COMMISSION OF INQUIRY INTO DNA PROJECT 13

Commissions of Inquiry Act 1950

SECOND STATEMENT OF VANESSA KATE IENTILE

I, **Vanessa Kate Ientile**, of C/- 1/300 Queen Street, Brisbane in the State of Queensland, primary school teacher, do solemnly and sincerely declare that:

1. On 24 October 2023, I provided a statement to Commissioner Bennett AC SC in response to Notice No. 4.001 (my **First Statement**).

2. On 25 October 2023, I was requested to provide a second statement answering the questions set out in Notice No. 4.003 issued by Commissioner Bennett AC SC under section 5(1)(d) of the *Commissions of Inquiry Act 1950* (Qld) (the **Second Notice**).

3. My responses are set out below to the Second Notice (my **Second Statement**).

4. My Second Statement is to be read together with my First Statement.

5. After providing my First Statement, at 12:07pm yesterday my legal representatives received an email, on my behalf, from a consultant with Epiq Global advising that my Online Review Book account had been set up and provided login details for me to access the account. Prior to receiving this email, I had not had access to the Online Review Book and had very limited documents/information available to me given that I am no longer a Queensland Health (**QH**) employee and had no independent means of accessing records that were relevant to my evidence.

6. The Online Review Book holds approximately 6,400 documents. In the time available, I have not read most of the documents in the Online Review Book for the purpose of preparing this statement.

7. As stated in paragraph 3 of my First Statement, I understand that this Commission of Inquiry is concerned with the report titled "Project 13: Report on the Verification of an Automated DNA IQ™ Protocol using the MultiPROBE II PLUS HT EX with Gripper Integration Platform" (**Project 13 Report**). For the reasons outlined in paragraph 67 of my First Statement, in my opinion the Project 13 Report appears to be an incomplete working draft. I also note from my limited review of the documents in the Online Review Book that there are multiple versions of the incomplete working draft of the Project 13 Report with differing dates. As such, in this statement I refer to the version of the Project

13 Report that is the subject of the Inquiry as a "Version of the Incomplete Project 13 Report".

Identification

Question 1: State:

(a) your full name;

8. My full name is Vanessa Kate Ientile.

(b) your qualifications, skills or experience relevant to forensic science and DNA; and

9. I hold a:

(a) Bachelor of Science, majoring in Molecular Biology/Biochemistry; and

(b) Graduate Diploma in Biotechnology.

(c) 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.

10. I have been employed in the following roles at Queensland Health Forensic and Scientific Services (**QHFSS**):

(a) Laboratory Technician at Queensland Health Scientific Services (**QHSS**) – Organics, from 1995 to 1997;

(b) Laboratory Technician at QHSS – Forensic Biology, from 1997 to 1999;

(c) Forensic Casework Scientist at QHSS – Forensic Biology, from 1999 to 2000;

(d) Forensic Scientist at QHSS – Forensic Biology (and holding the role of Quality Officer and Supervisor, Analytical Section), from 2000 to 2003;

(e) Senior Scientist at QHSS – Forensic Biology, from 2003 to 2004; and

(f) Managing Scientist at QHFSS, from 2004 to 11 August 2008 (during that period I did act in the role of Forensic Manager at QHSS, from 2 January 2007 to 9 February 2007 and then again from 30 April 2007 to 28 September 2007).

<u>Amendments to my First Statement</u>

11. My First Statement was prepared based solely on my recollection and belief and in the absence of the employment records I had requested from QH.

12. Since furnishing my First Statement to the Commission, QH has provided me with a copy of the employment records I had requested.

13. My employment records show that:

    (a) I acted in the position of Manager, Forensic Sciences from 2 January 2007 to 9 February 2007, then again from 30 April 2007 to 28 September 2007; and

    (b) I ceased employment in the role of Managing Scientist at QHFSS on 11 August 2008.

14. I have reflected those amendments in paragraph 10 above.

15. The records from QH also show that I:

    (a) attended a conference in Prague and made a number of laboratory visits in London, Ottawa and Washington DC from 19 April 2008 to 9 May 2008; and

    (b) attended a Biology Specialist Advisory Group meeting and then took some annual leave in New Zealand from 29 July 2008 to 6 August 2008.

16. In the absence of any records being made available to me to confirm, and to the best of my recollection and belief, I returned to the DNA Analysis Laboratory (**Laboratory**) on 7 and 8 August 2008 before starting the new role as AUSCARE Project Manager on 12 August 2008.

17. In December 2011, I took maternity leave and on 15 April 2012, I ceased employment with QH, accepting a Voluntary Separation Package.

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.

18. As I ceased employment with QHFSS on 11 August 2008 and have not worked in DNA since then, I am unable to give evidence in relation to the period from 12 August 2008.

Accordingly, my answer to this question relates to the period from 24 October 2007 to 11 August 2008.

19. I have no independent recollection of any iteration of the DNA IQ™ Protocol used by QHFSS from 24 October 2007 until 11 August 2008.

20. I do not recall performing any of these methods, and nor do I believe that I would have performed any of these methods because of the managerial nature of the roles I held during 2007 and 2008.

21. I believe that the positive control samples used in the Laboratory at the time were prepared from blood and cell samples collected from me. To the best of my recollection and belief, the blood and cell samples were collected from me because I did not perform any of the testing methods that were undertaken in the Laboratory at the time.

