959 Storage Guidelines for Forensic DNA Analysis
QIS 24256 Sequence Checking with the STORstar Instrument
QIS 24469 Batch functionality in AUSLAB
QIS 24919 Forensic DNA Analysis Workflow Procedure

17 AMENDMENT HISTORY

Version	Date	Author/s	Amendments
R0	23 Oct 2007	B. Gallagher, T. Nurthen, C. Iannuzzi, V. Hlinka, G. Lundie, I Muharam.	First Issue
R1	12 Dec 2007	M Harvey, C Iannuzzi, A McNevin	Reviewed and updated after initial training
R2	19 March 2008	M Harvey, B Andersen, C Iannuzzi, A McNevin	Addition of Off-deck Lysis procedure, Retention of fully automated method as Appendix, addition of reagent record tables into Appendix
R3	April 2008	QIS2 Migration Project	Headers and Footers changed to new CaSS format. Amended Business references from QHSS to FSS, QHPSS to CaSS and QHPS to Pathology Queensland
4	13 March 2009	QIS2 migration	Version incremented by one on migration to QIS2
5	03 June 2009	M Aguilera, B Micic, C Iannuzzi, A. Cheng, V. Hlinka, I. Muharam, G. Lundie, C. Weber	Major changes to reflect new procedure. Updated to reflect changes in procedure as an outcome of internal and external audits. Created ver.6.4 ODL in MPII Platforms. Minor changes in procedures using 4titude 4seal heat sealer to seal plates.
6	29 June 2009	A McNevin, K Lancaster	Removed references to retaining lysate and beads, fixed minor formatting errors. Created ver6.5 ODL in MPII Platforms. Substrates now to be retained in 2mL tube
7	30 September 2010	M.Cipollone, M.Mathieson	Major changes made RE: room numbers to reflect the move to Block 3. Re-formatted entire SOP. New Appendices added which outline all reagent volumes. All equipment and associated asset numbers have been included. Use of the electronic diary now included as an additional section. Preparation of reagents within the clean room (3188) now to be done prior to starting each process. Storage of worksheets updated. New software version 6.6 on automated extraction robots. S/N Retention Boxes now stored in Manual Ext Room. Associated Documents and hyperlinks updated. Consumables and Equipment table added for Manual DNA IQ.
8	24 May 2012	A. Speirs M. Cipollone	Major revision of document. Revision of sections 1-3 to improve clarity. Updated Tables 1 to 7. Removed redundant sections on locating

Version	Date	Author/s	Amendments
			samples and creating batches. Removed procedure for retain supernatant Off-Deck Lysis and thus associated Appendix. Table of Contents amended to reflect all changes. Moved the Manual DNA IQ procedures before the Off-Deck Lysis procedure. Re-formatted spacing and alignments of headings. Re-numbered Tables in Appendices. Updated sections to include which relevant Appendix to use in regards to reagents. Minor grammatical and spelling errors fixed.
9	4 Dec 2013	B. Andersen, M. Cipollone	Updated Section 7 of the Manual DNA IQ Retain Supernatant procedure to reflect differences in list inserting between P+ and PP21 samples. Updated Section 6 to clarify 9PLEX and XPLEX test codes. New template applied and minor formatting and wording changes.

18 APPENDICES

18.1 REAGENT VOLUMES FOR MANUAL DNA IQ™ (NO RETAIN SUPERNATANT)

Table 8 - Reagent volumes for manual method

Reagent	Volume for 12 samples (mL)	Volume for 24 Samples (mL)
TNE buffer	4.0	8.0
Proteinase K (20mg/mL)	0.216	0.432
Sarcosyl (40%)	0.108	0.216
Lysis-DTT Buffer		
DNA IQ™ Lysis Buffer	10	20
DTT (1M)	0.1	0.2
DNA IQ™ Resin solution		
Lysis-DTT Buffer	0.645	1.29
DNA IQ™ Resin	0.105	0.210
DNA IQ™ 1x Wash Buffer	4.0	8.0
DNA IQ™ Elution Buffer	1.4	2.8

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

18.2 REAGENT VOLUMES FOR MANUAL DNA IQ™ (RETAIN SUPERNATANT)

Table 9 - Reagent volumes for manual method (retain supernatant)

Reagent	Volume per sample (uL)	Volume for 12 samples (mL)	Volume for 24 Samples (mL)
TNE buffer	450	5.4	10.8
Proteinase K (20mg/mL)	14	0.168	0.336
Sarcosyl (40%)	7	0.084	0.168
Lysis-DTT Buffer			
DNA IQ™ Lysis Buffer	660	10	20
DTT (1M)	6.6	0.1	0.2
DNA IQ™ Resin solution			
Lysis-DTT Buffer	43	0.645	1.29
DNA IQ™ Resin	7	0.105	0.210
DNA IQ™ 1x Wash Buffer	300	4.0	8.0
DNA IQ™ Elution Buffer	100	1.4	2.8

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

18.3 REAGENT VOLUMES FOR THE OFF DECK LYSIS PROCEDURE

Table 10 - Reagent volumes for off-deck procedure

Reagent	Volume for 48 samples (mL)	Volume for 39 Samples (mL)
Extraction Buffer		
TNE buffer	16	12
Proteinase K (20mg/mL)	0.864	0.648
Sarcosyl (40%)	0.432	0.324
Lysis-DTT Buffer		
DNA IQ™ Lysis Buffer	N/A	N/A
DTT (1M)	N/A	N/A
DNA IQ™ Resin solution		
Lysis-DTT Buffer	N/A	N/A
DNA IQ™ Resin	N/A	N/A
DNA IQ™ 1x Wash Buffer	N/A	N/A
DNA IQ™ Elution Buffer	N/A	N/A

Note: Do not directly pipette from reagent stock bottles. Stock reagents should be decanted into working containers where appropriate.

18.4 REAGENT VOLUMES FOR AUTOMATED DNA IQ™

Table 11 - Reagent volumes for automated procedure

Reagent	Volume for 96 samples (mL)	Volume for 76 samples (mL)	Volume for 48 samples (mL)
Extraction Buffer (300µL/sample)			
TNE buffer	N/A	N/A	N/A
Proteinase K (20mg/mL)	N/A	N/A	N/A
Sarcosyl (40%)	N/A	N/A	N/A
Lysis-DTT Buffer			
DNA IQ™ Lysis Buffer	90	70	50
DTT (1M)	0.9	0.700	0.5
DNA IQ™ Resin solution			
Lysis-DTT Buffer	6.0	6.0	3
DNA IQ™ Resin	1.0	1.0	0.5
DNA IQ™ 1x Wash Buffer	35.0	30.0	18
DNA IQ™ Elution Buffer	14.0	12.0	8

Note: Do not directly pipette from reagent stock bottles. Stock reagents should be decanted into working containers where appropriate.

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

1 PURPOSE AND SCOPE

This document outlines the automated and manual procedures for extracting DNA from reference and casework samples using the DNA IQ™ kit (Promega Corp., Madison, WI, USA) within Forensic DNA Analysis Unit. The automated procedure within this document utilises the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (MPII) (PerkinElmer BioDiscovery, Downers Grove, IL, USA).

Reference samples and casework samples must be extracted on separate batches. If casework and reference batches are to be extracted on the same MPII, the instrument and all associated lab-ware must be decontaminated between operations.

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

2 DEFINITIONS

DTT	1,4 Dithiothreitol
EDTA	Ethylenediaminetetraacetatic acid
Extracts	Samples that have undergone DNA extraction
Lysates	Samples that have undergone off-deck lysis but not DNA extraction
MPII	MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform
Paramagnetic	Becomes magnetic with the application of a magnetic force
Pro K	Proteinase K
Samples	Sample substrates in tubes awaiting a DNA extraction process
Sarcosyl	N-Lauroylsarcosine
TNE	Tris, NaCl and EDTA buffer

3 PRINCIPLE

3.1 DNA IQ™ KIT

The DNA IQ™ system (Promega Corp., Madison, WI, USA) is designed to extract and purify DNA from a number of different substrates and has been optimised for trace DNA samples. An in-house validation was performed using a modified version of the PerkinElmer automated protocol.

Cell lysis is performed using DNA IQ™ Lysis Buffer containing Dithiothreitol (DTT). 1,4 Dithiothreitol is a reducing agent used in extractions to break disulfide bonds of proteins. The Lysis Buffer used is a proprietary buffer containing chaotropic salts required for binding of DNA to the magnetic beads. According to the MSDS (Promega Corporation 2006), the Lysis Buffer contains 50-75% guanidinium thiocyanate, < 2.5% EDTA, < 2% 3-[(3-Choalamidopropryl)dimethylammonio] propanesulfonic and < 2% polyethylene glycol tert-octylphenyl ether.

The foundation of the DNA IQ™ system is the magnetic bead resin which contains novel paramagnetic particles covered with silica. The resin has a negative charge at basic and near neutral pH. However, the addition of Lysis Buffer changes the pH and salt concentration of the solution, resulting in the silica on the magnetic beads becoming positively charged and able to bind free DNA.

The magnetic bead resin has a DNA binding capacity of 100ng and binding is more efficient with samples containing small amounts of DNA rather than samples with large amounts of DNA. As a result, small sample size is necessary to ensure efficient recovery of DNA.

Inhibitors are removed from the DNA through several washing steps. The first washing step is performed using Lysis Buffer to ensure the DNA is bound to the magnetic bead resin and to wash out inhibitors. The next three washing steps incorporate the use of DNA IQ™ Wash Buffer. This buffer contains an alcohol/aqueous mixture which dehydrates the DNA to ensure it is not eluted during washing, while the aqueous phase washes out inhibitors.

