Notice number: 9.003

COMMISSION OF INQUIRY INTO DNA PROJECT 13

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

STATEMENT OF BREANNA GALLAGHER

I, Breanna Lee Gallagher, care of MinterEllison, 1 Eagle Street, Brisbane, QLD 4000, Policy Officer at Australian Government Department of Health and Aged Care, do solemnly and sincerely declare that:

1. On 24 October 2024, I provided a statement responding to Notice 9.001 "Requirement to Give Information in a Written Statement".

2. On 24 October 2023, I was requested to provide a statement responding to Notice 9.003 "Requirement to Give Information in a Written Statement".

3. This written statement is provided in response to Notice 9.003.

Breanna Lee Gallagher

Identification

Question 1(a) - State your full name

4. My name is Breanna Lee Gallagher.

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

5. In 2005, I graduated with a Bachelor of Applied Science majoring in Biotechnology from the Queensland University of Technology in Queensland, Australia.

6. I currently work in the field of genomics, in particular implementing genomic technology in the health care system as a Policy Officer for the Australian Government Department of Health and Aged Care.

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

7. On or around March 2006, I was employed as an Operational Officer with Queensland Health Forensic and Scientific Services (**QHFSS**). This was my first role after graduating from university.

8. Approximately six months after I commenced as an Operational Officer, QHFSS conducted an expression of interest for the role of a junior Project Scientist position, to be appointed to a team at QHFSS's discretion.

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9. After providing my expression of interest for a Project Scientist position, I was employed as Project Scientist in the Automation/LIMS Implementation Project Team at QHFSS and remained in this role until my employment with QHFSS ended in approximately May 2008. During that time I was employed on numerous fixed term contracts for varying contract periods. I cannot recall the details about my employment contracts or when they were renewed.

10. I have not been employed or otherwise engaged with Queensland Health and/or Forensic Science Queensland.

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.

11. My recollection is the Automation/LIMS Implementation Project Team at QHFSS included:

(a) Project Lead – Thomas Nurthen;

(b) three scientists at that time - Cecilia Iannuzzi, Yojtech Hlinka and Iman Muharam; and

(c) two junior Project Scientists, being Generosa Lundie and myself.

12. As I understood it the Automation/LIMS Implementation Project Team was tasked to develop an automated testing method for extracting DNA from samples for genomic and genetic sampling on an automated platform (**Project**), being one of the four devices manufactured by Perkin Elmer (**Automated Device**). I do not recall this project being specifically described as 'Project 13'.

13. I recall the process of developing an automated testing method I have referred to in paragraph 12 involved the following:

(a) The analytical team at QHFFS conducted DNA extraction using a manual method, being the manual and human-led process. The results of testing for extracting DNA from samples using the manual method would be the comparator for the automated method the Automation/LIMS Implementation Project Team was developing. I do not recall if this manual method had a title.

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(b) Conducting extraction processes for extracting DNA from samples on an Automated Device.

(c) The verification process and validation process of the working automated testing method. These processes are standard scientific methods employed by all laboratories to show accuracy and repeatability of results. Information relating to the verification and validation of the automated testing method would be provided to National Association of Testing Authorities as evidence that the laboratory had complied with International Standards required for forensic laboratories.

(d) Comparing the results of conducting extraction processes on an Automated Device versus the manual method referred to at paragraph 13(a) above, to determine whether the laboratory could increase its capacity to test numerous types of samples and ensure that yield of DNA from samples was comparable to that of the manual method.

14. My duties and responsibilities as Project Scientist were:

(a) assisting with the work described at paragraph 13(b) and 13(c) including considering protocols for cleaning the Automated Devices;

(b) assisting the team lead, Thomas Nurthen, to try to determine how to implement the tracking of large batches of samples through the Auslab pathology system used by the Laboratory to record DNA results of processed samples;

(c) contributing to the drafting of version 1 of the 'DNA IQ™ Method of Extracting DNA from Casework and Reference Samples' which is identified on page 14 as Revision 0 dated 23 October 2007, attached and marked as Exhibit **BG-01** to this Statement (**Version 1**).

15. My duties and responsibilities as Project Scientist did not include the work described at paragraph 13(a) above. I was not involved in it nor was I trained in a manual DNA method or protocol. In the absence of information or documents in relation to this method, I do not know if a manual method or protocol was able to be, or was, run on an Automated Device.

16. I cannot recall if my duties and responsibilities included the work described at paragraph 13(d) above, nor do I recall who was responsibilities for that comparison or the method for that comparison.

17. On 25 October 2023, my legal representatives provided a copy of the following versions of a document with document number 24897 (followed by the version number) in each footer:

(a) Version 1;

(b) the 'DNA IQ™ Method of Extracting DNA from Casework and Reference Samples' which is identified on page 17 as Revision 1 with the date 12 December 2007, attached and marked as Exhibit **BG-02** to this Statement (**Version 2**);

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(c) the 'DNA IQ™ Method of Extracting DNA from Casework and Reference Samples' which is identified on page 21 under 'Amendment History' as Revision 2 with the date 19 March 2008, attached and marked as Exhibit **BG-03** to this Statement (**Version 3**);

(d) the 'DNA IQ™ Method of Extracting DNA from Casework and Reference Samples', which is identified on page 21 under 'Amendment History' as Revision 3 dated April 2008, attached and marked as Exhibit **BG-04** to this Statement (**Version 4**).

18. Each of Version 1, Version 2, Version 3 and Version 4 are iterations of a method that I recall the Laboratory referred to as the 'standard operating procedure' (**Standard Operating Procedure**).

19. The Standard Operating Procedure is the iteration of the protocol developed as a result of the process described at paragraph 13, as at the stated date of that revision of the standard operating procedure. Processes in the Laboratory referred to in the Standard Operating Procedure were then run in accordance with the most up to date version of that Standard Operating Procedure. In doing so, the Automation/LIMS Implementation Project Team or the analytical team would gather feedback on the Standard Operating Procedure and identify whether any modifications to the Standard Operating Procedure were needed in the next published iteration of the Standard Operating Procedure to address that feedback.

20. I have never held a copy of an iteration of the document 24897 titled DNA IQ™ Method of Extracting DNA from Casework and Reference Samples after my employment at QHFSS ended, other than:

(a) Version 1, Version 2, Version 3, Version 4 from 25 October 2023 when my legal representatives provided same to me; and

(b) the Exhibit BG-1 attached to my Statement dated 24 October 2023 when my legal representatives provided it to me on 24 October 2023.

21. I do not recall any specific details in relation to when each document referred to in subparagraphs 17(a) to 17(d) was drafted or approved other than by reference to the Amendment History section in each document.

22. My duties and responsibilities as Project Scientist included contributing to the drafting of Version 1 in relation to the automated protocols developed by the Automation/LIMS Implementation Project Team.

23. I was not solely responsible for drafting Version 1. I helped to draft the parts of Version 1 relating to the elements of the automated testing method being developed and subsequently agreed within the Automation/LIMS Implementation Project Team as part of the Project as described at paragraphs 12, 13(b) and 13(c).

24. Based on my review of the listed Author/s under the Amendment History section of Version 2 (page 17), Version 3 (page 21) and Version 4 (page 21), I do not recall preparing those iterations of document 24897.

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25. Having now had reference to Version 2, Version 3 and Version 4, I believe the Automation/LIMS Implementation Project Team followed the processes in any versions that followed Version 4 of document 24897 as updated from time to time.

26. I do not know if QHFSS continued to use Version 4 from the time my employment ended in May 2008, nor whether QHFSS used another automated method.

27. I do not know whether Version 4, or a following version the Standard Operating Procedure, is a part of, or is, the final method that was included within the 2008 Report.

28. I do not know if the 'DNA IQ™ protocol' referred to in Question 2 of the Notice 9.003 is the same as the Standard Operating Procedure.

29. For the purpose of my response to subparagraphs 2(a) to (e) inclusive:

    (a) I have assumed that the 'DNA IQ™ protocol' referred to in Question 2 of the Notice 9.003 is a reference to the Standard Operating Procedure;

    (b) my ability to provide descriptions of those matters as contained in the Standard Operating Procedure is limited to:

        (i) the iterations in Version 1, Version 2, Version 3 and Version 4 for the reason in paragraph 20 above; and

        (ii) the process of conducting extraction processes for extracting DNA from samples on an Automated Device.

30. I cannot describe any parts of a 'DNA IQ™ protocol' in connection with the manual method described at paragraphs 13(a) for the reasons at paragraph 15 above, other than to assume the Standard Operating Procedure refers to a manual method to account for a scenario in which there is no electricity and an automated device could not be used to follow the automated method in the Standard Operating Procedure.

(a) In seriatim, all steps comprising the protocol

31. My understanding of *'in seriatim, all steps comprising the protocol'* is a reference to all of the steps that comprised the DNA IQ™ protocol.

32. The steps comprising the protocol are outlined in Version 1, Version 2, Version 3 and Version 4.

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

33. The resin and reagent volumes used for DNA are:

    (a) in Version 1 at '*Table 2. Table of reagent volumes'* (page 4, under 4. Reagents and Equipment – 4.1 Reagents);

    (b) in Version 2 – '*Table 2. Table of reagent volumes'* (page 5 under 4. Reagents and Equipment – 4.1 Reagents);

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(c) in Version 3 - '*Table 2. Table of reagent volumes*' (page 5, under 4. Reagents and Equipment – 4.1 Reagents);

(d) in Version 4 - '*Table 2. Table of reagent volumes*' (page 5, under 4. Reagents and Equipment – 4.1 Reagents).

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

34. If the 'volume of the sample containing the DNA at the end of the extraction' is a reference to:

(a) the Elution buffer volume in a Bank-It tube which stored final extracts of DNA to keep it stable:

(i) for Version 1 – a final volume of 100µL following the 'double elution step' (page 2, fourth bullet point under the heading 'DNA IQ™ Kit');

(ii) for Version 2 – the same as Version 1 (page 3, fourth bullet point under the heading 'DNA IQ™ Kit');

(iii) for Version 3 – the same as Version 1 (page 3, sixth bullet point under the heading 'DNA IQ™ Kit');

(iv) for Version 4 – the same as Version 1 (page 3, sixth bullet point under the heading 'DNA IQ™ Kit');

however, it is likely that the actual final volume of Elution buffer volume would have varied based from sample to sample (e.g. 90µL or 110µL).

(b) the volume of DNA solution extracted from samples on an automated device using an automated protocol, this is not specified in Version 1, Version 2, Version 3 or Version 4.

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

35. The temperature used during the lysis step of the extraction process is specified as follows:

(a) Version 1 specifies the temperature for the plate, also referred to as a heating tile, only under the heading 'Setting up the EP-A or EP-B MPIIs' at step 9 (page 8):

> *9. Ensure that the DPC Shaker and Heater Controller Box are switched on.*
> *For EP-A: Tile 3 should be at F19 (50^{0}C), Tile 1 at F22 (85^{0}C).*
> *For EP-B: Tile 1 should be at F19 (50^{0}C), Tile 2 at F22 (85^{0}C).*

(b) Version 2 specifies the same step regarding the temperature for the plate at step 13 (page 10) and inserts the real temperature of the plate at Steps 24 (page 11) and 31 (page 12):

> *13. Ensure that the DPC Shaker and Heater Controller Box are switched on.*

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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.

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".

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.

I believe the 'real temp' information in steps 24 and 31 of Version 2 is a reference to real temperature, and I believe this was always the target real temperature when applying Version 1 because the real temperature appearing on the screen of the automated device would not have risen above 65^{0}C, however was inadvertently omitted from Version 1.

(c) As Version 3 introduced the off-deck lysis procedure such that the lysis procedure completed on the bench manually rather than on an automated device, the temperature used during the lysis step is specified under:

(i) the heading '7. Off-Deck Lysis Procedure (No retained supernatant) in Step 9:

9. Incubate in a hotblock at 37°C for 45minutes (note temperature on worksheet).

(ii) the heading '8. Off-Deck Lysis Procedure (retained supernatant) in Step 13:

13. Incubate in hotblock at 37°C for 45 minutes (note temperature on worksheet).

I do not know the difference between 'no retained supernatant' and 'retained supernatant'.