22. Based on my review of the documents available to me at the time of preparing this statement, I believe this request is referring to the versions of the Standard Operating Procedure (**SOP**) Document Number 24897 from the Quality Information System (**QIS**).[1]

23. The versions of SOP 24897 that I have reviewed for the purposes of preparing my Second Statement include:

    (a) SOP 24897 V1 Automated DNA IQ™ Method of Extracting DNA from Blood and Cell Substrates, valid from 24/10/2007 (exhibit "**VI-4**" to my First Statement);

    (b) SOP 24897 V2 DNA IQ™ Method of Extracting DNA from Blood and Cell Substrates, valid from 11/01/2008 (exhibit JH-41 to Justin Howe's statement dated 6 October 2022 that was tendered into the COI-1 at page WIT.0016.0188.0439, a copy of which is exhibited to this statement as "**VI-10**");

    (c) SOP 24897 V3 DNA IQ™ Method of Extracting DNA from Casework and Reference Samples, valid from 27/03/2008 (exhibit "**VI-8**" to my First Statement);

    (d) SOP 24897 V4 DNA IQ™ Method of Extracting DNA from Casework and Reference Samples, valid from 21/05/2008 (exhibit ARM-117 to Allan McNevin's statement dated 13 October 2022 that was tendered into the COI-1

[1] SOPs are managed in the QIS. An author creates a document and uploads it to QIS where it is allocated a document number and version control starts. When a document was uploaded, it appeared on a list to be approved. Approving a document moved the document from draft to active in the system and that version became the current version of the SOP for use in the Laboratory. All staff who used that SOP were then notified that a new version was approved and active and the previous version was superseded.

at page WIT.0040.0077.1618, a copy of which is exhibited to this statement as "**VI-11**"); and

(e) SOP 24897 V5 DNA IQ™ Method of Extracting DNA from Casework and Reference Samples, undated (exhibit ARM-118 to Allan McNevin's statement dated 13 October 2022 that was tendered into the COI-1 at page WIT.0040.0077.1651, a copy of which is exhibited to this statement as "**VI-12**").

24. I am also aware of another version of this SOP, being SOP 24897 V6 DNA IQ™ Method of Extracting DNA from Casework and Reference Samples, that was valid from 13/08/2009 (exhibit "**VI-9**" to my First Statement). I have not considered this version of the SOP in preparing my Second Statement as it was approved after I ceased work in the Laboratory on 11 August 2008.

25. As SOP 24897 V4 and V5 were created in relation to a QIS data migration process where a new QIS platform was implemented for use across QHFSS, and the changes related to document headers and footers and business references only, I do not address these versions further in my Second Statement. As such, this statement focuses on SOP 24897V1, V2 and V3.

26. The DNA IQ™ Protocol is described in detail in each of the three SOP versions. Based on my review of the three SOP versions I refer to in paragraph 23(a), (b) and (c), I have created a simplified summary of each protocol.

27. In each of the three versions of the SOP 24897, the reagent volumes for the Extraction Buffer, Lysis Buffer, DNA IQ Resin, Wash Buffer and Elution Buffer are listed in Table 2. The volumes for the components for each of these buffers appear to be the same in each version of the SOP.

<u>24897 V1</u>

28. In SOP 24897 V1, the detailed steps comprising the protocol are displayed in Figure 1 and appear to be a copy of the winprep program file - DNA IQ Extraction file_Ver1.1.mpt. I have attempted to identify the steps relating to Question 2 (a)-(e) from this figure in the SOP.

29. I provide the following summary of my understanding of the steps comprising the protocol in SOP 24897 V1 (based on my reading of the document):

(a) To each tube, add 500uL Extraction Buffer.

(b) Incubate at 37°C for 45 minutes.

(c) Add 50uL Resin.

(d) Add 957uL of Lysis Buffer.

(e) Incubate on shaker for 5 minutes (temperature of incubation is not apparent).

(f) Plate is moved to the magnet and 1600uL of supernatant is removed.

(g) Add 125uL of Lysis Buffer, shaking at room temperature for 1 minute. Plate is moved to the magnet and Lysis Buffer is removed.

(h) Add Wash Buffer (volume is not apparent), shaking for 1 minute. Plate is moved to the magnet and Wash Buffer is removed.

(i) Step f with 100uL of Wash Buffer is repeated 2 more times (total of 3 washes).

(j) Excess Wash Buffer is removed by air drying at room temperature for 5 minutes.

(k) Add 60uL of Elution Buffer.

(l) Incubate at 65°C for 6 minutes. Plate is moved to the magnet and supernatant is removed to new storage tubes (DNA Extract).

(m) Repeat steps (k) and (l).

<u>24897 V2</u>

30. In SOP 24897 V2, the steps comprising the protocol are listed in Section 7 Procedure as a summary of the DNA IQ Extraction winprep program file (v1.3). I have attempted to identify the steps relating to Question 2 (a)-(e) from this section in the SOP.

31. I do not have access to any records that allow me to compare v1.1 and v1.3 winprep program files, and nor do I recall having seen these file printouts previously. I am therefore not able to confirm whether there are any differences between these programs.

32. I provide the following summary of my understanding of the steps comprising the protocol in SOP 24897 V2 (based on my reading of the document):

(a) Add 500uL of Extraction Buffer to Slicprep plate wells. Cover with seal.

(b) Incubate at 37°C for 45 minutes.

(c) Add 50uL Resin Solution and 957uL of Lysis Buffer. Platform performs automated mixing and shaking at room temperature for 5 minutes.

(d) Plate is moved to the magnet and 1600uL of supernatant is removed.

(e) Add 125uL of Lysis Buffer, shaking at room temperature for 1 minute. Plate is moved to the magnet and supernatant is removed.

(f) Add 100uL of Wash Buffer, shaking at room temperature for 1 minute. Plate is moved to the magnet and supernatant is removed.

(g) Step f with 100uL of Wash Buffer is repeated 2 more times (total of 3 washes).