The DNA IQ™ Elution Buffer changes the salt content which, in conjunction with heating to 65 °C, allows the DNA to be released from the magnetic beads. The Elution Buffer is a low ionic strength buffer that reduces the affinity of the DNA for the silica that covers the magnetic beads by re-hydration of the phosphate backbone. To prevent PCR inhibition caused by the presence of very small DNA fragments within extracts the DNA IQ™ System selectively isolates DNA fragments greater than 80bp.

3.2 OFF-DECK LYSIS PROCEDURE

Samples must undergo a pre-extraction Off-Deck Lysis step before processing with the DNA IQ™ reagents on the MPII instrument.

The Extraction Buffer used for the Off-Deck Lysis is designed to digest cellular material and release DNA. It contains TNE buffer (Tris, NaCl, EDTA, pH 8.0), Sarcosyl and Pro K.

TNE acts as a basic buffer with EDTA chelating ions in solution.

Sarcosyl is a detergent that releases cellular material from the substrate.

Pro K is an enzyme from the fungus *Engyodontium album* (formerly *Tritirachium album*). As a highly active endopeptidase, it cleaves peptide bonds adjacent to the carboxyl group of N-substituted (blocked alpha amino) hydrophobic, aliphatic and aromatic amino acids. Pro K is added to the extraction buffer to digest cellular material, helping the DNA to be released. It also digests proteins that interfere with the DNA binding capacity of the resin and rapidly inactivates enzymatic activity that could potentially degrade DNA (e.g. nucleases).

3.3 MULTIPROBE® II HT EX PLUS WITH GRIPPER™ INTEGRATION PLATFORM

Within Forensic DNA Analysis, DNA extractions are performed on casework or reference samples using two MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms (MPII Ext-A or MPII Ext-B) located in Room 3191.

Each platform uses a computer-controlled Cartesian XYZ liquid handling system designed for the efficient automation of sample preparation. Liquid transfer is performed by an 8-tip system with VersaTip® and VariSpan™ options. The VersaTip® option allows the use of both fixed and disposable tips (conductive and non-conductive). The VariSpan™ option permits variable probe spacing between each of the individual probes so that a wide range of lab-ware such as micro plates, tubes, vials and reagent troughs can be accessed. Each sample probe is capable of independent motion in the Z direction due to independent Z drives.

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

For automated DNA extraction using the DNA IQ™ kit, a plate map is utilised to provide the necessary information for correct volumes, and locations for pipetting. It also contains information regarding batch and plate identification, sample positions, lab numbers of samples, and lists all the samples in the batch, providing the total number of samples. The program will fail to work without a plate map.

4 REAGENTS AND EQUIPEMENT

4.1 REAGENTS

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

Table 1 Reagents with storage room and location

Reagent	Room	Location
Amphyl (0.2 and 1%)	3191	Sink
DNA IQ™ Elution Buffer	3188	In-use tray or shelf
DNA IQ™ Lysis Buffer	3188	In-use tray or shelf
DNA IQ™ Resin	3188	In-use tray or shelf
DNA IQ™ 2x Wash Buffer	3188	In-use tray or shelf
DTT (1M)	3188	Freezer
70 % v/v ethanol	3188, 3189, 3191	Sink or bench
AnalR 100 % v/v Ethanol	3188	Shelf
0.5 % v/v CleanTech Bleach	3188, 3189, 3191	Sink or bench
2- Propanol	3188	Shelf
Nanopure water	3188, 3194	Sink
40 % w/v Sarcosyl	3188	In-use tray or shelf
Pro K (20 mg/mL)	3188	Freezer
TNE	3188	In-use tray or shelf
5 % v/v Trigene Advance	3191, 3188	Sink

4.1.1 Extraction Buffer

Prepare Extraction Buffer just prior to commencing the Manual DNA IQ™ or Off-Deck Lysis procedures.

Warning: The TNE, Pro K and Sarcosyl contained in the Extraction buffer may cause irritation or damage to eyes if contact occurs, may cause irritation to skin and respiratory system, or sensitisation if inhaled. Please use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling.

1. Use the correct table in the appendices (Section 18) for the required procedure to determine the reagent volumes.
2. Remove the appropriate aliquot/s of 20 mg/mL Pro K from the freezer and thaw. Vortex mix and briefly centrifuge before use.
3. Ensure that the 40 % w/v Sarcosyl is completely dissolved and appears clear in the stock solution. If not dissolved, invert the container a few times and leave at room temperature.
4. In the biohazard cabinet, aliquot the required amount of TNE buffer into a 50 mL tube. Do not use directly from the stock bottle, use a smaller working aliquot instead.
5. Add the appropriate volume of 20 mg/mL Pro K and 40% w/v Sarcosyl to the TNE buffer and invert gently to mix.
6. Label the tube with **"Extraction Buffer"**, your initials and the date.

7. Note down all reagent lot numbers.

4.1.2 Lysis Buffer with DTT Solution

Lysis Buffer is supplied with the DNA IQ™ kit. The Lysis Buffer with DTT solution is prepared just prior to commencing the Manual or Automated DNA IQ™ procedures.

Warning: Lysis Buffer is harmful if swallowed or inhaled, and causes severe skin burns and eye damage. DTT is also harmful if swallowed and irritating to eyes, respiratory system and skin. DTT is a reducing agent that destroys disulfide bonds. Please use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling.

1. Use the correct table in the appendices (Section 18) for the required procedure to determine the reagent volumes.
2. Remove the appropriate aliquot/s of DTT from the freezer and thaw. Vortex mix and centrifuge briefly before use.
3. In the biohazard cabinet, add the required volume of Lysis Buffer to a sterilised glass Schott bottle or 50mL tube.
4. Add the required volume of DTT to the Lysis Buffer and gently swirl to mix.
5. Label the container with **"Lysis Buffer + DTT"**, your initials and the date.
6. Note down all reagent lot numbers.

4.1.3 DNA IQ™ Resin

DNA IQ™ Resin is supplied with the DNA IQ™ kit. The resin solution is prepared using the Lysis Buffer with DTT solution (from section 4.1.2) just prior to commencing the Manual or Automated DNA IQ™ procedures.

Warning: Resin may cause an allergic skin reaction. Lysis Buffer is harmful if swallowed or inhaled, and causes severe skin burns and eye damage. DTT is also harmful if swallowed and irritating to eyes, respiratory system and skin. DTT is a reducing agent that destroys disulfide bonds. Please use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling.

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

1. Use the correct table in the appendices (Section 18) for the required procedure to determine the reagent volumes.
2. In the biohazard cabinet, aliquot the required amount of Lysis Buffer with DTT solution (from 4.1.2) into a 10, 5 or 2 mL tube.
3. Thoroughly vortex mix the DNA IQ™ resin and immediately add the required volume to the Lysis Buffer with DTT solution.
4. Mix by gentle inversion.
5. Label the tube with **"Resin"**, your initials and the date.
6. Note down all reagent lot numbers.

4.1.4 1x Wash Buffer

Note: 2x Wash Buffer is supplied with the DNA IQ™ kit.

Warning: The 2 x Wash Buffer, 2-Propanol and Ethanol is flammable and hazardous. It may cause serious eye irritation, drowsiness or dizziness if vapours are inhaled. Please use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling. It is recommended that this reagent is not to be prepared / handled by pregnant staff.

To prepare 1x Wash Buffer:

1. In a biohazard cabinet, add 35 mL of *AnalR* 100 % Ethanol to the 2x Wash Buffer bottle.
2. Add 35 mL of 2- Propanol to the 2x Wash Buffer bottle.
3. Update the information on the bottle to indicate that it now contains 1x Wash Buffer and when and by whom it was prepared by. Update the 1x Wash Buffer reagent audit trail in AUSLAB by:

    - From the main page press <2> "Sample Processing"
    - Press <8> "Materials Processing"
    - Press <2> "Consumable Inventory"
    - Highlight "DNA IQ Wash Buffer" in the list and press <enter>
    - Highlight the correct lot number and press <SF8> "Consumable Lot Audit"
    - Add audit entry that specifies the 1x Wash Buffer aliquot (e.g. A1) and the lot numbers of the ethanol and isopropyl alcohol used.

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

1. Use the correct table in the appendices (Section 18) for the required procedure to determine the reagent volume.
2. In the biohazard cabinet, aliquot the required amount of 1x Wash Buffer into a 50 or 10 mL tube.
3. Label the tube with **"Wash Buffer"**, your initials and the date.
4. Note down the reagent lot number.

4.1.5 Elution Buffer

Elution Buffer is supplied with the DNA IQ™ kit.

1. Use the correct table in the appendices (Section 18) for the required procedure to determine the reagent volume.
2. In the biohazard cabinet, aliquot the required amount of 1x Wash Buffer into a 50 or 10 mL tube.
3. Label the tube with **"Wash Buffer"**, your initials and the date.
4. Note down the reagent lot number.

4.2 EQUIPMENT

4.2.1 Equipment and consumables required for Manual DNA IQ™ and Off-Deck Lysis

Table 2 outlines the equipment and location required for Manual DNA IQ™ or Off-Deck Lysis procedures.