(d) The temperatures in Version 4 are the same as Version 3, specified under:

(i) the heading '7. Off-Deck Lysis Procedure (No retained supernatant) in Step 9:

9. Incubate in a hotblock at 37°C for 45minutes (note temperature on worksheet).

(ii) the heading '8. Off-Deck Lysis Procedure (retained supernatant) in Step 12:

Incubate in hotblock at 37°C for 45 minutes (note temperature on worksheet).

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

36. The washing steps during the extraction process are specified under the heading 'DNA IQ™ Kit' in Version 1 as follows (page 2):

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

37. My understanding of the extract in paragraph 36 above is three (3) 'washes' followed the lysis step of the extraction process. That is, I understand the lysis step as a step forming part of the extraction process where the lysis buffer would break cell wall to free the DNA from the original sample substrate, then sample was run through extraction columns to bind the DNA to the resin beads and then a wash was employed to wash away other proteins or inhibitors, but also during the extraction process.

38. The procedure in relation to the number of 'washes' employed following the lysis step of the extraction process:

(a) In Version 2 (page 3) is the same as Version 1 as follows:

> *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.*

(b) In Version 3 (page 3) is the same as Version 2, except to remove the '1x' before 'Wash Buffer':

> *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.*

(c) In Version 4 (page 3) is the same as Version 3:

> *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*

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during washing by keeping the DNA dehydrated, and aqueous phase washes out the inhibitor.

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

39. If the DNA IQ™ Protocol is a reference to the Standard Operating Procedure, I am not aware of the changes to the Standard Operating Procedure recorded in Version 2, Version 3 and Version 4 or the reasons for those changes other than by reference to:

(a) the summary of the updates to the Standard Operating Procedure which are modifications to the 'PerkinElmer automated protocol' to 'incorporate the work practices used in DNA Analysis FSS' outlined at:

(i) Version 1 – the five bullet points in the first paragraph under heading 'DNA IQ™ Kit' (page 2):

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.*

(ii) Version 2 – the five bullet points in the first paragraph under heading 'DNA IQ™ Kit' (pages 2 to 3) which are the same as in Version 1;

(iii) Version 3 – the seven bullet points in the first paragraph under heading 'DNA IQ™ Kit' (pages 2 to 3):

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:

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- *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.*

(iv) Version 4 – the seven bullet points in the first paragraph under heading 'DNA IQ™ Kit' (pages 2 to 3):

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.*

(b) the 'Amendments' column under the Amendment History section of each document.

(c) That the nature of amendments made to the manufacturer's protocol related to:

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(i) The cleaning of samples. I recall the automated protocol being developed by the Automation/LIMS Implementation Project Team introduced an additional cleaning step to that in the manufacturer's automated protocol.

(ii) The use of buffers in the extraction process. I recall the Automation/LIMS Implementation Project Team observed that particular brands of buffers performed more suitably and optimally then buffers provided by manufacturers in an extraction kit.

(iii) The brand of reagent used (but not the type of reagent). I recall the Automation/LIMS Implementation Project Team noticed the DNA extraction results differed for the types of reagents, including that a *Qiagen* reagent was producing higher yield of DNA for a sample than *Promega*. I cannot recall further details regarding the reagents which were purchased or at what volume.

(iv) Temperature of the plates using the heating device provided by the manufacturer of the Automated Device. The temperature of the heating device impacted the temperature of the sample with the plate. I recall issues with the yield of DNA extraction arose when we proceeded with extraction with the pre-set temperatures for the heating device outlined in the manufacturer's protocol because the samples would not increase to the necessary temperature.

(v) the length of time the pipette tips would hover over a plate containing samples during the extraction process. The purpose of this was to ensure there was a sufficient length of time to allow bubbles to drip off of pipette tips to avoid contamination risk. This was not a change to the DNA IQ™ Kit and rather a change to the electronic testing protocol installed in the computer software that was associated with the automated platform.

(d) As to paragraph 69 of my Statement dated 24 October 2023, I wish to clarify:

(i) having now referred to Version 1, Version 2, Version 3 and Version 4, my recollection of the specifics of each amendment are as described in paragraph 39 of this Statement.

(ii) I repeat and refer to paragraph 29(b) above and do not recall the specifics of the final protocol.

40. I cannot recall with clarity the reasons for the changes in the respective bullet points, however below I describe what I believe would have been the reasons for those changes based on my own knowledge at the time of making this Statement.

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

41. Based on my review of Version 1, this was introduced in Version 1.

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42. I believe the substrate refers to the original material from which the Laboratory would try to extract a sample from for testing – for example, cloth, clothing material, cigarette papers or swabs taken by the Laboratory or Queensland Police Service.

43. Before the Slicprep™ 96 device (Promega) was used for removing substrate from lysate, the original substrate material would remain in each sample well during processing, however the Automation/LIMS Implementation Project team found keeping the substrate in the wells could cause the blocking of the pipette tips during the automated process. Buffer liquid would also be left behind in the substrate, so not all the liquid containing the DNA would be removed from the well. By using the Slicprep™ 96 device, which allowed for the substrate material to be removed from the plate that contained the sample, it allowed the liquid containing the DNA to be processed without issue.

44. Having reviewed the Standard Operating Procedure, I believe the Slicprep™ 96 device (Promega) was a two plate device, with the top plate made of wells/tubes containing substrate material, which sat within a bottom plate in which the sample liquid would be retained. The device in this configuration sat on the Automated Device. Following the Standard Operating Procedure, the extraction buffer was then added to the relevant tube to remove the DNA from the substrate. This may have been followed by the lysis step, after which the plate was centrifuged (spun down) to remove all liquid from the top plate of the Slicprep™ 96 device into the bottom plate. That became the sample used during the extraction process that followed on that Slicprep™ 96 device (Promega) plate.

45. I believe a reason for introducing the use of the Slicprep™ 96 device (Promega) was used for removing substrate from lysate was that the Laboratory would have determined through trial and error that the decision to use the Slicprep™ 96 device (Promega) for removing substrate from lysate would improve the overall outcome of the extraction process so that the sample was as suitable as possible for subsequent DNA analysis.

The increase of extraction buffer volume to 500µL for use with the Slicprep™ 96 device

46. Based on my review of Version 1, this was introduced in Version 1.

47. I do not clearly recall the decision to implement an increase of extraction buffer volume to 500µL for use with the Slicprep™ 96 device.

48. I assume the decision to increase the extraction buffer volume was to increase the chances of obtaining a DNA sample from the substrate when it sat in the extraction buffer liquid.

49. I cannot recall what the extraction buffer volume was before this modification was made.

The increase of Lysis Buffer volume to 957µL proportional to the increase of Extraction Buffer volume, according to the manufacturer's instructions.

50. Based on the words *'according to the manufacturer's instructions'* I assume the PerkinElmer automated protocol (being a manufacturer's protocol) included formulas in relation to what was the proportional increase of lysis buffer volume required for a certain increase in extraction buffer volume.

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51. Accordingly, I believe this modification was made to increase the Lysis Buffer volume stated in the manufacturer's protocol, with the proportional increase in Extraction buffer volume to be determined in accordance with those calculations in the PerkinElmer automated protocol.

Double Elution step, with an Elution buffer volume of 60µL for a final volume of 100µL.

52. Based on my review of Version 1:

    (a) this was introduced in Version 1.

    (b) I believe that after a wash step to wash away inhibitors on the tubes of the Slicprep™ 96 device, this 'double elution step' occurred by pouring 60µL of elution buffer volume into the tube and repeating this. Because it was not always possible to remove all of the liquid buffer from the tubes, the final volume was not necessarily 120µL. I assume this is the reason why the Standard Operating Procedure refers to a final volume of 120µL.

53. I cannot recall what the PerkinElmer automated protocol (being a manufacturer's protocol) outlined in relation to elution and whether it required one or two steps.

The use of NUNC Bank-It tubes for storage of final extracts.

54. Based on my review of Version 1, this was introduced in Version 1.

55. I cannot recall why the Standard Operating Procedure introduced the use of NUNC Bank-It tubes for storage of final extracts.

56. I cannot recall what the PerkinElmer automated protocol said about storage of final extracts.

57. It is possible the assume the PerkinElmer automated protocol specified in the instructions for use their own product for storage of final extracts. The Laboratory may have decided to use the NUNC Bank-It tubes product. I cannot recall the reason for this.

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.

58. Based on my review of Versions 1, 2 and 3, the off-deck lysis procedure was introduced in Version 3.

59. I believe this to be what has been referred to in the Standard Operating Procedure as the 'off-deck lysis' process (referred to at paragraph 35(d) above) which was introduced in Version 3 based on my review of Versions 1, 2 and 3.

60. While I do not recall the exact reasoning as to why from Version 3 the lysis process was no longer done on the automated platform as per Versions 1 and 2, in reviewing Versions 1, 2, 3 and 4, I assume it to be that the heat plate for the lysis steps referred to in step 9 of Version 1 and step 13 for Version 2 did not get to temperature. I also make this assumption with reference to Figure 2 in Version 2 (Figure 2. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of

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Extraction on Extraction Platform A.) which shows only one heating tile rather than two heating tiles, that the reason for taking the lysis procedure off-deck related to the temperatures of the heating blocks for the lysis process.

61. I do not recall why the off-deck lysis process was done using tubes and spin baskets, nor the use of a 96-deepwell plate.

62. I note that Section 12, Validation of Version 3 notes I am an author of two publications in relation to the Off-Deck Lysis however I do not recall contributing to those publications, or my contribution into the creation of the off-deck lysis procedure outlined from Version 3.

The provision of initial incubation with TNE Buffer and retention of a portion thereof for further testing (retained supernatant testing).

63. Based on my review of Versions 1, 2 and 3, this was introduced in Version 3.

64. I do not recall why there was a provision of initial incubation with TNE buffer and retention of a portion thereof for further testing.

Other changes to the Standard Operating Procedure from Versions 1, Version 2 and Version 4 not already addressed above

65. In addition to those outlined in paragraph 39 above, based on my review of Version 1, Version 2, Version 3 and Version 4 I have identified the following processes implemented from Version 2, being a change to Version 1 and accordingly modifications to the 'PerkinElmer automated protocol' to 'incorporate the work practices used in DNA Analysis FSS' for the purpose of answering this Question 3:

    (a) Version 2:

        (i) Added the following Reagents in Section 4.1:

            (A) 'AnalR 100% Ethanol'. I cannot recall why this reagent was added.

            (B) 20% SDS. I believe this was to clarify that the reagent was already used as at Version 1 because Version 1 refers to '20% SDS' in Section 4.1, Table 2 (Reagent storage locations) and within the extractions (for example, under the heading Extraction Buffer).

        (ii) Made the changes outlined at paragraph 35(b) to add the real temperature of the plate.

        (iii) Regarding the summary of the amendments under Section 15, Amendment History as 'Reviewed and updated after initial training', I believe this is a reference to necessary changes as a result of feedback from the Analytical team regarding the Standard Operating Procedure, after they were trained in the procedure by a person in the Automation/LIMS Implementation Project Team. The training was provided to those who had previously routinely used the manual method only to process extractions. I did not deliver this

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

Breanna Lee Gallagher Witness

training during my employment at QHFSS. This is consistent with my understanding of how many forensic laboratories operate.

(b) Version 4 added 40% Sarcosyl to the list of Reagents in Section 4.1. I do not recall this reagent or why it was added.

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.

66. As for Question 3, I understand that the DNA IQ™ protocol is the Standard Operating Procedure described at paragraph 19 and the 'protocol issued by the manufacturer' is a reference to the PerkinElmer automated protocol.

67. My understanding of the differences between the Standard Operating Procedure and manufacturer protocol and the reasons for those changes are outlined in my response to Question 3.

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)[1]?

[1] Tendered at the Commission of Inquiry into Forensic DNA Testing in Queensland as exhibit 129.95, FSS:0001.0084.1444: https://www.dnainquiry.qld.gov.au/public-hearings/exhibits.aspx#m4.

68. On 20 October 2023, my legal representatives provided a copy of the 'Project 13. Report on the Verification of an Automated DNA IQ™ Protocol using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform' dated August 2008 on page 1 (**2008 Report**) to me. A copy of the 2008 Report is attached and marked as Exhibit **BG-05** to this Statement. I do not recall having seen the 2008 Report prior to being provided with a copy on 20 October 2023.