(h) Excess Wash Buffer is removed by air drying at room temperature for 5 minutes.

(i) Add 60uL of Elution Buffer.

(j) Incubate at 65°C for 6 minutes. Plate is moved to the magnet and supernatant is removed to new storage tubes (DNA Extract).

(k) Repeat steps (i) and (j).

24897 V3

33. The protocol in SOP 24897 V3 describes that the initial lysis steps were to occur "off-deck" or were performed manually before the remainder of the steps were performed on the automated platform.

34. There are two options available depending on whether a small volume (150uL) of the supernatant is required to perform presumptive testing for α-amylase.

35. The report titled "Project 21: A Modified DNA IQ™ Method Consisting of Off-Deck Lysis to Allow Supernatant Retention for Presumptive Identification of α-Amylase" (**Project 21 Report**) refers to the development of these two options. A copy of the Project 21 Report is exhibited to this statement as "**VI-13**".

36. I provide the following summary of my understanding of the steps comprising the protocol in SOP 24897 V3 for the Off-Deck Lysis with no retained supernatant (based on my reading of the document):

(a) Add 500uL of Extraction Buffer to each sample.

(b) Incubate at 37°C for 45 minutes.

(c) Transfer lysate to new tube.

(d) Incubate lysates at 65°C for 10 minutes.

(e) Store lysates at 4 °C until extraction process completed on automated platform.

37. I provide the following summary of my understanding of the steps comprising the protocol in SOP 24897 V3 for the Off-Deck Lysis with retained supernatant (based on my reading of the document):

(a) Add 650uL of TNE Buffer to each sample (Extraction Buffer with no Proteinase K or SDS added).

(b) Incubate at room temperature for 30 minutes.

(c) Remove 150uL of the supernatant into a new tube for presumptive testing.

(d) Add 25uL Proteinase K and 12.5uL of SDS to each tube containing the remaining 500uL of TNE Buffer.

(e) Incubate at 37°C for 45 minutes.

(f) Transfer lysate to new tube.

(g) Incubate lysates at 65°C for 10 minutes.

(h) Store lysates at 4 °C until extraction process completed on automated platform.

38. I provide the following summary of my understanding of the steps comprising the protocol in SOP 24897 V3 and completed on the automated platform for the lysates from paragraphs 36 and 37 (based on my reading of the document):

(a) Add 50uL Resin Solution and 957uL of Lysis Buffer. Platform performs automated mixing and shaking at room temperature for 5 minutes.

(b) Plate is moved to the magnet and 1600uL of supernatant is removed.

(c) Add 125uL of Lysis Buffer, shaking at room temperature for 1 minute. Plate is moved to the magnet and supernatant is removed.

(d) Add 100uL of Wash Buffer, shaking at room temperature for 1 minute. Plate is moved to the magnet and supernatant is removed.

(e) Step (d) with 100uL of Wash Buffer is repeated 2 more times (total of 3 washes).

(f) Excess Wash Buffer is removed by air drying at room temperature for 5 minutes.

(g) Add 60uL of Elution Buffer.

(h) Incubate at 65°C for 6 minutes. Plate is moved to the magnet and supernatant is removed to new storage tubes (DNA Extract).

(i) Repeat steps (g) and (h).

39. In SOP 24897 V3, the steps comprising the automated component of the protocol are listed in Section 9 MPII Extraction Procedure as a summary of the DNA IQ Extraction winprep program file- DNA IQ Extraction_Ver 2_ODL.mpt.

40. I do not have access to any records that allow me to compare the different versions of the winprep program files, and nor do I recall having seen these file printouts previously. I am therefore not able to confirm whether there are any differences between these programs referred to in SOPs 24897 V1, V2 or V3.

41. Based on my review of the SOPs 24897 V1, 24897 V2 and 24897 V3, I have created the following table to describe my responses to Question 2(b) to (e):

	SOP24897 V1	**SOP24897 V2**	**SOP24897 V3 (off-deck lysis)**	**SOP24897 V3 (off-deck lysis with retained supernatant)**
Extraction Buffer volume	500uL	500uL	500uL	650uL (TNE Buffer only)[2]
Temperature during lysis step	37°C	37°C	37°C	37°C
Lysis Buffer Volume	957uL	957uL	957uL	957uL
Temperature during lysis step	Not stated	Room temperature	Room temperature	Room temperature
Resin Volume	50uL	50uL	50uL	50uL
Lysis Buffer Volume (wash)	125uL	125uL	125uL	125uL
Wash Buffer volume	Not stated	100uL per wash	100uL per wash	100uL per wash
Number of washes following lysis step	1 x lysis buffer 3 x wash buffer	1 x lysis buffer 3 x wash buffer	1 x lysis buffer 3 x wash buffer	1 x lysis buffer 3 x wash buffer
Elution Buffer	2 x 60uL	2 x 60uL	2 x 60uL	2 x 60uL
Volume of the sample containing DNA at the end of the extraction	100uL	100uL	100uL	100uL

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

42. Based on my review of SOPs 24897 V1, 24897 V2 and 24897 V3, I can identify a change to the DNA IQ Protocol in SOP 24897 V3 with the introduction of the Off-Deck Lysis steps. This change is described in the Project 21 Report (exhibited as "**VI-13**").

43. I have no independent recollection of any specific details in relation to the Project 21 Report. Based on my review of the Project 21 Report, it appears that the method was changed to perform the first step using the Extraction Buffer manually rather than on the automated platform. The abstract to the Project 21 Report states this was to allow

[2] 650uL of TNE Buffer only added, incubated at room temperature for 30 minutes. 150uL of supernatant removed and 25uL of Proteinase K and 12.5uL of 20% (w/v) SDS added.

extraction of different sample types (such as fluff swab heads) and to retain a supernatant for samples where presumptive testing may be required.