Table 2 Equipment with asset number and location for each procedure

Equipment	Location	Procedure
Fridge	3189	Manual DNA IQ™; Off-deck Lysis
Freezers	3189 / 3190	Manual DNA IQ™; Off-deck Lysis
Biological safety cabinet class II x **4**	3188 x 1 3189 x 3	Manual DNA IQ™; Off-deck Lysis
PCR cabinet x 1	3189	Manual DNA IQ™; Off-deck Lysis
96 well tube racks	3189	Manual DNA IQ™; Off-deck Lysis
Vortex x 4	3189	Manual DNA IQ™; Off-deck Lysis
Hot block x 4	3189 / 3191 / 3194	Manual DNA IQ™; Off-deck Lysis
Centrifuge x 4	3189/3194	Manual DNA IQ™; Off-deck Lysis
Mini/Micro centrifuges x 4	3189	Manual DNA IQ™; Off-deck Lysis
Finnpipettes 100 – 1000 µL	3189	Manual DNA IQ™; Off-deck Lysis
Finnpipette 20 – 200 µL	3189	Manual DNA IQ™
Thermo mixer x 2	3189	Manual DNA IQ™
Magnetic rack	3189	Manual DNA IQ™
Multitube Shaker	3191	Manual DNA IQ™

Table 3 outlines the consumables and location required for Manual DNA IQ™ or Off-Deck Lysis procedures.

Table 3 Consumables and location for each procedure

Consumables	Location	Procedure
5 mL, 10 mL and 50mL sterile tubes	3188 / 3189	Manual DNA IQ™; Off-deck Lysis
1000 µL ART or clip tips	3188 / 3189	Manual DNA IQ™; Off-deck Lysis
1.5mL and/or 2mL tubes	3188 / 3189	Manual DNA IQ™; Off-deck Lysis
Spin baskets and 2 mL collection tubes	3189	Manual DNA IQ™; Off-deck Lysis
Inoculation sticks	3189	Manual DNA IQ™; Off-deck Lysis
300 µL ART, 200 µL One-touch tips, 200µL Clip tips	3188 / 3189	Manual DNA IQ™
Nunc Bank-it™ tubes	3189	Manual DNA IQ™; Off-deck Lysis
Nunc Bank-it™ caps	3189	Off-deck lysis
Sharps bin	3188 / 3189	Manual DNA IQ™; Off-deck Lysis

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

4.2.2 Equipment and consumables required for Automated DNA IQ™

Table 4 outlines the equipment with asset number and location required for Automated DNA IQ™.

Table 4 Equipment with asset number and location for Automated DNA IQ™

Equipment	Location
STORstar (A)	3190
STORstar (B)	3194
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (EXT MPII-A)	3191
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (EXT MPII-B)	3191
DPC shaker x 2	3191
Automated temperature controller, heat block tiles and heat block adapters x 2	3191
Milli-Q Integral 3 (A10) water purification system	3194
Eppendorf 5804 centrifuge Labogene Scanspeed 1248	3194 / 3191
Fridge	3191
Freezer	3189
MixMate	319 1/ 3194
Capit-All automated decapper	3191
4titude 4seal sealer	3191

Table 5 outlines the consumables and location required for Automated DNA IQ™.

Table 5 Consumables and location for Automated DNA IQ™

Consumables	Location
175µL Clear Non-Conductive Filter RoboRack tips – Pre-Sterilised	3191
MβP Pure 1000µL Tips – Pre-Sterilised	3191
Abgene 96-deep well plate	3191
Axygen 2mL deep well storage plate	3191
96 well Half Skirt PCR Microplate	3191
4titude Piercing Film	3191
12 Channel plate	3191
Nunc Bank-it™ tubes	3191
Nunc Bank-it™ caps	3191
Sterile 50mL Falcon tubes	3188
Sterile 10mL or 5mL tubes	3188
Autoclaved 100mL glass bottles	3188
Aluminium sealing film	3191
300uL ART tips, one-touch or clip tips	3189
1000µL ART tips or Clip tips	3189

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

5 SAFETY

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

The MPII instruments, labware, biohazard cabinets and centrifuges should be wiped with 5% v/v Trigene Advance **only**, followed by 70 % v/v ethanol.
Work benches and non-metallic equipment should be wiped with 0.5 % v/v CleanTech bleach, followed by 70 % v/v ethanol.

Warning: Do not place any part of the body inside the cabinet while the MPII is running a procedure.

If it is necessary to access the deck, pause the program using the computer software or the emergency STOP button located on the front of the instrument.

Warning: Tris base, EDTA, Sarcosyl (contained in the Extraction buffer which is prepared) and Lysis Buffer are irritants and hazardous. DTT is a reducing agent that destroys disulfide bonds. Wash Buffer (which contains 2 x Wash Buffer, 2-Propanol and Ethanol) is flammable and hazardous. Handle carefully and wear appropriate PPE. It is recommended that Wash Buffer is not prepared / handled if pregnant, and therefore the procedures using these reagents should not be performed by pregnant women.

Lysis Buffer contains guanidinium thiocyanate (GuSCN) which is toxic and can be harmful if inhaled, swallowed or comes in contact with skin. Left over Lysis Buffer-DTT is to be disposed of in a brown Winchester bottle in the ventilated cupboard in Room 3191. Never

dispose down the sink. If spillage occurs, absorb with liquid-binding material (such as sand or kitty litter) and dispose in a biohazard bin.

Do not combine bleach with Lysis Buffer and do not wipe areas of Lysis Buffer spillage with bleach, as the two reagents produce cyanide gas when combined. Handle carefully and wear appropriate PPE, including safety glasses, when handling Lysis Buffer. If Lysis Buffer is spilt onto PPE (eg. Gloves, gowns), remove and discard the contaminated item/s in a biohazard bin.

6 SAMPLING AND SAMPLE PREPARATION

6.1 SAMPLE LOCATIONS

Samples waiting to be extracted are stored in freezers as detailed in Table 6.

Table 6 Sample storage locations

Sample type	Storage Device	Storage Location
Urgent/High/Medium Priority Samples	Freezer	3190 or Block 6 walk-in freezer*
Lysates in 1.0mL Nunc Bank-it™ tubes	Freezer	3190
Extracts in 1.0mL Nunc Bank-It™ tubes	Freezer	3194 A

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

6.2 QC SAMPLES

One negative reagent control and one positive blood control (from a known donor) must be registered for all extraction batches. Additionally, off-deck lysis batches require five blank controls as detailed in Table 7.

Table 7 Extraction Quality Controls

Batch Type	Control
Off-deck lysis	Positive control (x1)
	Negative control (x1)
	Blank control (x5)
Manual DNA IQ™	Positive control (x1)
	Negative control (x1)
Retain supernatant DNA IQ™	Positive control (x1)
	Negative control (x1)

6.3 REGISTRATION OF QC SAMPLES

Register control samples according to the standard laboratory procedure QIS 24919 Forensic DNA Analysis Workflow Procedure. CQ samples for off-deck lysis are to be registered with a 9PLEX test code. QC samples for a DNA IQ Maxwell or a Retain Supernantant DNA IQ extraction batch are to be registered using the XPLEX test code.

6.4 CREATION OF EXTRACTION BATCHES

Create extraction batches according to the standard laboratory procedure QIS 24919 Forensic DNA Analysis Workflow Procedure. All samples that are registered using a 9PLEX test code will be extracted using the automated DNA IQ method with the exception of tapelifts that will be processed using the DNA IQ Maxwell procedure.

6.5 LOCATING SAMPLES

Locate samples according to the standard laboratory procedure QIS 23959 Storage Guidelines for Forensic DNA Analysis.

6.6 SEQUENCE CHECKING AUTOMATED EXTRACTION BATCHES

Sequence check lysates in Nunc Bank-It tubes for automated extraction batches according to the standard laboratory procedure QIS 24256 Procedure for the use of the STORstar unit for automated sequence checking.

6.7 ELECTRONIC WORKFLOW DIARY

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

7 MANUAL METHODS FOR EXTRACTION USING DNA IQ™

7.1 MANUAL DNA IQ™ (NO RETAIN SUPERNTANT)

Note: All manual extractions are to be performed within a biohazard cabinet in room 3189.

1. Prepare fresh reagents within the biohazard hood of the clean room (3188) prior to commencing the procedure. Refer to Section 4.1 for preparation of reagents. Use Table 8 in Appendix 18.1 for reagent volumes.

Warning: The 2 x Wash Buffer, 2-Propanol and Ethanol is flammable and hazardous. It may cause serious eye irritation, drowsiness or dizziness if vapours are inhaled. Please use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling. It is recommended that this reagent is not to be prepared / handled if pregnant, and therefore this procedure should not be performed by pregnant women.

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

3. Label for each sample:
   - Original sample tube;
   - Spin basket (if required);
   - 2mL tube; and
   - Nunc™ Bank-It™ tube.

Note: A spin basket is required for swabs, fabric, paper and cigarette butts. Samples that do not require a spin basket are tape-lifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

4. Have a second operator perform a sequence check of all tubes. Record the sequence check in AUSLAB.

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

6. Vortex and incubate at 37 °C on the Thermomixer at 1400 rpm for 45 minutes.

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

8. Transfer the substrate to spin basket (if required) or transfer the lysate to an appropriately labelled 2.0 mL tube. Retain the original tube containing the substrate if no spin basket is used.

9. Centrifuge the spin basket at 15800 xg for 2 minutes.

10. Transfer the lysate from the original sample tube and the flow-through from the collection tube of the spin basket to an appropriately labelled 2 mL tube. Transfer the substrate from the spin basket back into the original sample tube.

    Note: If original sample tube is an appropriate 2 mL tube, transfer the substrate into the new 2 mL tube and add the flow-through from the spin basket to the lysate still in the original sample tube.