69. I was not the author of the Abstract appearing in the 2008 Report.

70. I believe my name was included as an author of the 2008 Report because I was a member of the Automation/LIMS Implementation Project Team.

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

Breanna Lee Gallagher Witness

Declared by the deponent, Breanna Lee Gallagher, this 27th day of October 2023 at Canberra, Australia, and I say that:

1. I have not conferred or had any discussion with other witnesses in preparing my statement.
2. All the facts and circumstances declared in my statement, are within my own knowledge and belief, except for the facts and circumstances declared from information only, and where applicable, my means of knowledge and sources of information are contained in this statement.
3. I make this solemn declaration conscientiously believing the same to be true and by virtue of the provisions of the *Oaths Act 1867*.
4. The statement was made in the form of an electronic document.
5. The statement was electronically signed by the signatory.
6. The statement was made, signed and witnessed under Part 6A *Oaths Act 1867*.
7. I understand that it is an offence to provide a false matter in a statement.

Signature of deponent [redacted]

And as a witness, I certify the following matters concerning the person who made this statement (the deponent):

1 I saw the face of the deponent.

2 I have confirmed the deponent's identity using the following identification document: 18+ Identification Card.

3 I have verified that the name of the deponent matches the name written on the statement.

Signature of witness: [redacted]

Blaire O'Loughlin-Mills

Australian legal practitioner, MinterEllison

Special witness under the *Oaths Act 1867*

I understand the requirements for witnessing a document by audio visual link and have complied with those requirements

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

Breanna Lee Gallagher Witness

EXHIBITS INDEX

Exhibits Index – Breanna Lee Gallagher Statement

Exhibit	Document Title
BG-01	DNA IQ™ Method of Extracting DNA from Casework and Reference Samples, Version 1
BG-02	DNA IQ™ Method of Extracting DNA from Casework and Reference Samples, Version 2
BG-03	DNA IQ™ Method of Extracting DNA from Casework and Reference Samples, Version 3
BG-04	DNA IQ™ Method of Extracting DNA from Casework and Reference Samples, Version 4
BG-05	Project 13. Report on the Verification of an Automated DNA IQ™ Protocol using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform

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

Breanna Lee Gallagher Witness

BG-01

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

1 PURPOSE AND SCOPE

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

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

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

2 DEFINITIONS

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

3 PRINCIPLE

Sample Pre-lysis

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

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

DNA IQ™ Kit

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

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

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

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

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

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

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

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

MultiPROBE® II HT EX Plus with Gripper™ Integration Platform

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

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

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

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

4 REAGENTS AND EQUIPMENT

4.1 Reagents

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

Table 1. Reagent storage locations.

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

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

Table 2. Table of reagent volumes.

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

Extraction Buffer

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

Lysis Buffer with DTT

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

DNA IQ™ Resin

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

1X Wash buffer

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

4.2 Equipment

Table 3. Equipment used and location.

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

5 SAFETY

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

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

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

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

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

6 SAMPLING AND SAMPLE PREPARATION

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

Table 4. Sample storage locations.

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

QC samples

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

Table 5. Extraction Quality Controls

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

Registration of QC

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

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

Create the Extraction Batch

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

Locating Samples

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

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

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

7 PROCEDURE

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

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

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

Setting up the EP-A or EP-B MPIIs

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

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

Figure 2 The WinPrep® icon.

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

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

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

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

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

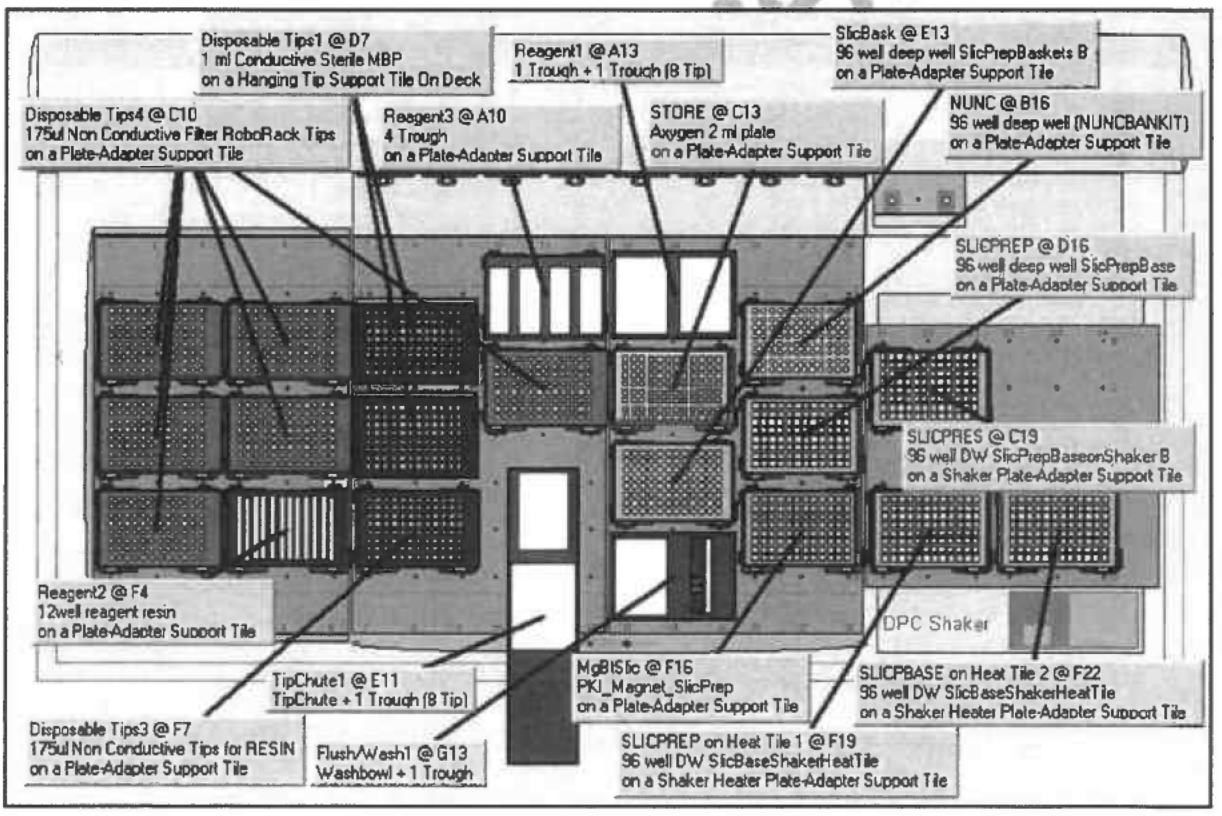


Figure 3. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Recording Reagent Details in AUSLAB

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

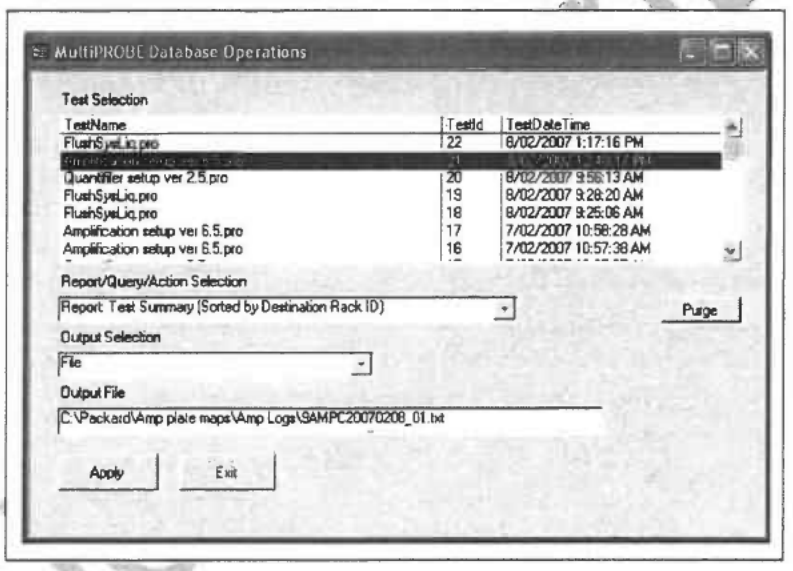
Finalising the MP II run

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

Importing the MP II log file into AUSLAB

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

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



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

Importing Extraction "Results" into AUSLAB

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

8 SAMPLE STORAGE

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

9 VALIDATION

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

10 QUALITY ASSURANCE/ACCEPTANCE CRITERIA

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

11 REFERENCES

1. Boom, R., Sol, C.J.A., Salimans, M.M.M., Jansen, C.L., Wertheim-van Dillen, P.M.E., & van der Noordaa, J., Rapid and Simple Method for Purification of Nucleic Acids. J. Clin. Microbiol., 1990. 28: p. 495-503.
2. Chen., C.W.T.J., C.A., Recovery of DNA Segments from Agarose Gels. Anal Biochem., 1980. 101: p. 339-341.
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7. Mandrekar, P., V., Flanagan, L., & Tereba, A., Forensic Extraction and Isolation of DNA Form Hair, Tissue and Bone. Profiles in DNA, 2002: p. 11.
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9. Marko, M.A., Chipperfield, R., & Birnboim, H.C., A Procedure for the Large Scale Isolation of Highly purified Plasmid DNA using alkaline extraction and binding to glass powder. Anal. Biochem., 1982. 121: p. 382-387.
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11. PerkinElmer, Automated DNA IQ™ System for Mixed Casework Sample DNA Isolation. MultiPROBE II Liquid Handling - Forensic Workstation Application Guide, 2004: p. 1-25.
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13. Promega, Protocols & Applications Guide. Chapter 9. rev. 7/06.
14. Promega, DNA IQ™ System -Small Casework Protocol. Promega Technical Bulletin #TB296 2006. Rev 4/06: p. 1-14.
15. Promega, DNA IQ™ System-Database Protocol. Promega Technical Bulletin #TB297, 2006. Rev 4/06: p. 1-14.
16. Promega, Tissue and hair Extraction Kit (for use with DNA IQ™) Protocol. Promega Technical Bulletin #TB307, 2006. Rev 5/06: p. 1-11.
17. Schiffner, L.A., Bajda, E. J., Prinz, M., Sebestyen, J., Shaler, R. & Caragine, T.A., Optimisation of a Simple, Automatable Extraction Method to Recover Sufficient DNA from Low Copy Number DNA Samples for Generation of Short Tandem Repeat Profiles. Croat Med J, 2005. 46(4): p. 578 -586.
18. Vandenberg, N., van Oorchot., R. A. H., & Mitchell, R. J., An evaluation of selected DNA extraction strategies for short tandem repeat typing. Electrophoresis, 1997. 18: p. 1624-1626.

12 STORAGE OF DOCUMENTS

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

13 ASSOCIATED DOCUMENTS

QIS 17120 Operational Practices in the DNA Dedicated Laboratories

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

14 AMENDMENT HISTORY

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

BG-02

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

1 PURPOSE AND SCOPE

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

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

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

2 DEFINITIONS

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

3 PRINCIPLE

Sample Pre-lysis

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

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

DNA IQ™ Kit

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

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

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

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

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

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

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

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

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

MultiPROBE® II HT EX Plus with Gripper™ Integration Platform

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

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

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

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

4 REAGENTS AND EQUIPMENT

4.1 Reagents

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

Table 1. Reagent storage locations.

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

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

Table 2. Table of reagent volumes.

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

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

Extraction Buffer

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

Lysis Buffer with DTT

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

DNA IQ™ Resin

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

1X Wash buffer

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

4.2 Equipment

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

Table 3. Equipment used and location.

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

Table 4. Consumables used for extraction

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

5 SAFETY

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

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

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

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

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

6 SAMPLING AND SAMPLE PREPARATION

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

Table 5. Sample storage locations.

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

QC samples

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

Table 6. Extraction Quality Controls

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

Registration of QC

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

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

Create the Extraction Batch

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

Locating Samples

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

Checking Samples

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

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

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

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

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

7 PROCEDURE

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

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

Summary of DNA IQ EXTRACTION winprep program (v 1.3)

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

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

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

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

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

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

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

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

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

Preparation of Reagents prior to extraction

1. Defrost Prot K and DTT

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

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

Setting up the EP-A or EP-B MPIIs

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

4. Turn on the instrument PC.

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

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

Figure 1 The WinPrep® icon.