44. I believe the difference between the two methods in SOP 24897 V3 is the addition of an extra 150uL of TNE buffer that allows 150uL of the supernatant to be retained.

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.

45. I have no independent recollection of the extent that the DNA IQ™ protocol changed in any respect from the DNA IQ™ protocol issued by the manufacturer. Furthermore, based on the limited review of the approximately 6,400 documents that were made available to me yesterday, I have not identified any records that allow me to be in a position to identify any change in the DNA IQ™ protocol. Therefore, if there were any such changes, I am not in a position to comment on the reason for the change.

Project 13

Question 5: Were you the author of the Abstract appearing in the "Project 13. Report on the Verification of an Automated DNA IQ Protocol using the Multiprobe II PLUS HT EX with Gripper Integration Platform", Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C., Ientile, V. Automation/LIMS Implementation Project, DNA Analysis FSS (August 2008)?

46. I have no independent recollection of the Version of the Incomplete Project 13 Report that is the subject of this Commission of Inquiry.

47. During my employment with QHFSS I have no independent recollection of ever seeing the Version of the Incomplete Project 13 Report that is the subject of this Inquiry.

48. I do not believe I was the author of the Abstract or any other part of the Version of the Incomplete Project 13 Report that is the subject of this Inquiry.

49. It is likely that I was listed with others, as an author of the Version of the Incomplete Project 13 Report that is the subject of this Inquiry because, at the time, it was common practice for the reporting scientists to list the Managing Scientist in the reports of each project that was undertaken (even if they were not directly involved).

50. While I do not believe I was involved in preparing or drafting the Version of the Incomplete Project 13 Report that is the subject of this Inquiry, I do not have access to the records to confirm that.

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

Deponent [redacted] ..... Taken by [redacted] ..

I declare that the contents of this statutory declaration are true and correct. Where the contents of this declaration are based on information and belief, the contents are true to the best of my knowledge and I have stated the source of that information and grounds for the belief.

I understand that it is a criminal offence to provide a false matter in a declaration, for example, the offence of perjury under section 123 of the Criminal Code.

DECLARED by

Vanessa Kate Ientile
at Brisbane

.....[redacted].....
[signature of declarant]

26 October 2023

In the presence of:

[redacted]
[signature of witness]

Thomas Daniel Robert Goodwin

Australian Legal Practitioner

Holding Redlich

26 October 2023

Schedule of Exhibits

Exhibit	Document title	Page
VI-10	Exhibit JH-41 to the statement of Justin Howes dated 6 October 2022, being Standard Operating Procedure titled: "DNA IQ™ Method of Extracting DNA from Blood and Cell Substrates 24897V2"	1-22
VI-11	Exhibit ARM-117 to the statement of Allan McNevin dated 13 October 2022, being Standard Operating Procedure titled: "DNA IQ™ Method of Extracting DNA from Casework and Reference Samples 24897V4"	23-55
VI-12	Exhibit ARM-118 to the statement of Allan McNevin dated 13 October 2022, being Standard Operating Procedure titled: "DNA IQ™ Method of Extracting DNA from Casework and Reference Samples 24897V5"	56-79
VI-13	Project 21: A Modified DNA IQ™ Method Consisting of Off-Deck Lysis to Allow Supernatant Retention for Presumptive Identification of α-Amylase	80-93

[redacted] .....

Vanessa Kate Tentile

Declarant

[redacted]

Thomas Daniel Robert Goodwin

Australian Legal Practitioner

VI-10
JH-41

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

Figure 1 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.