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

12. Add 50 µL of DNA IQ™ Resin-Lysis Buffer-DTT solution to the lysate. Pipette mix or vortex the solution frequently to keep a homogenous suspension of resin within the solution.

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

14. Remove from the Multitube shaker, vortex and immediately place into the magnetic stand. Separation will occur instantly.

    Note: If resin does not form a distinct pellet on the side of the tube, or if the magnetic beads have re-suspended while in the stand, vortex the tube and quickly place back in the stand.

15. Carefully remove and discard the liquid, ensuring that the resin is not disturbed. Remove from the magnetic stand.

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

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

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

18. Add 100 µL of 1X Wash Buffer, vortex and place into the magnetic stand. Once separation has occurred remove and discard the liquid. Remove the tube from the magnetic stand.

19. Repeat step 18 twice for a total of three Wash Buffer washes. Ensure that all of the liquid has been removed after the last wash. Remove the tube from the magnetic stand.

20. Uncap the tubes and place the lids down onto a clean rediwipe within the biohazard cabinet. Allow the resin to air-dry in the hood for 15 minutes and then recap the tubes.

    Note: Do not allow the resin to dry for more than 20 minutes as this may inhibit DNA elution.

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

22. Incubate in the Thermomixer at 65°C for 3 minutes and then continue to incubate for a further 3 minutes shaking at 1100 rpm. If no thermomixer is available, incubate samples within a hot block and vortex mix at the start and end of the second 3 minute incubation. Remove samples.

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

24. Carefully transfer the DNA extract to the corresponding labelled Nunc™ Bank-It™ tube.

25. Remove from the magnetic stand and repeat steps 21-23 once. The final volume after the second elution is approximately 95 µL of DNA extract.

26. Store the DNA extracts in temporary storage located within the Pre-PCR sorting room 3194-A in the upright freezer with equipment number 30512883.

27. Store tubes containing substrates in Spin Basket boxes located within the Pre-PCR sorting room 3194-A in the upright freezer with equipment number 30512883.

7.2 PROCEDURE FOR MANUAL DNA IQ™ (RETAIN SUPERNATANT)

Note: All manual extractions are to be performed within a biohazard cabinet in room 3189.

1. Prepare fresh reagents within the biohazard hood of the clean room (3188) prior to commencing the procedure. Refer to Section 4.1 for preparation of reagents. Use Table 9 in the Appendix 18.2 for reagent volumes.

Warning: The 2 x Wash Buffer, 2-Propanol and Ethanol is flammable and hazardous. It may cause serious eye irritation, drowsiness or dizziness if vapours are inhaled. Please use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling. It is recommended that this reagent is not to be prepared / handled if pregnant, and therefore this procedure should not be performed by pregnant women.

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

3. Label for each sample:
   - Original sample tube;
   - 2 mL tube for sample transfer and processing;
   - 1.5mL tube labelled 'SUP' for supernatant;
   - Spin basket (if required); and
   - Nunc™ Bank-It™ tube.

   Note: A spin basket is required for swabs, fabric, paper and cigarette butts. Samples that do not require a spin basket are tape-lifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

4. Have a second operator perform a sequence check of all tubes. This person must enter the sequence check in AUSLAB.

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

6. Incubate at room temperature for 30 minutes.

7. Vortex, then centrifuge at 15800 xg for 3 minutes.

8. Remove 150 µL of supernatant and place into the respective 1.5 mL tube labelled with 'SUP' (for further testing).

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

10. Vortex, then incubate at 37°C on the Thermomixer at 1400 rpm for 45 minutes. If no thermomixer available, incubate samples within a hot block, vortex mix at the start and end of the incubation and at least one during the incubation.

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

12. Transfer substrate to spin basket if required or transfer the lysate to an appropriately labelled 2.0mL tube. Retain original tube containing the substrate in if no spin basket used.

13. Centrifuge spin basket at 15800 g for 2 minutes.

14. Transfer the substrate in the spin basket into a new and labelled 2mL tube to be retained. Transfer the flow through (lysate) back into the original sample tube.

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

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

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

18. Remove from the Multitube shaker, vortex and immediately place into the magnetic stand. Separation will occur instantly.

    Note: If resin does not form a distinct pellet on the side of the tube, or if the magnetic beads have re-suspended while in the stand, vortex the tube and quickly place back in the stand.

19. Carefully remove and discard the solution, ensuring that the resin is not disturbed. Remove from the magnetic stand.

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

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

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

22. Add 100µL of 1X Wash Buffer, vortex and place into the magnetic stand. Once separation has occurred remove and discard the Wash Buffer. Remove from the magnetic stand.

23. Repeat the Wash Buffer step (step 23) two times for a total of three washes. Ensure that all of the solution has been removed after the last wash. Remove from the magnetic stand.

24. Uncap the tubes and place the lids down onto a clean rediwipe. Air-dry the resin in the hood for 15 minutes and then recap tubes

    Note: Do not dry for more than 20 minutes, as this may inhibit the elution of DNA.

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

26. Incubate in the Thermomixer at 65°C for 3 minutes and then continue to incubate for a further 3 minutes shaking at 1100 rpm. If no thermomixer available, incubate samples within a hot block, vortex mix at the start and end of 2nd 3 minute incubation. Remove samples.

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

28. Carefully transfer the DNA extract to the corresponding labelled Nunc™ Bank-It™ tube.

29. Remove from the magnetic stand and repeat the Elution Buffer steps (step 26-29). The final volume after the double elution is approximately 95µL of DNA extract.

30. Store the DNA extracts in temporary storage located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

31. Store tubes containing substrates in Spin Basket boxes located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

32. Store supernatants in the S/N Retention boxes located within the Extraction sorting room in the Orford Freezer 30512883 (Room 3190).

33. Ensure that the negative extraction control from the batch is also inserted onto the **'Saliva List':**

    - Log into Auslab
    - 3 for Patient Enquiry
    - Type/Scan the lab number for the negative extraction control
    - From the 9Plex page or the XPLEX Page press Shift F12
    - A prompt will appear 'Enter List Name'
    - Type Saliva or use the F1 lookup list.
    - Press Enter

8 OFF-DECK LYSIS PROCEDURE

1. All reagents are to be made fresh within the clean room (3188) prior to commencing each process. Use Table 10 in Appendix 18.3 for reagent volumes.

2. All manual extractions are to be performed within a biohazard cabinet in Room 3189

3. For each sample label:
   - Original sample tube
   - Spin basket as required
   - 1.0mL Nunc Bank-It™ tube
   - 1.5mL or 2.0mL tube

   Note: Samples that require a spin basket are: swabs, fabric, paper and cigarette butts. Samples that do not require a spin basket are: tapelifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

4. Have a second operator sequence check the tubes and enter the sequence check in AUSLAB.

5. Add 300µL of Extraction Buffer and vortex briefly. Ensure that substrates are immersed in the Extraction Buffer.

6. Incubate for 45 minutes at 37 degrees on a hotblock. It is best practice to vortex the samples intermittently throughout the duration of the incubation period.

7. Remove from the hotblock. Vortex and pulse spin samples to remove condensation from the lids.

8. Transfer substrate to spin basket if required or transfer the lysate to an appropriately labelled 1.5mL or 2mL tube. Retain original tube containing the substrate if no spin basket is used.

9. Centrifuge spin baskets at 15800 g for 2 minutes.

10. Transfer the substrate in the spin basket to a new and appropriately labelled 1.5mL or 2mL tube and retain.Transfer the flow through back to original lysis tube.

11. Vortex lysate, then incubate on hotblock at 65°C for 10 minutes. Record temperature on worksheet.

12. Centrifuge at 15800 g for 1 minute.

13. Transfer 300µL of lysate to the corresponding Nunc Bank-It™ tube.

Note: If more than 300µL of lysate is present, retain the remaining lysate in the 1.5mL tube. In AUSLAB, write a specimen note for that sample (eg. Extra lysate retained from sample XXXXXXXXX.). Store the retained 1.5mL lysate tube in appropriate box in freezer.

14. In AUSLAB, enter reagent and hotblock temperature details and complete the batch.

15. Store lysates in temporary storage boxes located within the extraction sorting room in the orford Freezer with equipment number 102384430 (Room 3190).

16. Store tubes containing substrates or extra lysate in Spin Basket boxes located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

9 AUTOMATED EXTRACTION OF LYSED SAMPLES

9.1 MPII EXTRACTION PROCEDURE

Automated setup of DNA IQ extractions in 96-deep well format is performed using the MultiPROBE® II PLUS HT EX EP-A and EP-B platforms located in Room 3191.

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

9.2 PREPARATION OF REAGENTS FOR THE AUTOMATED EXTRACTION PROCESS

All reagents are to be made fresh within the clean room (3188) prior to commencing each process. Use Table 11 in the Appendix 18.4 for reagent volumes.

Warning: The 2 x Wash Buffer, 2-Propanol and Ethanol is flammable and hazardous. It may cause serious eye irritation, drowsiness or dizziness if vapours are inhaled. Please use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling. It is recommended that this reagent is not to be prepared / handled if pregnant, and therefore this procedure should not be performed by pregnant women.

9.3 SUMMARY OF DNA IQ™ EXTRACTION VERSION 6.7_ODL

9.3.1 Transfer of lysates from Nunc Bank-It™ tubes into the Abgene 96-deep well plate

Lysates from the off-deck lysis protocol, contained in individual Nunc Bank-It™ tubes, are transferred automatically into an Abgene 96-deep well plate prior to commencing automated sample processing. Instead of a 96-deep well plate, the use of individual Nunc Bank-It™ tubes for storage of lysates removes the need for an adhesive film and therefore reduces the risk of well-to-well contamination from the action of peeling back an adhesive plate cover.