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

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

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

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

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

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

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

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

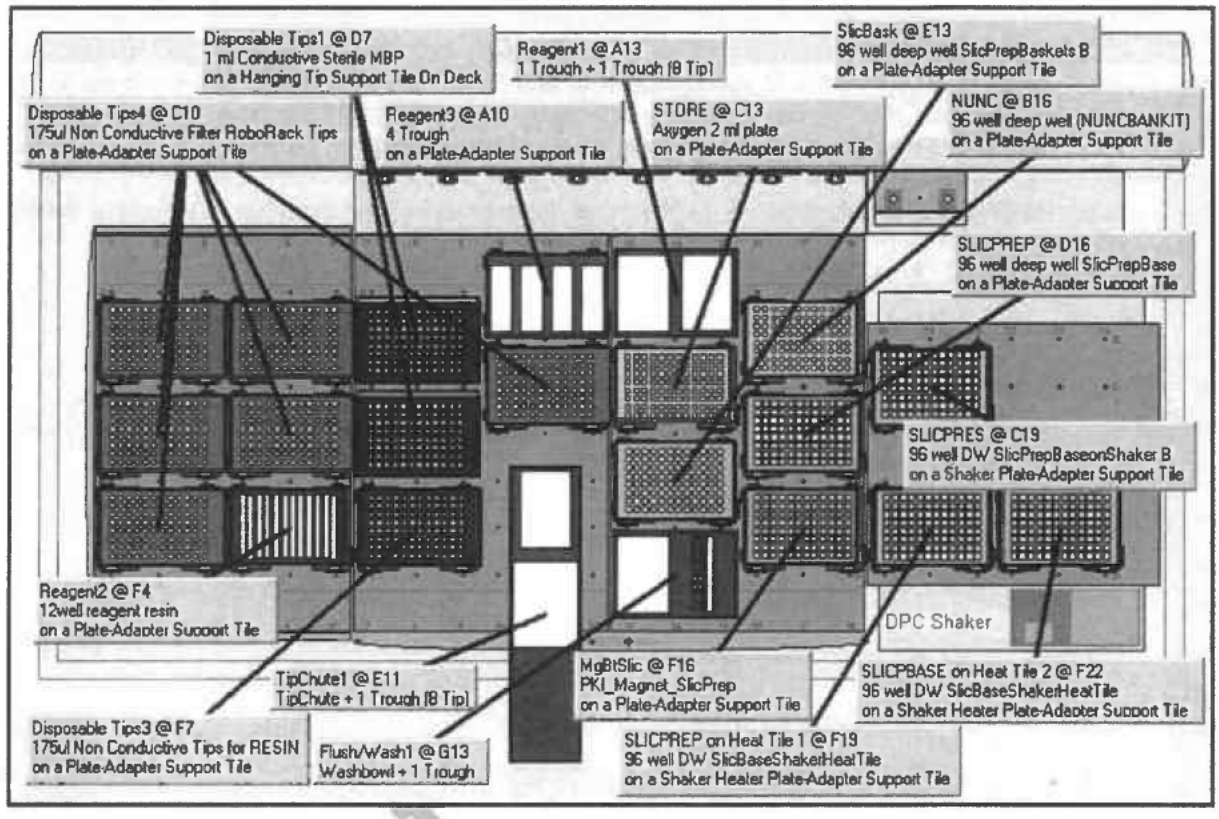


Figure 2. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Finalising the MP II run

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

Recording Reagent Details and other information in AUSLAB

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

Importing the MP II log file into AUSLAB

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

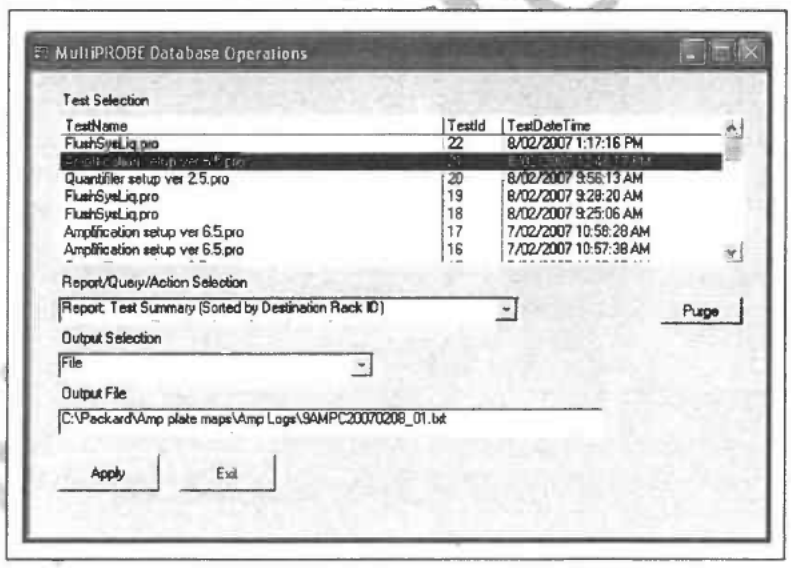


Figure 4. The MultiPROBE log database for collecting MP II run information

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

61. Press **[Esc]**.

Importing Extraction "Results" into AUSLAB

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

8 SAMPLE STORAGE

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

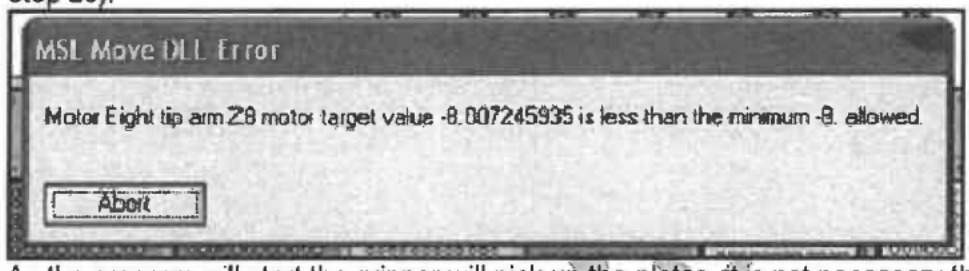
9 TROUBLESHOOTING

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

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

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

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



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

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

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

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

9. If the message:

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

10 VALIDATION

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

11 QUALITY ASSURANCE/ACCEPTANCE CRITERIA

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

12 REFERENCES

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

13 STORAGE OF DOCUMENTS

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

14 ASSOCIATED DOCUMENTS

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

15 AMENDMENT HISTORY

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

16 APPENDIX

16.1 Reagents Calculation Tables

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

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

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

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

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

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

16.2 Manual method for extraction using DNA IQ™

16.2.1 Sampling and Sample Preparation

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

Table 3. Sample storage locations.

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

QC samples

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

Table 4. Extraction Quality Controls

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

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

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

Create the Extraction Batch

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

Locating Samples

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

When all samples have been located:

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

Sequence Check the tubes

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

16.2.2 Procedure

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

16.2.3 Sample storage

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

BG-03

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

1 PURPOSE AND SCOPE

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

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

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

2 DEFINITIONS

Samples	Samples awaiting DNA extraction
Lysates	Samples that have had the Lysis step performed, but have not yet completed the entire extraction process
DNA Extracts	Samples that have had a DNA extraction processes performed
DNA IQ™ Resin	Magnetic Resin Beads used to bind DNA
MP II	MultiPROBE® II PLUS HT ex Platform
DTT	1,4 Dithiothreitol
Pro K	Proteinase K
SDS	Sodium Dodecyl Sulphate
TNE	Tris, NaCl and EDTA Buffer
EDTA	Ethylenediaminetetraacetate
EP-A	Extraction Platform A
EP-B	Extraction Platform B

3 PRINCIPLE

Sample Pre-lysis

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

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

DNA IQ™ Kit

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

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

- The increase of Extraction Buffer volume to 500µL for use with the Slicprep™ 96 device.
- The use of tubes and spin-baskets for the off-deck lysis of samples prior to extraction on MPII. Use of a 96-deepwell plate for completion of extraction on MPII.
- The provision of initial incubation with TNE Buffer and retention of a portion thereof for further testing (retained supernatant testing).
- The increase of Lysis Buffer volume to 957µL proportional to the increase of Extraction Buffer volume, according to the manufacturer's instructions.
- Double Elution step, with an Elution Buffer volume of 60µL for a final volume of 100µL.
- The use of NUNC Bank-It tubes for storage of final extracts.

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

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

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

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

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

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

MultiPROBE® II HT EX PLUS with Gripper™ Integration Platform

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

sample probe is capable of independent motion in the Z direction due to independent Z drives.

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

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

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

4 REAGENTS AND EQUIPMENT

4.1 Reagents

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

Table 1. Reagent storage locations.

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

Please see Table 2 for the volume of reagents for a full plate or half plate. Refer to "*Receipt, Storage and Preparation of Chemicals, Reagents and Kits*" (QIS 17165) for

preparation of the TNE Buffer. All reagents can be made on the bench in Room 6122, except for the Lysis Buffer with DTT which needs to be made in a fume hood. DNA IQ reagents are prepared by staff performing the DNA IQ method.

Table 2. Table of reagent volumes.

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

NOTE: For batches not equal to either 96 or 48 samples samples, refer to Appendix 1 "*18.1 Reagents Calculation Tables*" Table 7 for batches of <48 samples, and Table 8 for <96 (but >48)

Extraction Buffer

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

Lysis Buffer with DTT

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

DNA IQ™ Resin

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

1X Wash Buffer

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

4.2 Equipment

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

Table 3. Equipment used and location.

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

Table 4. Consumables used for extraction

Consumables	Location
175uL Clear Non-Conductive Filter Tips – Pre-sterilized	6127
MβP Pure 1000uL Tips – Pre-Sterilized	6127
SlicPrep™ 96 device plate	6122
ABgene 96-deepwell plate	6120
Axygen 2mL Deep Well storage plate	6127
1.5ml or 2ml Eppendorf tubes with Spin baskets	6120
12 Channel plate	6127
Nunc tubes	6120
Nunc Caps	6127
Sterile 50mL Falcon tubes	6122
Sterile 10mL tubes	6122
Autoclaved 100mL glass bottles	6122
Autoclaved 250mL glass bottles	6122
Aluminium sealing film	6127
1000uL disposable tips	6120

5 SAFETY

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

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

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

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

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

6 SAMPLING AND SAMPLE PREPARATION

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

Table 5. Sample storage locations.

Sample type	Storage Device	Storage Location
Urgent/High/Medium Priority Samples	Freezer	6117-2
Medium Priority Samples	Walk in Freezer or Freezer in 6117	6109 or 6117-5
Low Priority Samples	Walk in Freezer or Freezer in 6117	6109 or 6117-5
Lysates in 1.5ml tubes	Fridge	6120
96 deep well plate containing lysates	Fridge	6127

QC samples

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

Table 6. Extraction Quality Controls

QC Name	UR Number	Description
Negative Control	FBOT277	Negative Extraction control – Empty well
Positive Control	FBOT279	Positive Extraction control – Known Donor dried blood swab

Registration of QC

1. Log into the **AUSLAB Main Menu**.
2. Select **1. Request Registration**.
3. Select **2. Full Reception Entry**.
4. Scan in barcode of control.
5. Enter the UR number as per Table 6 and press **[Enter]**.
6. Enter the appropriate Specimen type (e.g. **Blood** for blood control).
7. Request a **9PLEX** test, when prompted to enter the processing comment, enter **EXTP** (Positive extraction control) or **EXTN** (Negative extraction control). Do no assign a priority.
8. Press **[F7]** ***Save*** to save the Billing details.
9. Enter **LAB** in the Billing code field and **t** in the date field and **FBQC** in the Loc/Client field.
10. Press **[F4]** ***Save twice*** to save the registration details.