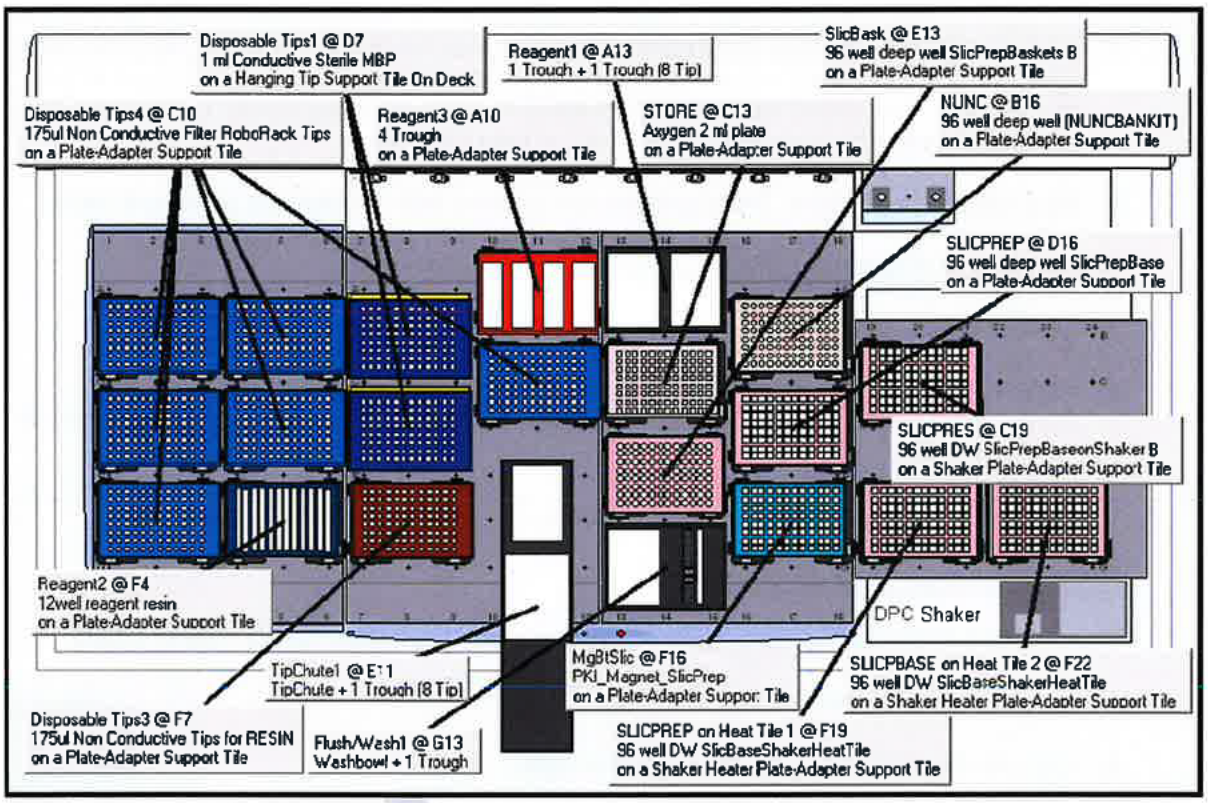


Figure 2. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

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

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

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

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

Finalising the MP II run

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

Recording Reagent Details and other information in AUSLAB

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

Importing the MP II log file into AUSLAB

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

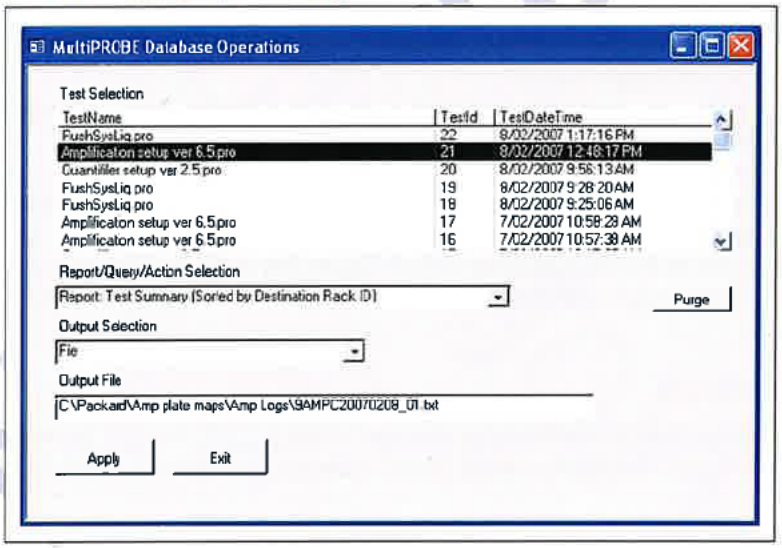


Figure 4. The MultiPROBE log database for collecting MP II run information

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.

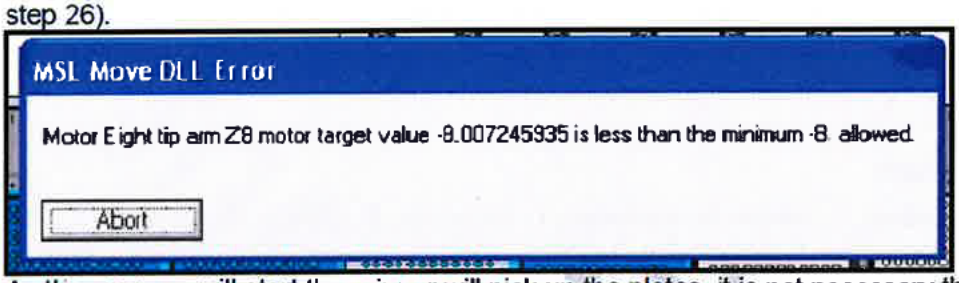
9 TROUBLESHOOTING

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.
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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.

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13 STORAGE OF DOCUMENTS

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

14 ASSOCIATED DOCUMENTS

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

15 AMENDMENT HISTORY

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

16 APPENDIX

16.1 Reagents Calculation Tables

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

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

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

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

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

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

16.2 Manual method for extraction using DNA IQ™

16.2.1 Sampling and Sample Preparation

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

Table 3. Sample storage locations.

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

QC samples

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

Table 4. Extraction Quality Controls

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

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

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

Create the Extraction Batch

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

Locating Samples

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

When all samples have been located:

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

Sequence Check the tubes

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

16.2.2 Procedure

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

16.2.3 Sample storage

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

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

1 PURPOSE AND SCOPE

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

This method applies to all DNA Analysis staff that are required to extract DNA from samples.

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

2 DEFINITIONS

Samples	Samples awaiting DNA extraction
Lysates	Samples that have had the Lysis step performed, but have not yet completed the entire extraction process
DNA Extracts	Samples that have had a DNA extraction processes performed
DNA IQ™ Resin	Magnetic Resin Beads used to bind DNA
MP II	MultiPROBE® II PLUS HT EX Platform
DTT	1,4 Dithiothreitol
Pro K	Proteinase K
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