9.3.2 Automated addition of DNA IQ™ Resin and Lysis Buffer

DNA IQ™ Resin is added automatically into the Abgene 96-deep well plate on the platform. The program uses a waste mode dispensing step to add 53µL DNA IQ™ Resin-Lysis Buffer solution to each well in either half the plate or the whole plate. Because of this, batch sizes are restricted to either 48 or 96 samples in order to maintain efficiency and economy. Two volumes of Lysis Buffer are then added to promote binding of DNA to the paramagnetic resin.

9.3.3 Mixing using a MixMate to bind DNA to resin

Manual intervention is required to seal the Abgene 96-deep well plate with a 4titude Pierce Seal film using the 4titude sealer pre-heated at 175 °C. The plate is then transferred onto a MixMate instrument for mixing at 1100rpm for 5 minutes, followed by centrifugation on an Eppendorf 5804 centrifuge at 3000rpm for 2 minutes. The seal is then carefully pierced with a 96 well PCR microplate and the Abgene plate is returned to the Applied Biosystems magnet on the MPII platform.

9.3.4 Removing lysis reagents for storage

At this point, most of the DNA is bound to the paramagnetic resin. With the positioning of the Abgene plate on the ABI magnet, DNA IQ™ Resin becomes immobile at the bottom of the plate. The lysis reagents from each well are transferred automatically to a storage plate on the MPII platform without disturbing the DNA IQ™ Resin.

9.3.5 Washing of the resin-DNA complex

Washing steps are performed to remove any inhibitors in solution. The first wash uses 125µL Lysis Buffer with shaking at room temperature for 1 minute on the DPC shaker to ensure that the DNA is bound to the paramagnetic resin. The plate is moved to the ABI magnet and the supernatant is transferred into the storage plate. The next three washes incorporate 100µL of 1x Wash Buffer with shaking at room temperature for 1 minute on the DPC shaker. During each wash cycle, the Abgene 96-deep well plate is moved to the ABI magnet and the supernatant is discarded into the tip chute.

9.3.6 Removing any excess of 1x Wash Buffer

The samples are allowed to air dry at room temperature for 5 minutes in order to remove all traces of Ethanol from the Wash Buffer. The presence of Ethanol may potentially inhibit both the elution process and also downstream PCR.

9.3.7 Elution of DNA from the resin-DNA complex

A double elution method is employed in this procedure. At each elution step, 60µL of Elution Buffer is added to each sample, followed by incubation at 65°C for 6 minutes (3 minutes without shaking followed by 3 minutes shaking on the DPC shaker). The Abgene plate is moved to the ABI magnet and the eluted solution (supernatant containing eluted DNA) is transferred to fresh Nunc™ Bank-It™ tubes.

9.3.8 Flushing of liquid pathway

As a decontamination measure, the liquid pathway is washed with Amphyl and Nanopure water at the conclusion of the automated extraction process.

9.4 SETTING UP THE MPII PLATFORMS FOR AUTOMATED DNA IQ™ PROCESSING

The following steps are carried out in the automated extraction room (Room 3191).

1. Remove the Nunc Bank-It™ tubes containing lysates from the fridge to allow to come to room temperature before commencing the extraction procedure.

   Note: If the lysates are frozen, remove them from the freezer and thaw in Room 3191.

2. Restart or turn on the instrument PC.
3. Log onto the network using the **Robotics** login.
4. Open WinPrep® by double clicking icon on the computer desktop (Figure 1).

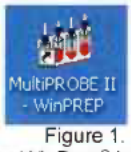

Figure 1.
The WinPrep® icon.

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):

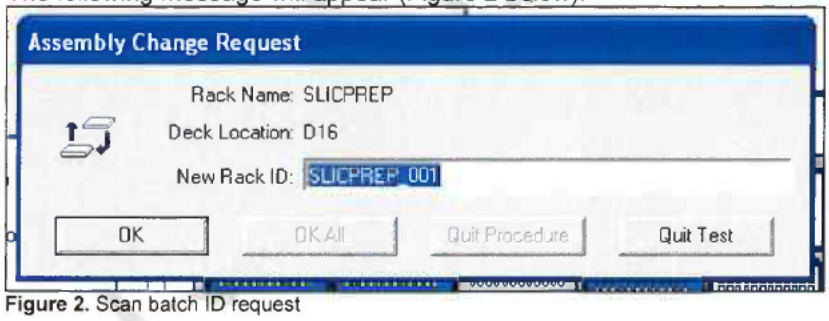

Figure 2. Scan batch ID request

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.
- 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

14 STORAGE OF DOCUMENTS

The off-deck lysis worksheets are to be stored within the extraction sorting room (3190) in the nominated intray. It is essential that ALL off-deck lysis worksheets go within this intray so the operational officers are aware that batches are ready and waiting to be combined for extraction on the automated robots.

All worksheets, after auto extraction, manual IQ and Retain supernatant batches have been completed, are stored within the Pre-PCR sorting room (3194-A) in an allocated in-tray for scanning by the operational officers.

15 REFERENCES

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16 ASSOCIATED DOCUMENTS

QIS 17165 Receipt, Storage and Preparation of Chemicals, Reagents and Test Kits
QIS 22857 Anti-Contamination procedure
QIS 23939 Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform
QIS 23959 Storage Guidelines for Forensic DNA Analysis
QIS 24256 Sequence Checking with the STORstar Instrument
QIS 24469 Batch functionality in AUSLAB
QIS 24919 Forensic DNA Analysis Workflow Procedure
QIS 17130 CE Quality Check
QIS 24012 Miscellaneous Analytical Section Tasks

17 AMENDMENT HISTORY

Version	Date	Author/s	Amendments
R0	23 Oct 2007	B. Gallagher, T. Nurthen, C. Iannuzzi, V. Hlinka, G. Lundie, I Muharam.	First Issue

Version	Date	Author/s	Amendments
R1	12 Dec 2007	M Harvey, C Iannuzzi, A McNevin	Reviewed and updated after initial training
R2	19 March 2008	M Harvey, B Andersen, C Iannuzzi, A McNevin	Addition of Off-deck Lysis procedure, Retention of fully automated method as Appendix, addition of reagent record tables into Appendix
R3	April 2008	QIS2 Migration Project	Headers and Footers changed to new CaSS format. Amended Business references from QHSS to FSS, QHPSS to CaSS and QHPS to Pathology Queensland
4	13 March 2009	QIS2 migration	Version incremented by one on migration to QIS2
5	03 June 2009	M Aguilera, B Micic, C Iannuzzi, A. Cheng, V. Hlinka, I. Muharam, G. Lundie, C. Weber	Major changes to reflect new procedure. Updated to reflect changes in procedure as an outcome of internal and external audits. Created ver.6.4 ODL in MPII Platforms. Minor changes in procedures using 4titude 4seal heat sealer to seal plates.
6	29 June 2009	A McNevin, K Lancaster	Removed references to retaining lysate and beads, fixed minor formatting errors. Created ver6.5 ODL in MPII Platforms. Substrates now to be retained in 2mL tube
7	30 September 2010	M.Cipollone, M.Mathieson	Major changes made RE: room numbers to reflect the move to Block 3. Re-formatted entire SOP. New Appendices added which outline all reagent volumes. All equipment and associated asset numbers have been included. Use of the electronic diary now included as an additional section. Preparation of reagents within the clean room (3188) now to be done prior to starting each process. Storage of worksheets updated. New software version 6.6 on automated extraction robots. S/N Retention Boxes now stored in Manual Ext Room. Associated Documents and hyperlinks updated. Consumables and Equipment table added for Manual DNA IQ.
8	24 May 2012	A. Speirs M. Cipollone	Major revision of document. Revision of sections 1-3 to improve clarity. Updated Tables 1 to 7. Removed redundant sections on locating samples and creating batches. Removed procedure for retain supernatant Off-Deck Lysis and thus associated Appendix. Table of Contents amended to reflect all

Version	Date	Author/s	Amendments
			changes. Moved the Manual DNA IQ procedures before the Off-Deck Lysis procedure. Re-formatted spacing and alignments of headings. Re-numbered Tables in Appendices. Updated sections to include which relevant Appendix to use in regards to reagents. Minor grammatical and spelling errors fixed.
9	4 Dec 2013	B. Andersen, M. Cipollone	Updated Section 7 of the Manual DNA IQ Retain Supernatant procedure to reflect differences in list inserting between P+ and PP21 samples. Updated Section 6 to clarify 9PLEX and XPLEX test codes. New template applied and minor formatting and wording changes.
10	April 2015	M. Aguilera M.Cipollone	Adjusted Table 9 for Retain Supernatant batches to indicated TNE, Prok and Sarcosyl are not to be combined at reagent prep stage and to be added to samples separately. Changed reference to Isopropyl Alcohol to 2-Propanol. Included warning for the preparation and use of the Wash Buffer for pregnancy, and amended Risk phrases for reagents used as per risk assessment completed by JSM. Updated equipment tables and removed asset numbers. Changed template to HSSA Fixed Document headers; Fixed formatting, removed steps no longer used; Added two more associated documents QIS 17130 CE Quality Check QIS 24012 Miscellaneous Analytical Section Tasks