Note 1: Quality controls should not have a DNA priority allocated at time of registration to ensure they are included in the top positions of a batch

Note 2: for DNA IQ Lysis batches with more than 46 samples (excluding controls) two sets of controls should be registered

Create the DNA IQ Lysis or Retain Supernatant Batch (as required)

1. Log into the **AUSLAB Main Menu**.
2. Select **5. Workflow management**.
3. Select **1. DNA workflow table**.
4. Highlight the appropriate batch type and press **[F5]** ***Batch Allocation***.
5. Press **[F6]** ***Create batch***.
6. Press **[F8]** ***Print menu***.
7. Press **[F7]** ***Print Sample Label***. (print 3 sets)
8. Press **[F8]** ***Print Worksheet***. (print 2 copies)
9. Press **[SF5]** ***Main menu***.
10. Press **[SF11]** ***Print***.

11. Press **[SF6]** ***Accept batch***.
12. Press **[Pause/Break]** to exit to the **Main Menu**.
13. Obtain worksheets **(FBLASER3)** and labels **(FBLABEL13-16)** from the Analytical Section printing bench **(Room 6117)**.

Locating Samples

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

7 OFF-DECK LYSIS PROCEDURE (NO RETAINED SUPERNATANT)

1. Print or obtain a copy of Appendix 2. "*18.2 Reagent & Batch details recording tables (DNA IQ™ Lysis Batch & Extraction Batch)*".

2. Separate the batch into two smaller batches of 48 samples, including one set of controls. (If only a single operator the second batch can be started during step 11)

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

3. Remove samples from 5mL storage tubes and label side with barcodes if necessary. If substrates are in a 0.5mL tube, transfer to a labelled 1.5mL tube. Label lid of each sample with position number within the lysis batch. Retain the original 5mL storage tube for substrate storage.

4. Prepare / assemble spin basket assembly or 1.5mL (or 2.0mL) tube and label side with a barcode and lid with position number as required.

Note: substrates from each sample need to be retained

a. Samples requiring a spin basket include swabs, fabric, paper and cigarette butts.
b. Samples requiring a 1.5mL include tapelifts, chewing gum, straws and toothbrush bristles.

5. Label the side of sterile 1.0mL Nunc Bank-It tubes with barcode.

6. Have a second operator sequence check all tubes and complete the sequence check in AUSLAB.

7. Prepare Extraction Buffer (store at 4°C when not in use).

8. Using a multi-stepper pipette add 500µL of Extraction Buffer and vortex briefly.

9. Incubate in a hotblock at 37°C for 45minutes (note temperature on worksheet).

10. Remove samples from hot block and vortex briefly then return to rack.

11. Increase temperature on hotblock to 65°C (preparation for second incubation step).

12. Transfer substrates to spin baskets if required using twirling sticks (if unable to remove with twirling sticks, use forceps. Forceps must be cleaned between each sample by rinsing in bleach followed by ethanol and flaming).

13. For samples not requiring spin baskets, transfer the lysate to the newly labeled 1.5mL tube. Then store original 1.5mL containing substrate in the original 5mL tube.

14. Centrifuge spin baskets at maximum speed (14000rpm) for 2 minutes.

15. Transfer flow through from spin baskets back to original lysis tube, retain spin basket in 5mL tube.

16. Vortex Lysates briefly, then incubate in hotblock at 65°C for 10 minutes (note temperature on worksheet)

17. Enter reagent details, temperatures etc. into AUSLAB.

18. Complete batch in AUSLAB.

19. Store lysates at 4°C (fridge in 6120).

20. Enter into completed batch in AUSLAB and determine the DNA IQ extraction batch ID the DNA lysates have progressed to.

21. Print the appropriate worksheets and 3 sets of batch labels for the DNA IQ extraction batch and note the lysis batch the samples have progressed from on the worksheets. Stamp as "Urgent" if necessary.

8 OFF-DECK LYSIS PROCEDURE (RETAINED SUPERNATANT)

1. Print or obtain a copy of Appendix 2. "*18.2 Reagent & Batch details recording tables (DNA IQ™ Lysis Batch & Extraction Batch)*".

2. Remove samples from 5mL storage tubes and label side with barcodes if necessary. If substrates are in a 0.5mL tube, transfer to a labelled 1.5mL tube. Label lid of each sample with position number. Retain 5mL tube for substrate storage.

3. Label the side of 1.5mL tubes with barcodes for retaining supernatant. Also label lid of 1.5mL tube indicating it contains supernatant.

4. Prepare spin basket assembly or a 1.5mL (or 2.0mL) tube and label side with a barcode and lid with position number as required. **Note:**
   a. Samples requiring a spin basket include swabs, fabric, paper and cigarette butts.
   b. Samples requiring a 1.5mL include tapelifts, chewing gum, straws and toothbrush bristles.

5. Label the side of sterile 1.0mL Nunc Bank-It tubes with barcode.

6. Have a second operator sequence check all tubes and complete the sequence check in AUSLAB.

7. Using a pipette add 650µL of TNE Buffer and vortex briefly.

8. Incubate at room temperature for 30 minutes.

9. During 30 minute incubation prepare Proteinase K and SDS solutions.

10. Vortex, then centrifuge for 3 minutes at maximum speed (14000rpm).

11. Remove 150µL of supernatant and place into labelled 1.5ml tube (for further testing).

12. Add 25µL of 20ng/µL (mg/mL) Proteinase K and 12.5µL 20% (w/v) SDS to each original sample tube containing TNE Buffer. Vortex briefly.

13. Incubate in hotblock at 37°C for 45 minutes (note temperature on worksheet).

14. Remove samples from hotblock, vortex briefly and return to rack.

15. Change settings on hotblock to temperature of 65°C (preparation for second incubation step).

16. Transfer substrates to spin baskets if required using twirling sticks (if unable to remove with twirling sticks, use forceps. Forceps must be cleaned between each sample by rinsing in bleach followed by ethanol and flaming).

17. For samples not requiring spin baskets, transfer the lysate to the newly labeled 1.5mL tube. Then store original 1.5mL containing substrate in the original 5mL tube.

18. Centrifuge spin baskets at maximum speed (14000rpm) for 2 minutes.

19. Transfer flow through from spin baskets back to original lysis tube, retain spin basket in 5mL.

20. Vortex Lysates briefly, then incubate in hotblock at 65°C for 10minutes (note temperature on worksheet).

21. Enter reagent details, temperatures etc. into AUSLAB.

22. Complete batch in AUSLAB.

23. Store supernatants in Freezer 6117-2 (-20°C).

24. Store lysates at 4°C (Fridge in 6120).

25. Enter into completed batch in AUSLAB and determine the DNA IQ extraction batch ID the DNA lysates have progressed to.

26. Print the appropriate worksheets and 3 sets of batch labels for the DNA IQ extraction batch and note the retained supernatant batch the samples have progressed from on the worksheets. Stamp as "Urgent" if necessary.

9 MPII EXTRACTION PROCEDURE

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

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

Summary of DNA IQ EXTRACTION Version 2 ODL

1. ***Binding of paramagnetic resin to DNA and further Lysis:*** add Resin solution (50µL) and Lysis Buffer (957µL). Automated mixing and shaking at room temperature for 5

minutes. (this occurs at steps 10-15 of the protocol)

2. ***Removing lysis reagents:*** Slicprep plate is moved to the PKI Magnet to separate beads. Removing of supernatant (1600µL) without disturbing resin, dispense this solution in the storage plate. (this occurs at steps 16-18 of the protocol)

3. ***Washing of the resin-DNA complex:*** To remove any inhibitors in solution. The first wash is with Lysis Buffer (125µL), shaking at room temperature for 1 minute. The plate is moved to the PKI Magnet and the supernatant is removed into the storage plate. The next three washes are with 1X Wash Buffer (100µL), shaking at room temperature for 1 minute. During each wash cycle, the plate is moved to the PKI Magnet and the supernatant is discarded. (this occurs at steps 21-59 of the protocol)

4. ***Removing any excess of 1X Wash Buffer:*** air dry at room temperature for 5 minutes. (this occurs at step 60 of the protocol)

5. ***Elution of DNA from the Resin-DNA complex:*** Add Elution Buffer (60µL) and incubate at 65 °C for 6 minutes (3 minutes no shaking and 3 minutes shaking). The plate is moved to the PKI Magnet. The eluted solution (supernatant) is removed to the Nunc tubes. Elution is repeated twice. (this occurs at steps 63-83 of the protocol)

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

Preparation of Reagents prior to extraction

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

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

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

To sequence check storage tubes and transfer DNA lysates to ABgene 96-deep well plates, please refer to method "*Procedure for the Use of the STORstar unit for automated sequence checking*" (QIS 24256).

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

Setting up the EP-A or EP-B MPIIs

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

3. Turn on the instrument PC.

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

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

Figure 1 The WinPrep® icon.

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

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

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

9. Open the Extraction setup MP II test file in WinPrep® by selecting:
   - **File**
   - **Open**, navigate to **C:\PACKARD\MULTIPROBE\BIN\QHSS PROTOCOLS**
   - Select "**DNA IQ Extraction_Ver 2_ODL.mpt**"
   - Click the "**Open**" button

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

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

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

    - The white WALLAC Isoplates (catalogue #1450-514) that are used to support the ABgene 96-deep well plate must be placed into positions **D16** and **C19**.

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

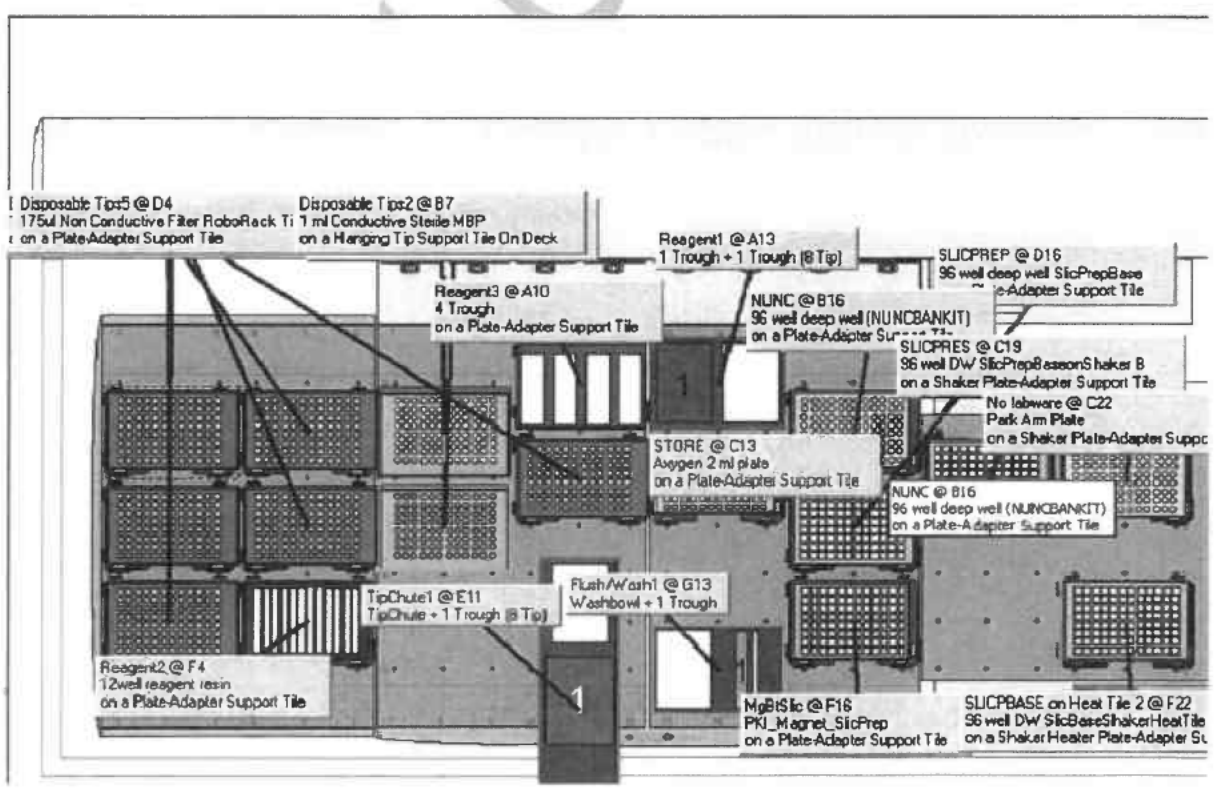

Figure 2. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

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

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

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

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

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

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

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

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

21. Message will appear (Figure 3 below):

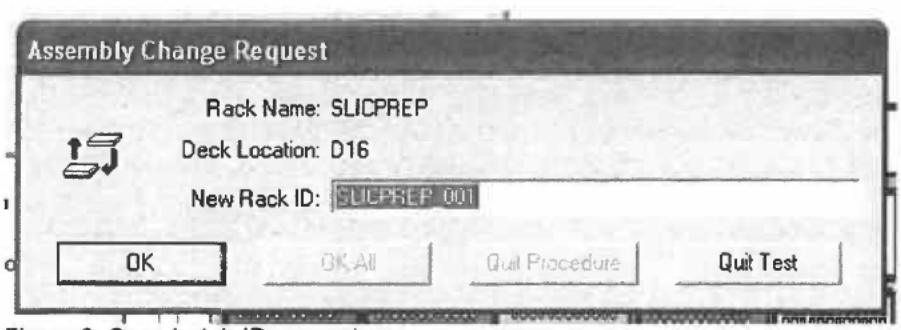

Figure 3. Scan batch ID request

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

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

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

24. Add the Resin to the 12 Channel plate. Pipette mix thoroughly. Then dispense 50uL of resin into each well of the ABgene 96-deep well plate containing Lysates.