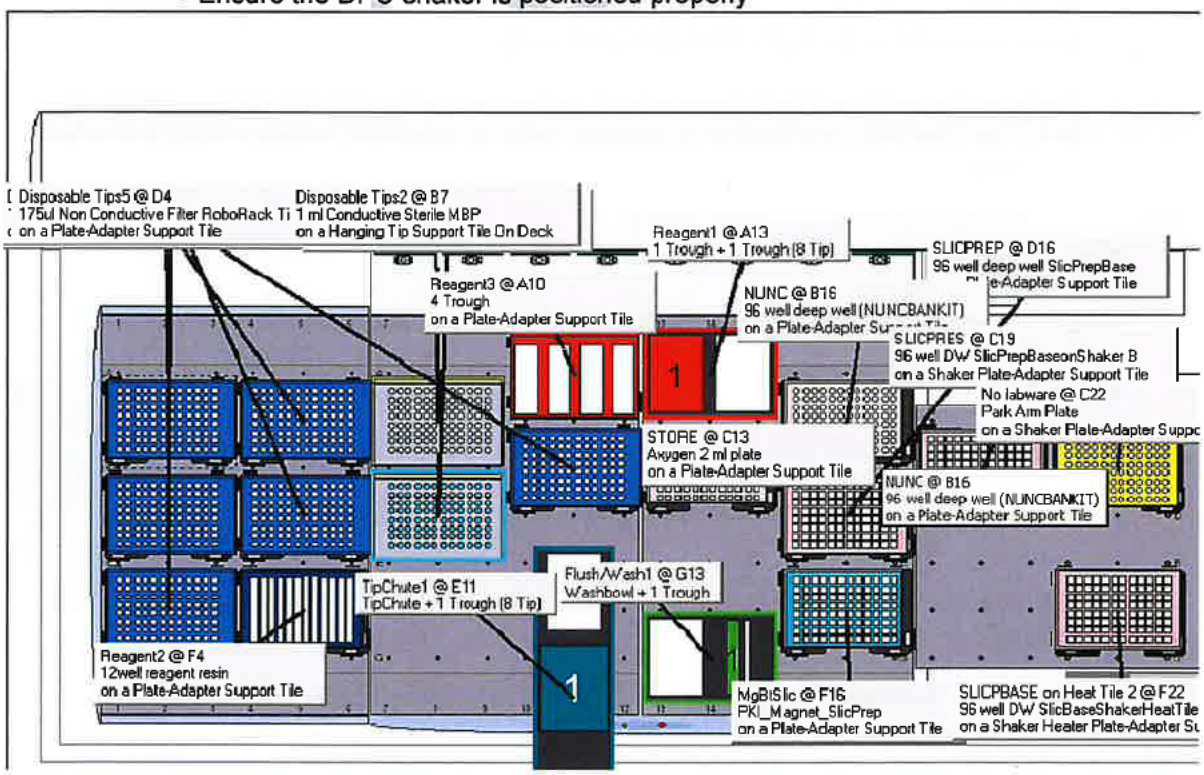

Figure 2. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

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):

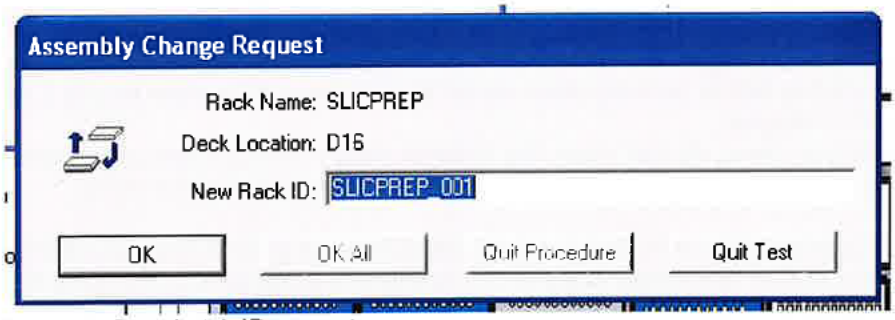

Figure 3. Scan batch ID request

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)

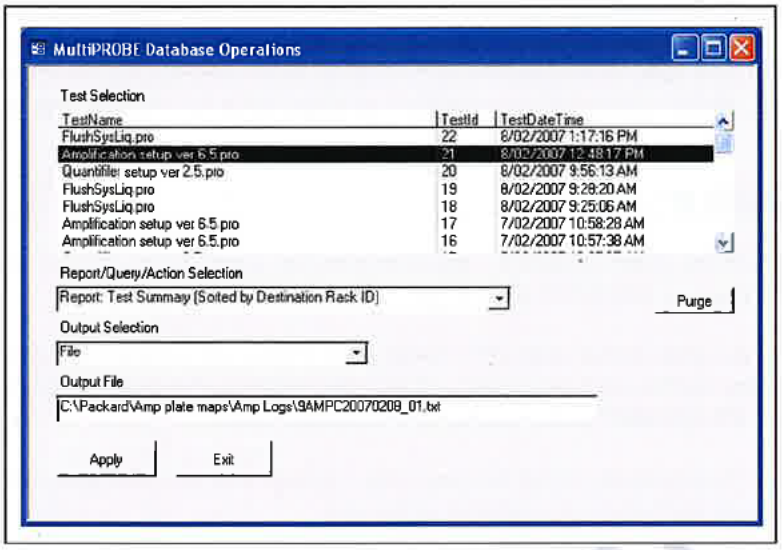

Figure 4. The MultiPROBE log database for collecting MP II run information

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).

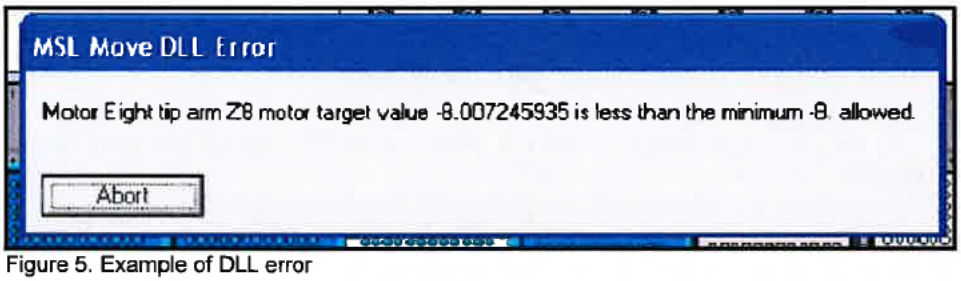

Figure 5. Example of DLL error

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.