18 APPENDICES

18.1 REAGENT VOLUMES FOR MANUAL DNA IQ™ (NO RETAIN SUPERNATANT)

Table 8 - Reagent volumes for manual method

Reagent	Volume for 12 samples (mL)	Volume for 24 Samples (mL)
TNE buffer	4.0	8.0
Proteinase K (20mg/mL)	0.216	0.432
Sarcosyl (40%)	0.108	0.216
Lysis-DTT Buffer		
DNA IQ™ Lysis Buffer	10	20
DTT (1M)	0.1	0.2
DNA IQ™ Resin solution		
Lysis-DTT Buffer	0.645	1.29
DNA IQ™ Resin	0.105	0.210
DNA IQ™ 1x Wash Buffer	4.0	8.0
DNA IQ™ Elution Buffer	1.4	2.8

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

18.2 REAGENT VOLUMES FOR MANUAL DNA IQ™ (RETAIN SUPERNATANT)

Table 9 - Reagent volumes for manual method (retain supernatant)

Reagent	Volume per sample (uL)	Volume for 12 samples (mL)
TNE buffer	450	5.4
Proteinase K (20mg/mL)	14	0.168
Sarcosyl (40%)	7	0.084
Lysis-DTT Buffer		
DNA IQ™ Lysis Buffer	660	10
DTT (1M)	6.6	0.1
DNA IQ™ Resin solution		
Lysis-DTT Buffer	43	0.645
DNA IQ™ Resin	7	0.105
DNA IQ™ 1x Wash Buffer	300	4.0
DNA IQ™ Elution Buffer	100	1.4

Note: The volume of Lysis-DTT Buffer calculated includes the volume used in the Resin-Lysis solution preparation. For retain supernatant batches, please ensure that TNE, Pro K, and Sarcosyl are not combined at reagent preparation stage and are to be added to samples separately.

18.3 REAGENT VOLUMES FOR THE OFF DECK LYSIS PROCEDURE

Table 10 - Reagent volumes for off-deck procedure

Reagent	Volume for 39 Samples (mL)
Extraction Buffer	
TNE buffer	12
Proteinase K (20mg/mL)	0.648
Sarcosyl (40%)	0.324

Note: Do not directly pipette from reagent stock bottles. Stock reagents should be decanted into working containers where appropriate.

18.4 REAGENT VOLUMES FOR AUTOMATED DNA IQ™

Table 11 - Reagent volumes for automated procedure

Reagent	Volume for 78 samples (mL)
Lysis-DTT Buffer	
DNA IQ™ Lysis Buffer	70
DTT (1M)	0.700
DNA IQ™ Resin solution	
Lysis-DTT Buffer	6.0
DNA IQ™ Resin	1.0
DNA IQ™ 1x Wash Buffer	30.0
DNA IQ™ Elution Buffer	12.0

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

1 PURPOSE AND SCOPE

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

Reference samples and casework samples must be extracted on separate batches.

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

2 DEFINITIONS

DTT	1,4 Dithiothreitol
EDTA	Ethylenediaminetetraacetatic acid
Extracts	Samples that have undergone DNA extraction
Lysates	Samples that have undergone off-deck lysis but not DNA extraction
Paramagnetic	Becomes magnetic with the application of a magnetic force
Pro K	Proteinase K
Samples	Sample substrates in tubes awaiting a DNA extraction process
Sarcosyl	N-Lauroylsarcosine
TNE	Tris, NaCl and EDTA buffer

3 PRINCIPLE

3.1 DNA IQ™ KIT

The DNA IQ™ system (Promega Corp., Madison, WI, USA) is designed to extract and purify DNA from a number of different substrates and has been optimised for trace DNA samples.

Cell lysis is performed using DNA IQ™ Lysis Buffer containing Dithiothreitol (DTT). 1,4 Dithiothreitol is a reducing agent used in extractions to break disulfide bonds of proteins. The Lysis Buffer used is a proprietary buffer containing chaotropic salts required for binding of DNA to the magnetic beads. According to the MSDS (Promega Corporation 2006), the Lysis Buffer contains 50-75% guanidinium thiocyanate, < 2.5% EDTA, < 2% 3-[(3-Choalamidopropryl)dimethylammonio] propanesulfonic and < 2% polyethylene glycol tert-octylphenyl ether.

The foundation of the DNA IQ™ system is the magnetic bead resin which contains novel paramagnetic particles covered with silica. The resin has a negative charge at basic and near neutral pH. However, the addition of Lysis Buffer changes the pH and salt concentration of the solution, resulting in the silica on the magnetic beads becoming positively charged and able to bind free DNA.

The magnetic bead resin has a DNA binding capacity of 100ng and binding is more efficient with samples containing small amounts of DNA rather than samples with large amounts of DNA. As a result, small sample size is necessary to ensure efficient recovery of DNA.

Inhibitors are removed from the DNA through several washing steps. The first washing step is performed using Lysis Buffer to ensure the DNA is bound to the magnetic bead resin and to wash out inhibitors. The next three washing steps incorporate the use of DNA IQ™ Wash Buffer. This buffer contains an alcohol/aqueous mixture which dehydrates the DNA to ensure it is not eluted during washing, while the aqueous phase washes out inhibitors.

The DNA IQ™ Elution Buffer changes the salt content which, in conjunction with heating to 65 °C, allows the DNA to be released from the magnetic beads. The Elution Buffer is a low ionic strength buffer that reduces the affinity of the DNA for the silica that covers the magnetic beads by re-hydration of the phosphate backbone. To prevent PCR inhibition caused by the presence of very small DNA fragments within extracts the DNA IQ™ System selectively isolates DNA fragments greater than 80bp.

3.2 OFF-DECK LYSIS PROCEDURE

Samples must undergo a pre-extraction Off-Deck Lysis step before processing with the DNA IQ™ reagents on the MPII instrument.

The Extraction Buffer used for the Off-Deck Lysis is designed to digest cellular material and release DNA. It contains TNE buffer (Tris, NaCl, EDTA, pH 8.0), Sarcosyl and Pro K.

TNE acts as a basic buffer with EDTA chelating ions in solution.

Sarcosyl is a detergent that releases cellular material from the substrate.

Pro K is an enzyme from the fungus *Engyodontium album* (formerly *Tritirachium album*). As a highly active endopeptidase, it cleaves peptide bonds adjacent to the carboxyl group of N-substituted (blocked alpha amino) hydrophobic, aliphatic and aromatic amino acids. Pro K is added to the extraction buffer to digest cellular material, helping the DNA to be released. It also digests proteins that interfere with the DNA binding capacity of the resin and rapidly inactivates enzymatic activity that could potentially degrade DNA (e.g. nucleases).

4 REAGENTS AND EQUIPEMENT

4.1 REAGENTS

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

Table 1 Reagents with storage room and location

Reagent	Room	Location
Amphyl (0.2 and 1%)	3191	Sink
DNA IQ™ Elution Buffer	3188	In-use tray or shelf
DNA IQ™ Lysis Buffer	3188	In-use tray or shelf
DNA IQ™ Resin	3188	In-use tray or shelf
DNA IQ™ 2x Wash Buffer	3188	In-use tray or shelf
DTT (1M)	3188	Freezer
70 % v/v ethanol	3188, 3189, 3191	Sink or bench
AnalR 100 % v/v Ethanol	3188	Shelf
0.5 % v/v Bleach	3188, 3189, 3191	Sink or bench
2- Propanol	3188	Shelf
Nanopure water	3188, 3194	Sink
40 % w/v Sarcosyl	3188	In-use tray or shelf
Pro K (20 mg/mL)	3188	Freezer
TNE	3188	In-use tray or shelf
5 % v/v Trigene Advance	3191, 3188	Sink

4.1.1 Extraction Buffer

Prepare Extraction Buffer just prior to commencing the Manual DNA IQ™ or Off-Deck Lysis procedures.

Warning: The TNE, Pro K and Sarcosyl contained in the Extraction buffer may cause irritation or damage to eyes if contact occurs, may cause irritation to skin and respiratory system, or sensitisation if inhaled. Please use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling.

1. Use the correct table in the appendices (Section 18) for the required procedure to determine the reagent volumes.
2. Remove the appropriate aliquot/s of 20 mg/mL Pro K from the freezer and thaw. Vortex mix and briefly centrifuge before use.
3. Ensure that the 40 % w/v Sarcosyl is completely dissolved and appears clear in the stock solution. If not dissolved, invert the container a few times and leave at room temperature.
4. In the biohazard cabinet, aliquot the required amount of TNE buffer into a 50 mL tube. Do not use directly from the stock bottle, use a smaller working aliquot instead.
5. Add the appropriate volume of 20 mg/mL Pro K and 40% w/v Sarcosyl to the TNE buffer and invert gently to mix.
6. Label the tube with **"Extraction Buffer"**, your initials and the date.
7. Note down all reagent lot numbers.

4.1.2 Lysis Buffer with DTT Solution

Lysis Buffer is supplied with the DNA IQ™ kit. The Lysis Buffer with DTT solution is prepared just prior to commencing the Manual DNA IQ™ procedures.

Warning: Lysis Buffer is harmful if swallowed or inhaled, and causes severe skin burns and eye damage. DTT is also harmful if swallowed and irritating to eyes, respiratory system and skin. DTT is a reducing agent that destroys disulfide bonds. Please use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling.

1. Use the correct table in the appendices (Section 18) for the required procedure to determine the reagent volumes.
2. Remove the appropriate aliquot/s of DTT from the freezer and thaw. Vortex mix and centrifuge briefly before use.
3. In the biohazard cabinet, add the required volume of Lysis Buffer to a sterilised glass Schott bottle or 50mL tube.
4. Add the required volume of DTT to the Lysis Buffer and gently swirl to mix.