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

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

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

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

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

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

31. Once the program is completed, a final User Message prompt appears asking to:
"**Remove plates and cover them with aluminium sealing film. Remove Nunc rack and recap Nunc tubes.**"
Once all plates are removed from the deck and sealed, place into small clipseal plastic bags. Click "**OK**" to proceed to the Amphyl wash step to decontaminate the system tubing.

Finalising the MP II run

32. Remove the resin-Lysis-DTT solution from the 12 channel plate in the glass Lysis-DTT bottle used. Discard the plate in the biohazard waste bin.

33. Remove Lysis Buffer with DTT (wear face shield) and dispose of left over reagent into the glass bottle used previously. Bring this bottle to the room 6122 and dispose in the brown Winchester bottle located in the fume hood.

34. Empty the contents of the Amphyl wash station into the sink and rinse the troughs with nanopure H_2O upon completion of the run.

35. Remove all labware from the deck and clean with 5% TriGene™ followed by 70% ethanol, and setup for the next run if necessary.

36. Remove the tip chute and funnel, rinse with warm tap water to help loosen and remove any residue inside the chute before cleaning with 5% TriGene and 70% ethanol. Hang the chute to dry on the rack beside the sink. If running another extraction straight after, replace the tip chute with another spare, clean chute.

37. Move the platemap to **C:\PACKARD\EXT PLATE MAPS** to the "**Completed Extractions**" folder.

Recording Reagent Details and other information in AUSLAB

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

Importing the MP II log file into AUSLAB

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

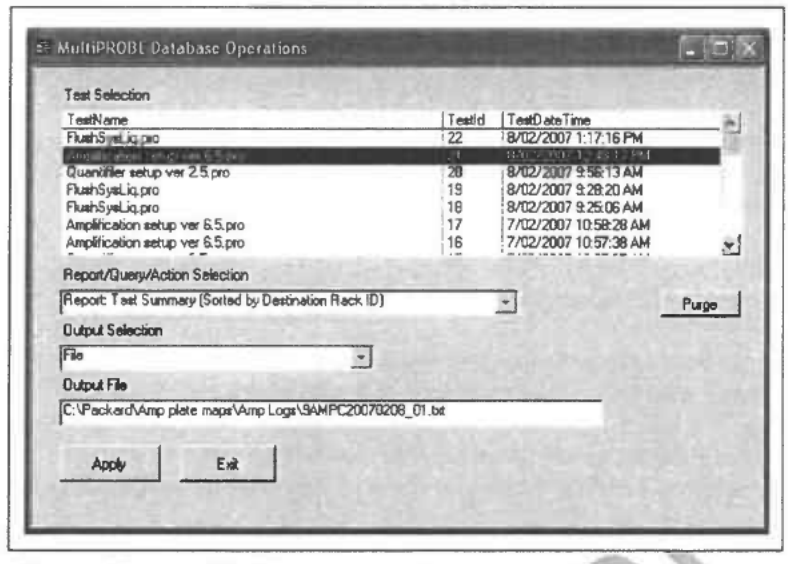

Figure 4. The MultiPROBE log database for collecting MP II run information

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

Importing Extraction "Results" into AUSLAB

59. Log into the **AUSLAB Main Menu**.
60. Select **5. Workflow Management**.
61. Select **2. DNA Batch Details.**
62. Scan the Extraction batch ID barcode located on the worksheet.
63. Press **[SF6]** ***Files***.
64. Press **[SF6]** ***Import Files***.
65. AUSLAB prompts "***Enter filename***"; enter batch name and extension and press **[Enter]**.(e.g. CWIQEXT20071115_01.txt)
66. AUSLAB prompts "***Is this a results file y/n?***" enter "**y**" and press **[Enter]**.
67. The file will be imported into AUSLAB and appear in the DNA file table.
68. Highlight entry and press **[Enter]**, for access to the DNA results table.
69. Page down through the table and check that all sample results have been imported.
70. Press **[SF8]** ***Table Sort Order***, this sorts the table so any samples that have failed Autovalidation are sorted to the top of the table.
71. For all samples that have failed check the **Processing Comments,** by entering into the sample.
72. **a)** If processing comments state sample is to be sent to another batch type **other** than quant. Return the sample to the correct next batch type – e.g. Microcon, NucleoSpin and pooling

b) Press **[Esc]** to exit back to the DNA results table.
c) Do not toggle accept.

73. **a)** If processing comment does not state next step for sample the sample will be processed as normal.
    b) Press **[Esc]** to exit back to the DNA results table.
    c) Highlight any entries to be changed and press **[SF7]** ***Toggle Accept***
74. Press **[F7]** ***Complete Batch***, all of the samples with a ***Yes*** in the Accept column will be transferred to the outstanding Quant Casework or Quant Reference lists.
75. File the Extraction worksheet into the relevant folder in Room 6117.

10 SAMPLE STORAGE

Please refer to "*Analytical Sample Storage*" (QIS 24255) for how to store the old original 5 mL sample tubes, the DNA extract NUNC tubes, ABgene 96-deep well and Axygen store plates.

11 TROUBLESHOOTING

1. If the barcode reader is not reading the barcodes of the Nunc tube rack, or the Slicprep Plate or the Store plate, manually scan the appropriate barcodes.
2. When reading the Nunc tube rack barcode, if the Gripper is not picking up or placing the Nunc tube rack properly on the deck, just manually place the rack properly on the plate adapter support tile.
3. When reading the Store plate barcode, if the Gripper is not picking up or placing the Store plate properly on the deck, just manually place the plate properly on the plate adapter support tile.
4. When reading the Slicprep plate barcode if the Gripper is not picking up the plate properly :
    a. if the plate was not properly placed on the plate adapter support tile with the Wallac Isoplate, just manually place the plate properly.
    b. if the plate was properly placed on the plate adapter support tile with the Wallac Isoplate on it: it means that the gripper needs to be initialised. Abort the run, Initialise the instrument and restart the run. If problem persists, shutdown the MPII and PC, restart and then initialise the whole instrument. Otherwise, contact your line manager.
    c. Calibrate relevant labware using the SlicPrep Calibration plate. This has preset standardised positions that need to be the same on all labware where the Slicprep plate is being moved. The same plate is used on both extraction platforms A and B.
    d. Check the calibrations against the run program DNAIQGripperTest.pro. This program moves the Slicprep across all the labware the gripper moves across. Start with the Slicprep at D16.

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

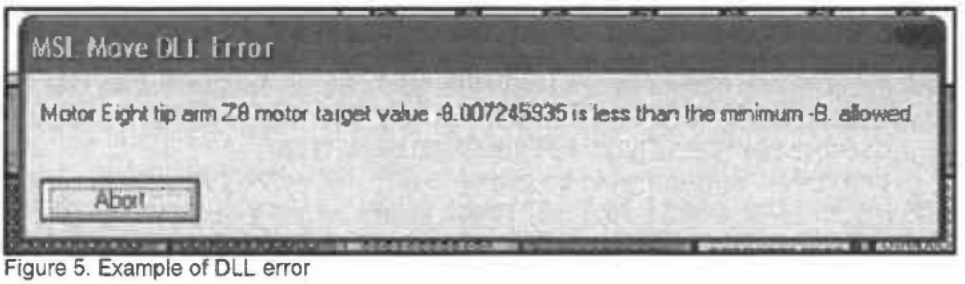

Figure 5. Example of DLL error

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

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

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

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

9. If the message Figure 6 below has appeared:

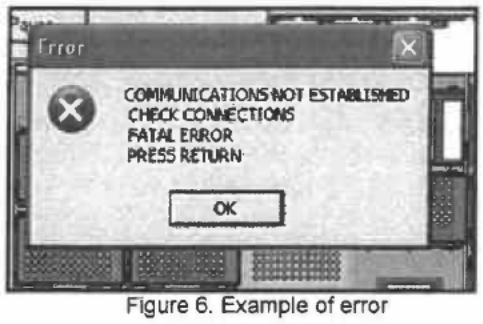

Figure 6. Example of error

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

12 VALIDATION

- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 9. Automation: Report on the Evaluation of DNA Extraction Chemistries." June 2007.
- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 11: Report on the Validation of the Automated Extraction Chemistry Kit using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Casework Platform." 2007.
- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 13: Report on the Verification of the Automated Extraction Chemistry Kit using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Casework Platform." 2007.
- Muharam I., Hlinka V., Gallagher, B., Lundie, G., Iannuzzi, C., Nurthen T., McNevin A., Ientile V. "Project 21: A Modified DNA IQ™ Method Consisting of Off-Deck Lysis to Allow Supernatant Retention for Presumptive Identification of α-Amylase" 2008
- Muharam I., Hlinka V., Gallagher, B., Lundie, G., Iannuzzi, C., Nurthen T., McNevin A., Ientile V. "Project 22: A Modified DNA IQ™ Method for Off-Deck Lysis Prior to Performing Automated DNA Extraction" 2008

13 QUALITY ASSURANCE/ACCEPTANCE CRITERIA

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

14 REFERENCES

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2. Chen., C.W.T.J., C.A., Recovery of DNA Segments from Agarose Gels. Anal Biochem., 1980. 101: p. 339-341.
3. Cowan, C., The DNA IQ™ System on the Tecan Freedom EVO® 100 Profiles in DNA. Profiles in DNA, 2006: p. 8-10.

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7. Mandrekar, P., V., Flanagan, L., & Tereba, A., Forensic Extraction and Isolation of DNA Form Hair, Tissue and Bone. Profiles in DNA, 2002: p. 11.
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10. Melzak, K.A., Sherwood, C.S., Turner, R.F.B. & Haynest, C.A., Driving forces for DNA Adsorption to Silica in Percholorate Solutions. J. Colloid. Interface Sci., 1996. 181: p. 635-644.
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15. Promega, DNA IQ™ System-Database Protocol. Promega Technical Bulletin #TB297, 2006. Rev 4/06: p. 1-14.
16. Promega, Tissue and hair Extraction Kit (for use with DNA IQ™) Protocol. Promega Technical Bulletin #TB307, 2006. Rev 5/06: p. 1-11.
17. Schiffner, L.A., Bajda, E. J., Prinz, M., Sebestyen, J., Shaler, R. & Caragine, T.A., Optimisation of a Simple, Automatable Extraction Method to Recover Sufficient DNA from Low Copy Number DNA Samples for Generation of Short Tandem Repeat Profiles. Croat Med J, 2005. 46(4): p. 578 -586.
18. Vandenberg, N., van Oorchot., R. A. H., & Mitchell, R. J., An evaluation of selected DNA extraction strategies for short tandem repeat typing. Electrophoresis, 1997. 18: p. 1624-1626.