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

15 STORAGE OF DOCUMENTS

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

16 ASSOCIATED DOCUMENTS

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

17 AMENDMENT HISTORY

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

18 APPENDIX

18.1 Reagents Calculation Tables

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

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

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

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

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

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

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

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

Lysis batch:

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

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

Extraction batch:

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

MultiPROBE Platform:	Operator:
Date and Start time:	

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

18.3 Fully automated method for extraction using DNA IQ™

18.3.1 Sampling and Sample Preparation

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

18.3.2 Procedure

Preparation of Reagents prior to extraction

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

Setting up the EP-A or EP-B MPIIs

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

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

Figure 7 The WinPrep® icon.

8. Ensure the **System Liquid reservoir is FULL** and fully submerged in the system liquid before every run and perform a Flush/Wash.
9. Ensure that the daily/weekly start-up has been performed before running any program. If WinPrep® has been closed or been idle for a long period of time initialise the MP II platform as described in QIS 23939.
10. Open the Extraction setup MP II test file in WinPrep® by selecting:
    - **File**
    - **Open**, navigate to **C:\PACKARD\MULTIPROBE\BIN\QHSS PROTOCOLS**
    - Select **"DNA IQ Extraction_Ver1.3.mpt."**
    - Click the "**Open**" button
11. Check the whole program there are no bold fonts (e.g. plates may lose gripability and the program will not run). See the line manager.
12. Decontaminate the required labware by wiping with 5% TriGene followed by 70% ethanol before placing the labware on to the designated grid positions as outlined in the virtual deck view within WinPrep® (Figure 2).
    - The white WALLAC Isoplates (catalogue #1450-514) that are used to support the SlicPrep™ 96 device plate must be placed into positions **E13**, **D16** and **C19**.

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

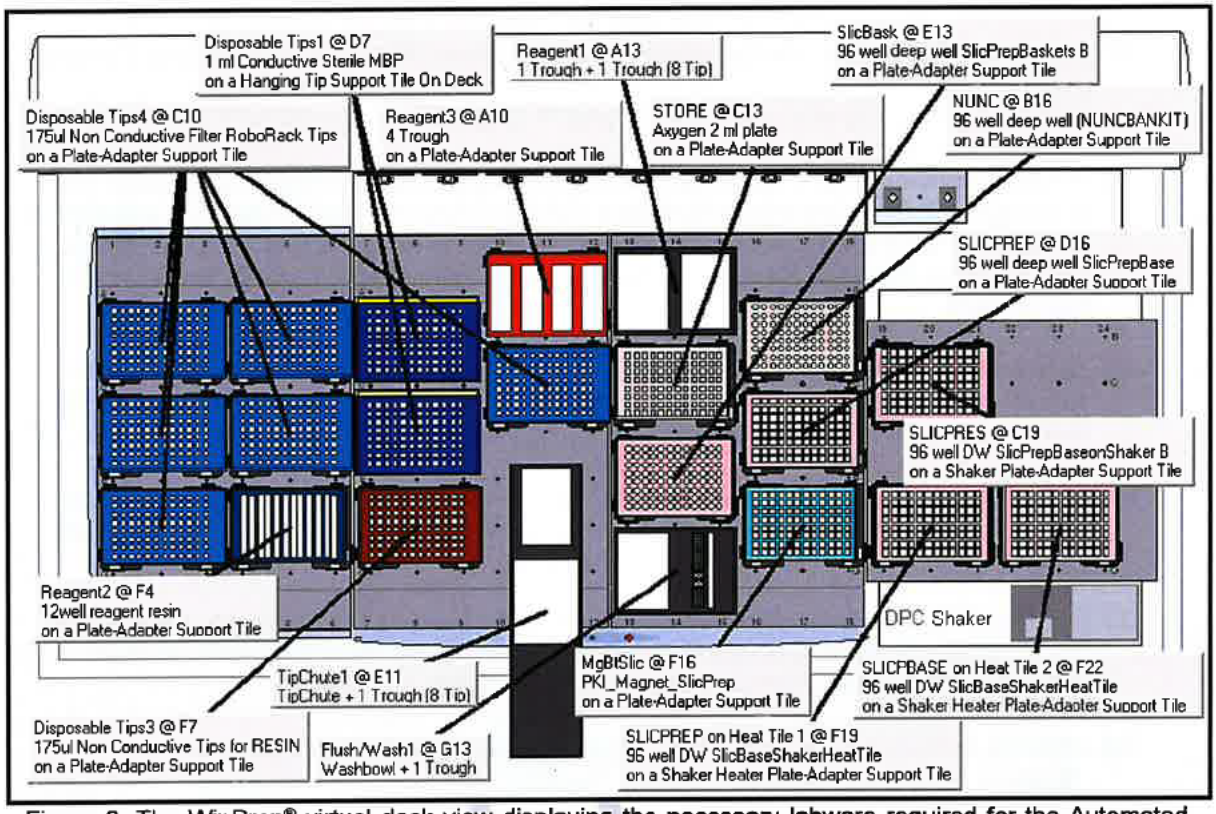

Figure 8. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

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

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

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

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

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

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

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

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

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

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

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

24. Once the Extraction Buffer has been added to the plate, a message will appear waiting for the heating tile to reach 50°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^{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)