5. Label the container with **"Lysis Buffer + DTT"**, your initials and the date.
6. Note down all reagent lot numbers.

4.1.3 DNA IQ™ Resin

DNA IQ™ Resin is supplied with the DNA IQ™ kit. The resin solution is prepared using the Lysis Buffer with DTT solution (from section 4.1.2) just prior to commencing the Manual DNA IQ™ procedures.

Warning: Resin may cause an allergic skin reaction. Lysis Buffer is harmful if swallowed or inhaled, and causes severe skin burns and eye damage. DTT is also harmful if swallowed and irritating to eyes, respiratory system and skin. DTT is a reducing agent that destroys disulfide bonds. Please use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling.

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

1. Use the correct table in the appendices (Section 18) for the required procedure to determine the reagent volumes.
2. In the biohazard cabinet, aliquot the required amount of Lysis Buffer with DTT solution (from 4.1.2) into a 10, 5 or 2 mL tube.
3. Thoroughly vortex mix the DNA IQ™ resin and immediately add the required volume to the Lysis Buffer with DTT solution.
4. Mix by gentle inversion.
5. Label the tube with **"Resin"**, your initials and the date.
6. Note down all reagent lot numbers.

4.1.4 1x Wash Buffer

Note: 2x Wash Buffer is supplied with the DNA IQ™ kit.

Warning: The 2 x Wash Buffer, 2-Propanol and Ethanol is flammable and hazardous. It may cause serious eye irritation, drowsiness or dizziness if vapours are inhaled. Please use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling. It is recommended that this reagent is not to be prepared / handled by pregnant staff.

To prepare 1x Wash Buffer:
1. In a biohazard cabinet, add 35 mL of *AnalR* 100 % Ethanol to the 2x Wash Buffer bottle.
2. Add 35 mL of 2- Propanol to the 2x Wash Buffer bottle.
3. Update the information on the bottle to indicate that it now contains 1x Wash Buffer and when and by whom it was prepared by. Update the 1x Wash Buffer reagent audit trail in AUSLAB by:

   - From the main page press <2> "Sample Processing"
   - Press <8> "Materials Processing"
   - Press <2> "Consumable Inventory"
   - Highlight "DNA IQ Wash Buffer" in the list and press <enter>
   - Highlight the correct lot number and press <SF8> "Consumable Lot Audit"
   - Add audit entry that specifies the 1x Wash Buffer aliquot (e.g. A1) and the lot numbers of the ethanol and isopropyl alcohol used.

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

1. Use the correct table in the appendices (Section 18) for the required procedure to determine the reagent volume.
2. In the biohazard cabinet, aliquot the required amount of 1x Wash Buffer into a 50 or 10 mL tube.
3. Label the tube with **"Wash Buffer"**, your initials and the date.
4. Note down the reagent lot number.

4.1.5 Elution Buffer

Elution Buffer is supplied with the DNA IQ™ kit.

1. Use the correct table in the appendices (Section 18) for the required procedure to determine the reagent volume.
2. In the biohazard cabinet, aliquot the required amount of 1x Wash Buffer into a 50 or 10 mL tube.
3. Label the tube with **"Wash Buffer"**, your initials and the date.
4. Note down the reagent lot number.

4.2 EQUIPMENT

4.2.1 Equipment and consumables required for Manual DNA IQ™

Table 2 outlines the equipment and location required for Manual DNA IQ™ procedures.

Table 2 Equipment with asset number and location for each procedure

Equipment	Location	Procedure
Fridge	3189	Manual DNA IQ™; Off-deck Lysis
Freezers	3189 / 3190	Manual DNA IQ™; Off-deck Lysis
Biological safety cabinet class II x **4**	3188 x 1 3189 x 3	Manual DNA IQ™; Off-deck Lysis
PCR cabinet x 1	3189	Manual DNA IQ™; Off-deck Lysis
96 well tube racks	3189	Manual DNA IQ™; Off-deck Lysis
Vortex x 4	3189	Manual DNA IQ™; Off-deck Lysis
Hot block **x 4**	3189 / 3191 / 3194	Manual DNA IQ™; Off-deck Lysis
Centrifuge x 4	3189/3194	Manual DNA IQ™; Off-deck Lysis
Mini/Micro centrifuges x 4	3189	Manual DNA IQ™; Off-deck Lysis
Finnpipettes 100 – 1000 µL	3189	Manual DNA IQ™; Off-deck Lysis
Finnpipette 20 – 200 µL	3189	Manual DNA IQ™
Thermo mixer x 2	3189	Manual DNA IQ™
Magnetic rack	3189	Manual DNA IQ™
Multitube Shaker	3191	Manual DNA IQ™

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

Table 3 Consumables and location for each procedure

Consumables	Location	Procedure
5 mL, 10 mL and 50mL sterile tubes	3188 / 3189	Manual DNA IQ™; Off-deck Lysis
1000 µL ART or clip tips	3188 / 3189	Manual DNA IQ™; Off-deck Lysis
1.5mL and/or 2mL tubes	3188 / 3189	Manual DNA IQ™; Off-deck Lysis
Spin baskets and 2 mL collection tubes	3189	Manual DNA IQ™; Off-deck Lysis
Inoculation sticks	3189	Manual DNA IQ™; Off-deck Lysis
300 µL ART, 200 µL One-touch tips, 200µL Clip tips	3188 / 3189	Manual DNA IQ™
Nunc Bank-it™ tubes	3189	Manual DNA IQ™; Off-deck Lysis
Nunc Bank-it™ caps	3189	Off-deck lysis
Sharps bin	3188 / 3189	Manual DNA IQ™; Off-deck Lysis

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

5 SAFETY

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

The MPII instruments, labware, biohazard cabinets and centrifuges should be wiped with 5% v/v Trigene Advance **only**, followed by 70 % v/v ethanol.
Work benches and non-metallic equipment should be wiped with 0.5 % v/v bleach, followed by 70 % v/v ethanol.

Warning: Do not place any part of the body inside the cabinet while the MPII is running a procedure.

If it is necessary to access the deck, pause the program using the computer software or the emergency STOP button located on the front of the instrument.

Warning: Tris base, EDTA, Sarcosyl (contained in the Extraction buffer which is prepared) and Lysis Buffer are irritants and hazardous. DTT is a reducing agent that destroys disulfide bonds. Wash Buffer (which contains 2 x Wash Buffer, 2-Propanol and Ethanol) is flammable and hazardous. Handle carefully and wear appropriate PPE. It is recommended that Wash Buffer is not prepared / handled if pregnant, and therefore the procedures using these reagents should not be performed by pregnant women.

Lysis Buffer contains guanidinium thiocyanate (GuSCN) which is toxic and can be harmful if inhaled, swallowed or comes in contact with skin. Left over Lysis Buffer-DTT is to be disposed of in a brown Winchester bottle in the ventilated cupboard in Room 3191. Never dispose down the sink. If spillage occurs, absorb with liquid-binding material (such as sand or kitty litter) and dispose in a biohazard bin.

Do not combine bleach with Lysis Buffer and do not wipe areas of Lysis Buffer spillage with bleach, as the two reagents produce cyanide gas when combined. Handle carefully and wear appropriate PPE, including safety glasses, when handling Lysis Buffer. If Lysis Buffer

is spilt onto PPE (eg. Gloves, gowns), remove and discard the contaminated item/s in a biohazard bin.

6 SAMPLING AND SAMPLE PREPARATION

6.1 SAMPLE LOCATIONS

Samples waiting to be extracted are stored in freezers as detailed in Table 6.

Table 6 Sample storage locations

Sample type	Storage Device	Storage Location
Urgent/High/Medium Priority Samples	Freezer	3190 or Block 6 walk-in freezer*
Lysates in 1.0mL Nunc Bank-it™ tubes	Freezer	3190
Extracts in 1.0mL Nunc Bank-It™ tubes	Freezer	3194 A

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

6.2 QC SAMPLES

One negative reagent control and one positive blood control (from a known donor) must be registered for all extraction batches. Additionally, off-deck lysis batches require five blank controls as detailed in Table 7.

Table 7 Extraction Quality Controls

Batch Type	Control
Off-deck lysis	Positive control (x1)
	Negative control (x1)
	Blank control (x5)
Manual DNA IQ™	Positive control (x1)
	Negative control (x1)
Retain supernatant DNA IQ™	Positive control (x1)
	Negative control (x1)

6.3 REGISTRATION OF QC SAMPLES

Register control samples according to the standard laboratory procedure QIS 24919 Forensic DNA Analysis Workflow Procedure. CQ samples for off-deck lysis are to be registered with a 9PLEX test code. QC samples for a DNA IQ Maxwell or a Retain Supernantant DNA IQ extraction batch are to be registered using the XPLEX test code.

6.4 CREATION OF EXTRACTION BATCHES

Create extraction batches according to the standard laboratory procedure QIS 24919 Forensic DNA Analysis Workflow Procedure.

6.5 LOCATING SAMPLES

Locate samples according to the standard laboratory procedure QIS 23959 Storage Guidelines for Forensic DNA Analysis.

6.6 EXCESS SUBSTRATE

If excess substrate is noticed before extraction buffer is added, remove the sample from the extraction batch and return to Evidence Recovery for sampling. If excess substrate is

identified after the extraction buffer has been added then proceed with extraction and notify Analytical HP5 for reporting to QPS via EXH.