15 STORAGE OF DOCUMENTS

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

16 ASSOCIATED DOCUMENTS

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

17 AMENDMENT HISTORY

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

		G. Lundie, I Muharam.	
1	12 Dec 2007	M Harvey, C Iannuzzi, A McNevin	Reviewed and updated after initial training
2	19 March 2008	M Harvey, B Andersen, C Iannuzzi, A McNevin	Addition of Off-deck Lysis procedure, Retention of fully automated method as Appendix, addition of reagent record tables into Appendix

18 APPENDIX

18.1 Reagents Calculation Tables

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

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

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

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

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

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

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

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

Lysis batch:

Samples located by:	
For samples 1-48	**For samples 49-96**
Operator:	Operator:
Sequence check performed by:	Sequence check performed by:
Transfer tubes sequence checked:	Transfer tubes sequence checked:

Extraction Buffer made by:	TNE Buffer Lot#:
20% SDS Lot#:	Proteinase K Lot#:
Comments:	

Extraction batch:

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

MultiPROBE Platform:	Operator:
Date and Start time:	

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

18.3 Fully automated method for extraction using DNA IQ™

18.3.1 Sampling and Sample Preparation

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

18.3.2 Procedure

Preparation of Reagents prior to extraction

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

Setting up the EP-A or EP-B MPIIs

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

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

Figure 7 The WinPrep® icon.

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

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

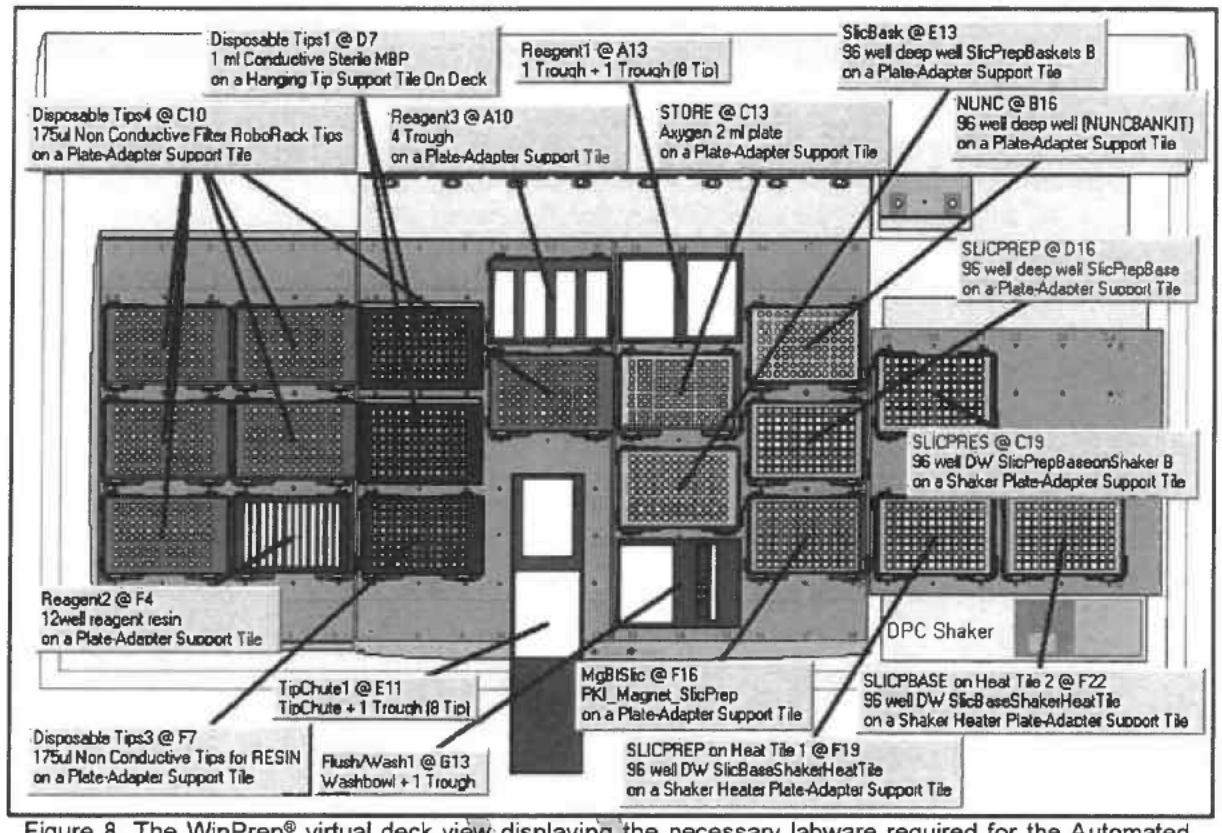

Figure 8. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Finalising the MP II run

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

Recording Reagent Details and other information in AUSLAB

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

Importing the MP II log file into AUSLAB

48. To extract the MP II log file, click on the Microsoft Access icon in the WinPrep® main menu to open the MultiPROBE log database.
49. Click on the relevant run Test ID in the Test Selection box. In the Report/Query/Action Selection dropdown menu, select "Report: Test Summary (Sorted by Destination Rack ID)"
50. In the Output Selection dropdown menu, select "File". Save the output file as *.csv format to **C:\PACKARD\EXT PLATE MAPS\EXT LOGS** with the same name as the AUSLAB batch ID and click "**Apply**". (refer to Figure 9. below)

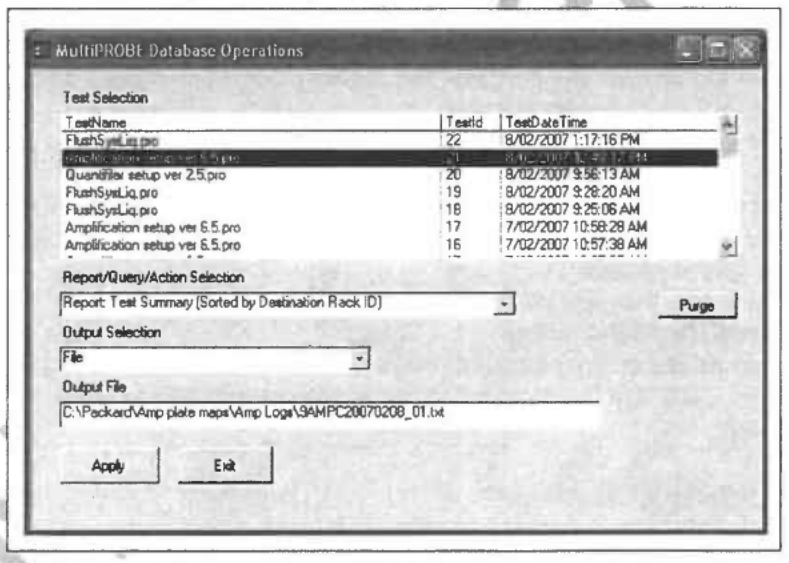

Figure 9. The MultiPROBE log database for collecting MP II run information

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

Importing Extraction "Results" into AUSLAB

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

18.3.3 Sample Storage

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

18.4 Manual method for extraction using DNA IQ™

18.4.1 Sampling and Sample Preparation

Samples waiting to be extracted are stored in freezers as described in Table 9.

Table 9. Sample storage locations.

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

QC samples

All extraction batches require two controls to be registered. These controls, UR numbers and positions are listed below in Table 10.

Table 10. Extraction Quality Controls

QC	UR Number	Extraction types
Neg Control	FBOT277	All
QC swab (blood)	FBOT279	Blood

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

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

Create the Extraction Batch

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

Locating Samples

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

When all samples have been located:

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

Sequence Check the tubes

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

18.4.2 Procedure

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

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

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

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

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

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

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

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

9. Add 500 µL of Lysis Buffer to each tube.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

24. Repeat step 20 to 22. The final volume after this elution should be approximately of 95 µL of DNA solution.

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

18.4.3 Sample storage

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

BG-04

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

1 PURPOSE AND SCOPE

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

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

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

2 DEFINITIONS

Samples	Samples awaiting DNA extraction
Lysates	Samples that have had the Lysis step performed, but have not yet completed the entire extraction process
DNA Extracts	Samples that have had a DNA extraction processes performed
DNA IQ™ Resin	Magnetic Resin Beads used to bind DNA
MP II	MultiPROBE® II PLUS HT EX Platform
DTT	1,4 Dithiothreitol
Pro K	Proteinase K
Sarcosyl	N-Lauroylsarcosine sodium
TNE	Tris, NaCl and EDTA Buffer
EDTA	Ethylenediaminetetraacetate
EP-A	Extraction Platform A
EP-B	Extraction Platform B

3 PRINCIPLE

Sample Pre-lysis

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

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

DNA IQ™ Kit

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

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

- The increase of Extraction Buffer volume to 500µL for use with the Slicprep™ 96 device.
- The use of tubes and spin-baskets for the off-deck lysis of samples prior to extraction on MPII. Use of a 96-deepwell plate for completion of extraction on MPII.
- The provision of initial incubation with TNE Buffer and retention of a portion thereof for further testing (retained supernatant testing).
- The increase of Lysis Buffer volume to 957µL proportional to the increase of Extraction Buffer volume, according to the manufacturer's instructions.
- Double Elution step, with an Elution Buffer volume of 60µL for a final volume of 100µL.
- The use of Nunc™ Bank-It™ tubes for storage of final extracts.

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

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

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

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

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

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

MultiPROBE® II HT EX PLUS with Gripper™ Integration Platform

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

sample probe is capable of independent motion in the Z direction due to independent Z drives.

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

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

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

4 REAGENTS AND EQUIPMENT

4.1 Reagents

1. DNA IQ™ System Kit – 400 sample Kit
   - Resin
   - Lysis Buffer (LB)
   - 2x Wash Buffer (2xWB)
   - Elution Buffer (EB)
2. Tris/Sodium chloride/EDTA Buffer (TNE)
3. Proteinase K (Pro K) 20mg/mL
4. Dithiothreitol (DTT) 1M
5. 5% TriGene
6. 70% Ethanol
7. 1% Amphyl
8. 0.2% Amphyl
9. Isopropyl alcohol
10. AnalR 100 %Ethanol
11. 40% Sarcosyl
12. Decon® 90 solution
13. Nanopure H_2O

Table 1. Reagent storage locations.

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

Please see Table 2 for the volume of reagents for a full plate or half plate. Refer to "*Receipt, Storage and Preparation of Chemicals, Reagents and Kits*" (QIS 17165) for

preparation of the TNE Buffer. All reagents can be made on the bench in Room 6122, except for the Lysis Buffer with DTT which needs to be made in a fume hood. DNA IQ reagents are prepared by staff performing the DNA IQ method.

Table 2. Table of reagent volumes.

Reagent (volume per sample)	Constituent (volume per sample)	Volume req'd for 96 Samples (mL)	Volume req'd for 48 Samples (mL)
Extraction Buffer (500 µL/sample	TNE Buffer 462.5µL	54	27
	Prot K (20 mg/mL)25.0 µL	2.9	1.5
	Sarcosyl (40 %) 12.5µL	1.5	0.7
Lysis Buffer (with DTT) (1.127mL/sample)	Lysis Buffer (no DTT)	130	66
	DTT (add to Lysis Buffer)	1.3	0.66
Lysis Buffer (with DTT) Reagent Trough	As above	125	63
DNA IQ RESIN Solution (50µL/sample)	Lysis Buffer (with DTT) (from above) 43µL	5.536	3
	DNA IQ RESIN 7µL	0.901	0.5
DNA IQ 1X Wash Buffer (300µl/sample)	See Below for preparation	35	18
DNA IQ Elution Buffer (120µl/sample)	Use directly from Kit	14	8

NOTE: For batches not equal to either 96 or 48 samples samples, refer to Appendix 1 "*18.1 Reagents Calculation Tables*" Table 7 for batches of <48 samples, and Table 8 for <96 (but >48)

Extraction Buffer

Prepare the buffer just before extraction in a 100mL sterile glass bottle according to Table 2. Use one aliquot of 20mg/mL Proteinase K (1.5mL) for a 48 sample run and two aliquots for a 96 sample run. Remove aliquot/s of Proteinase K from the freezer, defrost, vortex and centrifuge before use. Ensure that the 40% (w/v) Sarcosyl is completely dissolved (clear) in the stock solution before making the Extraction Buffer. If not dissolved, invert the container a few times and leave longer at room temperature.