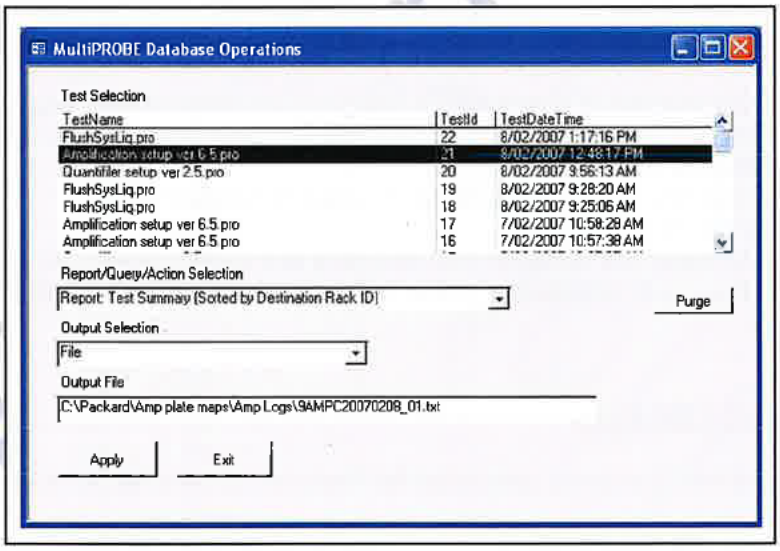

Figure 9. The MultiPROBE log database for collecting MP II run information

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 65ºC allows the DNA to be released from the magnetic beads. The Elution Buffer is a low ionic strength buffer that reduces the affinity of the DNA for the silica that covers the magnetic beads, by re-hydration of the phosphate backbone.

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

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

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

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

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

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

4. REAGENTS AND EQUIPMENT

4.1. Reagents

- DNA IQ™ System Kit (400 sample kit)
  - DNA IQ™ Resin
  - Lysis Buffer (LB)
  - 2x Wash Buffer (2xWB)
  - Elution Buffer (EB)
- TNE (10mM Tris, 100mM NaCl, 1mM EDTA, pH 8.0)
- Proteinase K (Pro K) 20mg/mL
- Dithiothreitol (DTT) 1M

- 5% TriGene
- 70% Ethanol
- 10% Bleach 7x Solution
- 1% Amphyl
- 0.2% Amphyl
- Isopropyl Alcohol
- AnalR 100% Ethanol
- 40% Sarcosyl
- Nanopure Water

These reagents are stored in locations as per Table 1.

Table 1. Reagent storage locations.

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

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

Table 2. Table of reagent volumes.

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

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

4.2. Extraction Buffer

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

1. Determine the required volumes of reagents by using Table 2.
2. Remove the required amount of 20mg/mL Proteinase K from the freezer and thaw. Vortex and centrifuge before use.

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

4.3. Lysis Buffer with DTT Solution

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

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

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

4.4. DNA IQ™ Resin

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

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

4.5. 1x Wash Buffer

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

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

4.6. Elution Buffer

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

5. EQUIPMENT

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

Table 3. Equipment used and location.

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

Table 4. Consumables used for extraction.

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

6. SAFETY

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

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

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

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

Lysis Buffer contains guanidinium thiocyanate (GuSCN) which is toxic and can be harmful if inhaled, swallowed or comes in contact with skin. Left over Lysis Buffer-DTT is disposed of in a brown Winchester bottle in the fume hood. Never dispose down the sink. If spillage

occurs, absorb with liquid-binding material (such as sand or kitty litter) and dispose in a biohazard bin. Do not combine bleach with Lysis Buffer and do not wipe areas of Lysis Buffer spillage with bleach, as the two reagents produce cyanide gas when combined. Handle carefully and wear appropriate PPE, including safety glasses, when handling Lysis Buffer. If Lysis Buffer is spilt onto PPE (eg. gloves, gowns), discard the PPE and obtain new PPE.

7. SAMPLING AND SAMPLE PREPARATION

7.1. Sample Locations

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

Table 5. Sample storage locations.

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

Note: Some Medium and Low Priority storage boxes may be located in the 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):

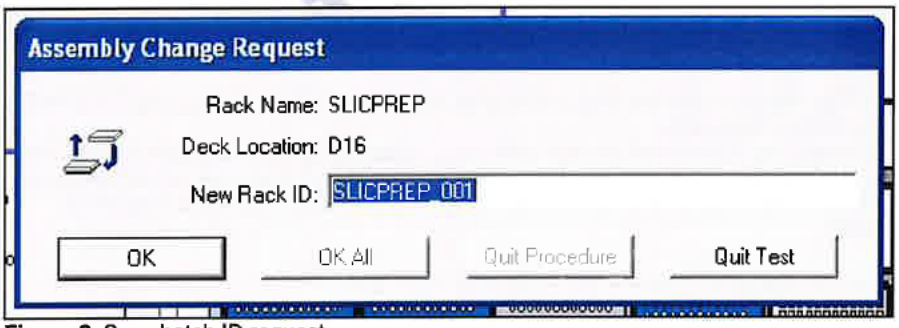

Figure 2. Scan batch ID request

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

2008

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

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Automation/LIMS Implementation Project, DNA Analysis FSS (February 2008)

1. Abstract

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

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

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

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

2. Aim

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

3. Equipment and Materials

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

- Eppendorf 5415C centrifuge

4. Methods

4.1 Sample collection

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

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

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

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

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

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

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

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

4.4 α-Amylase presumptive screening

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

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4.5 DNA quantitation

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

4.6 PCR amplification

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

4.7 Capillary electrophoresis and fragment analysis

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

5. Results and Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

6. Conclusion and Recommendations

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

7. Acknowledgements

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