6.7 ELECTRONIC WORKFLOW DIARY

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

7 MANUAL METHODS FOR EXTRACTION USING DNA IQ™

7.1 MANUAL DNA IQ™ (NO RETAIN SUPERNTANT)

Note: All manual extractions are to be performed within a biohazard cabinet in room 3189.

1. Prepare fresh reagents within the biohazard hood of the clean room (3188) prior to commencing the procedure. Refer to Section 4.1 for preparation of reagents. Use Table 8 in Appendix 18.1 for reagent volumes.

Warning: The 2 x Wash Buffer, 2-Propanol and Ethanol is flammable and hazardous. It may cause serious eye irritation, drowsiness or dizziness if vapours are inhaled. Please use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling. It is recommended that this reagent is not to be prepared / handled if pregnant, and therefore this procedure should not be performed by pregnant women.

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

3. Label for each sample:
   - Original sample tube;
   - Spin basket (if required);
   - 2mL tube; and
   - Nunc™ Bank-It™ tube.

Note: A spin basket is required for swabs, fabric, paper and cigarette butts. Samples that do not require a spin basket are tape-lifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

4. Have a second operator perform a sequence check of all tubes. Record the sequence check in AUSLAB.

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

6. Vortex and incubate at 37 °C on the Thermomixer at 1400 rpm for 45 minutes.

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

8. Transfer the substrate to spin basket (if required) or transfer the lysate to an appropriately labelled 2.0 mL tube. Retain the original tube containing the substrate if no spin basket is used.

9. Centrifuge the spin basket at 15800 xg for 2 minutes.

10. Transfer the lysate from the original sample tube and the flow-through from the collection tube of the spin basket to an appropriately labelled 2 mL tube. Transfer the substrate from the spin basket back into the original sample tube.

    Note: If original sample tube is an appropriate 2 mL tube, transfer the substrate into the new 2 mL tube and add the flow-through from the spin basket to the lysate still in the original sample tube.

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

12. Add 50 µL of DNA IQ™ Resin-Lysis Buffer-DTT solution to the lysate. Pipette mix or vortex the solution frequently to keep a homogenous suspension of resin within the solution.

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

14. Remove from the Multitube shaker, vortex and immediately place into the magnetic stand. Separation will occur instantly.

    Note: If resin does not form a distinct pellet on the side of the tube, or if the magnetic beads have re-suspended while in the stand, vortex the tube and quickly place back in the stand.

15. Carefully remove and discard the liquid, ensuring that the resin is not disturbed. Remove from the magnetic stand.

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

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

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

18. Add 100 µL of 1X Wash Buffer, vortex and place into the magnetic stand. Once separation has occurred remove and discard the liquid. Remove the tube from the magnetic stand.

19. Repeat step 18 twice for a total of three Wash Buffer washes. Ensure that all of the liquid has been removed after the last wash. Remove the tube from the magnetic stand.

20. Uncap the tubes and place the lids down onto a clean rediwipe within the biohazard cabinet. Allow the resin to air-dry in the hood for 15 minutes and then recap the tubes.

    Note: Do not allow the resin to dry for more than 20 minutes as this may inhibit DNA elution.

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

22. Incubate in the Thermomixer at 65°C for 3 minutes and then continue to incubate for a further 3 minutes shaking at 1100 rpm. If no thermomixer is available, incubate samples within a hot block and vortex mix at the start and end of the second 3 minute incubation. Remove samples.

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

24. Carefully transfer the DNA extract to the corresponding labelled Nunc™ Bank-It™ tube.

25. Remove from the magnetic stand and repeat steps 21-23 once. The final volume after the second elution is approximately 95 µL of DNA extract.

26. Store the DNA extracts in temporary storage located within the Pre-PCR sorting room 3194-A in the upright freezer with equipment number 30512883.

27. Store tubes containing substrates in Spin Basket boxes located within the Pre-PCR sorting room 3194-A in the upright freezer with equipment number 30512883.

7.2 PROCEDURE FOR MANUAL DNA IQ™ (RETAIN SUPERNATANT)

Note: All manual extractions are to be performed within a biohazard cabinet in room 3189.

1. Prepare fresh reagents within the biohazard hood of the clean room (3188) prior to commencing the procedure. Refer to Section 4.1 for preparation of reagents. Use Table 9 in the Appendix 18.2 for reagent volumes.

Warning: The 2 x Wash Buffer, 2-Propanol and Ethanol is flammable and hazardous. It may cause serious eye irritation, drowsiness or dizziness if vapours are inhaled. Please use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling. It is recommended that this reagent is not to be prepared / handled if pregnant, and therefore this procedure should not be performed by pregnant women.

2. Turn on the Eppendorf Thermomixer and set to 37 °C and 1400 rpm.

3. Label for each sample:
   - Original sample tube;
   - 2 mL tube for sample transfer and processing;
   - 1.5mL tube labelled 'SUP' for supernatant;
   - Spin basket (if required); and
   - Nunc™ Bank-It™ tube.

   Note: A spin basket is required for swabs, fabric, paper and cigarette butts. Samples that do not require a spin basket are tape-lifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

4. Have a second operator perform a sequence check of all tubes. This person must enter the sequence check in AUSLAB.

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

6. Incubate at room temperature for 30 minutes.

7. Vortex, then centrifuge at 15800 xg for 3 minutes.

8. Remove 150 µL of supernatant and place into the respective 1.5 mL tube labelled with 'SUP' (for further testing).

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

10. Vortex, then incubate at 37°C on the Thermomixer at 1400 rpm for 45 minutes. If no thermomixer available, incubate samples within a hot block, vortex mix at the start and end of the incubation and at least one during the incubation.

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

12. Transfer substrate to spin basket if required or transfer the lysate to an appropriately labelled 2.0mL tube. Retain original tube containing the substrate in if no spin basket used.

13. Centrifuge spin basket at 15800 g for 2 minutes.

14. Transfer the substrate in the spin basket into a new and labelled 2mL tube to be retained. Transfer the flow through (lysate) back into the original sample tube.

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

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

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

18. Remove from the Multitube shaker, vortex and immediately place into the magnetic stand. Separation will occur instantly.

    Note: If resin does not form a distinct pellet on the side of the tube, or if the magnetic beads have re-suspended while in the stand, vortex the tube and quickly place back in the stand.

19. Carefully remove and discard the solution, ensuring that the resin is not disturbed. Remove from the magnetic stand.

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

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

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

22. Add 100µL of 1X Wash Buffer, vortex and place into the magnetic stand. Once separation has occurred remove and discard the Wash Buffer. Remove from the magnetic stand.

23. Repeat the Wash Buffer step (step 23) two times for a total of three washes. Ensure that all of the solution has been removed after the last wash. Remove from the magnetic stand.

24. Uncap the tubes and place the lids down onto a clean rediwipe. Air-dry the resin in the hood for 15 minutes and then recap tubes

    Note: Do not dry for more than 20 minutes, as this may inhibit the elution of DNA.

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

26. Incubate in the Thermomixer at 65°C for 3 minutes and then continue to incubate for a further 3 minutes shaking at 1100 rpm. If no thermomixer available, incubate samples within a hot block, vortex mix at the start and end of 2nd 3 minute incubation. Remove samples.

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

28. Carefully transfer the DNA extract to the corresponding labelled Nunc™ Bank-It™ tube.

29. Remove from the magnetic stand and repeat the Elution Buffer steps (step 26-29). The final volume after the double elution is approximately 95µL of DNA extract.

30. Store the DNA extracts in temporary storage located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

31. Store tubes containing substrates in Spin Basket boxes located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

32. Store supernatants in the S/N Retention boxes located within the Extraction sorting room in the Orford Freezer 30512883 (Room 3190).

33. Ensure that the negative extraction control from the batch is also inserted onto the **'Saliva List':**

    - Log into Auslab
    - 3 for Patient Enquiry
    - Type/Scan the lab number for the negative extraction control
    - From the 9Plex page or the XPLEX Page press Shift F12
    - A prompt will appear 'Enter List Name'
    - Type Saliva or use the F1 lookup list.
    - Press Enter

8 QUALITY ASSURANCE/ACCEPTANCE CRITERIA

- Positive and negative (reagent blank) controls are included in each extraction batch. However, for off deck-lysis batches, an additional five negative (reagent blank) controls are included. These controls are processed as normal samples through to completion.
- If any results are obtained from the reagent blank, either at quantification or during the CEQ check, then the possible source of the contamination is investigated. The samples extracted with this control are thoroughly checked and repeated if required. This is covered in QIS 17130 CE Quality Check and QIS 24012 Miscellaneous Analytical Section Tasks

9 VALIDATION

- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 9. Automation: Report on the Evaluation of DNA Extraction Chemistries." June 2007.
- Muharam I., Hlinka V., Gallagher, B., Lundie, G., Iannuzzi, C., Nurthen T., McNevin A., Ientile V. "Project 21: A Modified DNA IQ™ Method Consisting of Off-Deck Lysis to Allow Supernatant Retention for Presumptive Identification of α-Amylase" 2008

10 STORAGE OF DOCUMENTS

All worksheets, after auto extraction, manual IQ and Retain supernatant batches have been completed, are stored within the Pre-PCR sorting room (3194-A) in an allocated in-tray for scanning by the operational officers.

11 REFERENCES

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- Marko, M.A., Chipperfield, R., & Birnboim, H.C., A Procedure for the Large Scale Isolation of Highly purified Plasmid DNA using alkaline extraction and binding to glass powder. Anal. Biochem., 1982. 121: p. 382-387.
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- Promega, 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.