Lysis Buffer with DTT

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

DNA IQ™ Resin

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

1X Wash Buffer

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

4.2 Equipment

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

Table 3. Equipment used and location.

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

Table 4. Consumables used for extraction

Consumables	Location
175uL Clear Non-Conductive Filter Tips – Pre-sterilized	6127
MβP Pure 1000uL Tips – Pre-Sterilized	6127
SlicPrep™ 96 device plate	6122
ABgene 96-deepwell plate	6120
Axygen 2mL Deep Well storage plate	6127
1.5ml or 2ml Eppendorf tubes with Spin baskets	6120
12 Channel plate	6127
Nunc™ Bank-it™ tubes	6120
Nunc™ Bank-it™ Caps	6127
Sterile 50mL Falcon tubes	6122
Sterile 10mL tubes	6122
Autoclaved 100mL glass bottles	6122
Autoclaved 250mL glass bottles	6122
Aluminium sealing film	6127
1000uL disposable tips	6120

5 SAFETY

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

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

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

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

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

6 SAMPLING AND SAMPLE PREPARATION

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

Table 5. Sample storage locations.

Sample type	Storage Device	Storage Location
Urgent/High/Medium Priority Samples	Freezer	6117-2
Medium Priority Samples	Walk in Freezer or Freezer in 6117	6109 or 6117-5
Low Priority Samples	Walk in Freezer or Freezer in 6117	6109 or 6117-5
Lysates in 1.5ml tubes	Fridge	6120
96 deep well plate containing lysates	Fridge	6127

QC samples

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

Table 6. Extraction Quality Controls

QC Name	UR Number	Description
Negative Control	FBOT277	Negative Extraction control – Empty well
Positive Control	FBOT279	Positive Extraction control – Known Donor dried blood swab

Registration of QC

1. Log into the **AUSLAB Main Menu.**
2. Select **1. Request Registration.**
3. Select **2. Full Reception Entry.**
4. Scan in barcode of control.
5. Enter the UR number as per Table 6 and press **[Enter]**.
6. Enter the appropriate Specimen type (e.g. **Blood** for blood control).
7. Request a **9PLEX** test, when prompted to enter the processing comment, enter **EXTP** (Positive extraction control) or **EXTN** (Negative extraction control). Do no assign a priority.
8. Press **[F7]** ***Save*** to save the Billing details.
9. Enter **LAB** in the Billing code field and **t** in the date field and **FBQC** in the Loc/Client field.
10. Press **[F4]** ***Save twice*** to save the registration details.

Note 1: Quality controls should not have a DNA priority allocated at time of registration to ensure they are included in the top positions of a batch

Note 2: for DNA IQ Lysis batches with more than 46 samples (excluding controls) two sets of controls should be registered

Create the DNA IQ Lysis or Retain Supernatant Batch (as required)

1. Log into the **AUSLAB Main Menu.**
2. Select **5. Workflow management.**
3. Select **1. DNA workflow table.**
4. Highlight the appropriate batch type and press **[F5]** ***Batch Allocation***.
5. Press **[F6]** ***Create batch***.
6. Press **[F8]** ***Print menu***.
7. Press **[F7]** ***Print Sample Label***. (print 3 sets)
8. Press **[F8]** ***Print Worksheet***. (print 2 copies)
9. Press **[SF5]** ***Main menu***.

10. Press **[SF11]** ***Print***.
11. Press **[SF6]** ***Accept batch***.
12. Press **[Pause/Break]** to exit to the **Main Menu**.
13. Obtain worksheets **(FBLASER3)** and labels **(FBLABEL13-16)** from the Analytical Section printing bench **(Room 6117)**.

Locating Samples

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

7 OFF-DECK LYSIS PROCEDURE (NO RETAINED SUPERNATANT)

1. Print or obtain a copy of Appendix 2. "*18.2 Reagent & Batch details recording tables (DNA IQ™ Lysis Batch & Extraction Batch)*".

2. Separate the batch into two smaller batches of 48 samples, including one set of controls. (If only a single operator the second batch can be started during step 11)

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

3. Remove samples from 5mL storage tubes and label side with barcodes if necessary. If substrates are in a 0.5mL tube, transfer to a labelled 1.5mL tube. Label lid of each sample with position number within the lysis batch. Retain the original 5mL storage tube for substrate storage.

4. Prepare / assemble spin basket assembly or 1.5mL (or 2.0mL) tube and label side with a barcode and lid with position number as required.

Note: substrates from each sample need to be retained

   a. Samples requiring a spin basket include swabs, fabric, paper and cigarette butts.
   b. Samples requiring a 1.5mL include tapelifts, chewing gum, straws, fingernail clippings and toothbrush bristles.

5. Label the side of sterile 1.0mL Nunc™ Bank-It™ tubes with barcode.

6. Have a second operator sequence check all tubes and complete the sequence check in AUSLAB.

7. Prepare Extraction Buffer.

8. Using a multi-stepper pipette add 500µL of Extraction Buffer and vortex briefly. Ensure substrate is fully immersed in extraction buffer.

9. Incubate in a hotblock at 37°C for 45minutes (note temperature on worksheet).

10. Remove samples from hot block and vortex briefly then return to rack.

11. Transfer substrates to spin baskets if required using twirling sticks (if unable to remove with twirling sticks, use forceps. Forceps must be cleaned between each sample by rinsing in bleach followed by ethanol and flaming).

12. For samples not requiring spin baskets, transfer the lysate to the newly labeled 1.5mL tube. Then store original 1.5mL containing substrate in the original 5mL tube.

13. Centrifuge spin baskets at maximum speed (14000rpm) for 2 minutes.

14. Transfer flow through from spin baskets back to original lysis tube, retain spin basket in 5mL tube.

15. Vortex Lysates briefly, then incubate in hotblock at 65°C for 10 minutes (note temperature on worksheet)

16. Enter reagent details, temperatures etc. into AUSLAB.

17. Complete batch in AUSLAB.

18. Store lysates at 4°C (fridge in 6120).

19. Enter into completed batch in AUSLAB and determine the DNA IQ extraction batch ID the DNA lysates have progressed to.

20. Print the appropriate worksheets and 3 sets of batch labels for the DNA IQ extraction batch and note the lysis batch the samples have progressed from on the worksheets. Stamp as "Urgent" if necessary.

8 OFF-DECK LYSIS PROCEDURE (RETAINED SUPERNATANT)

1. Print or obtain a copy of Appendix 2. "*18.2 Reagent & Batch details recording tables (DNA IQ™ Lysis Batch & Extraction Batch)*".

2. Remove samples from 5mL storage tubes and label side with barcodes if necessary. If substrates are in a 0.5mL tube, transfer to a labelled 1.5mL tube. Label lid of each sample with position number. Retain 5mL tube for substrate storage.

3. Label the side of 1.5mL tubes with barcodes for retaining supernatant. Also label lid of 1.5mL tube indicating it contains supernatant.

4. Prepare spin basket assembly or a 1.5mL (or 2.0mL) tube and label side with a barcode and lid with position number as required. **Note:**
   a. Samples requiring a spin basket include swabs, fabric, paper and cigarette butts.
   b. Samples requiring a 1.5mL include tapelifts, chewing gum, straws, fingernail clippings and toothbrush bristles.

5. Label the side of sterile 1.0mL Nunc™ Bank-It™ tubes with barcode.

6. Have a second operator sequence check all tubes and complete the sequence check in AUSLAB.

7. Using a pipette add 650µL of TNE Buffer and vortex briefly.

8. Incubate at room temperature for 30 minutes.

9. Vortex, then centrifuge for 3 minutes at maximum speed (14000rpm).

10. Remove 150µL of supernatant and place into labelled 1.5ml tube (for further testing).

11. Add 25µL of 20ng/µL (mg/mL) Proteinase K and 12.5µL 40% (w/v) Sarcosyl to each original sample tube containing TNE Buffer. Vortex briefly.

12. Incubate in hotblock at 37°C for 45 minutes (note temperature on worksheet).

13. Remove samples from hotblock, vortex briefly and return to rack.

14. Transfer substrates to spin baskets if required using twirling sticks (if unable to remove with twirling sticks, use forceps. Forceps must be cleaned between each sample by rinsing in bleach followed by ethanol and flaming).

15. For samples not requiring spin baskets, transfer the lysate to the newly labeled 1.5mL tube. Then store original 1.5mL containing substrate in the original 5mL tube.

16. Centrifuge spin baskets at maximum speed (14000rpm) for 2 minutes.

17. Transfer flow through from spin baskets back to original lysis tube, retain spin basket in 5mL.

18. Vortex Lysates briefly, then incubate in hotblock at 65°C for 10minutes (note temperature on worksheet).

19. Enter reagent details, temperatures etc. into AUSLAB.

20. Complete batch in AUSLAB.

21. Store supernatants in Freezer 6117-2 (-20°C).

22. Store lysates at 4°C (Fridge in 6120).

23. Enter into completed batch in AUSLAB and determine the DNA IQ extraction batch ID the DNA lysates have progressed to.

24. Print the appropriate worksheets and 3 sets of batch labels for the DNA IQ extraction batch and note the retained supernatant batch the samples have progressed from on the worksheets. Stamp as "Urgent" if necessary.

9 MPII EXTRACTION PROCEDURE

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

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

Summary of DNA IQ EXTRACTION Version 4.1 ODL

1. ***Binding of paramagnetic resin to DNA and further Lysis:*** add Resin solution (50µL) and Lysis Buffer (957µL). Automated mixing and shaking at room temperature for 5 minutes. (this occurs at steps 10-15 of the protocol)

2. ***Removing lysis reagents:*** Slicprep plate is moved to the PKI Magnet to separate beads. Removing of supernatant (1600µL) without disturbing resin, dispense this solution in the storage plate. (this occurs at steps 16-18 of the protocol)

3. ***Washing of the resin-DNA complex:*** To remove any inhibitors in solution. The first wash is with Lysis Buffer (125µL), shaking at room temperature for 1 minute. The plate is moved to the PKI Magnet and the supernatant is removed into the storage plate. The next three washes are with 1X Wash Buffer (100µL), shaking at room

temperature for 1 minute. During each wash cycle, the plate is moved to the PKI Magnet and the supernatant is discarded. (this occurs at steps 21-59 of the protocol)

4. ***Removing any excess of 1X Wash Buffer:*** air dry at room temperature for 5 minutes. (this occurs at step 60 of the protocol)

5. ***Elution of DNA from the Resin-DNA complex:*** Add Elution Buffer (60µL) and incubate at 65 °C for 6 minutes (3 minutes no shaking and 3 minutes shaking). The plate is moved to the PKI Magnet. The eluted solution (supernatant) is removed to the Nunc™ Bank-It™ tubes. Elution is repeated twice. (this occurs at steps 63-83 of the protocol)

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

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

To sequence check storage tubes and transfer DNA lysates to ABgene 96-deep well plates, please refer to method "*Procedure for the Use of the STORstar unit for automated sequence checking*" (QIS 24256).

ENSURE the Nunc™Bank-It™ tube rack is labelled with the AUSLAB Batch ID and barcode on the right hand side of the plate.

Preparation of Reagents & Lysates prior to extraction

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

2. Record the Lot numbers of all the reagents used onto the worksheet (printed from appendix 2 and in the AUSLAB batch audit entry.

3. Remove the deep well plate containing Lysates from either storage (either freezer or fridge as case may be) to allow to come to room temperature before starting extraction procedure.

Setting up the EP-A or EP-B MPIIs

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

4. Turn on the instrument PC.

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

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

Figure 1 The WinPrep® icon.

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

8. Ensure the **System Liquid reservoir is FULL** and tubing is fully submerged in the system liquid before every run and perform a Flush/Wash.

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

10. Open the Extraction setup MP II test file in WinPrep® by selecting:
    - **File**
    - **Open**, navigate to **C:\PACKARD\MULTIPROBE\BIN\QHSS PROTOCOLS**
    - Select "**DNA IQ Extraction_Ver 4.1_ODL.mpt**"
    - Click the "**Open**" button

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

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

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

    - The white WALLAC Isoplates (catalogue #1450-514) that are used to support the ABgene 96-deep well plate must be placed into positions **D16** and **C19**.

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

    - Ensure the DPC shaker is positioned properly

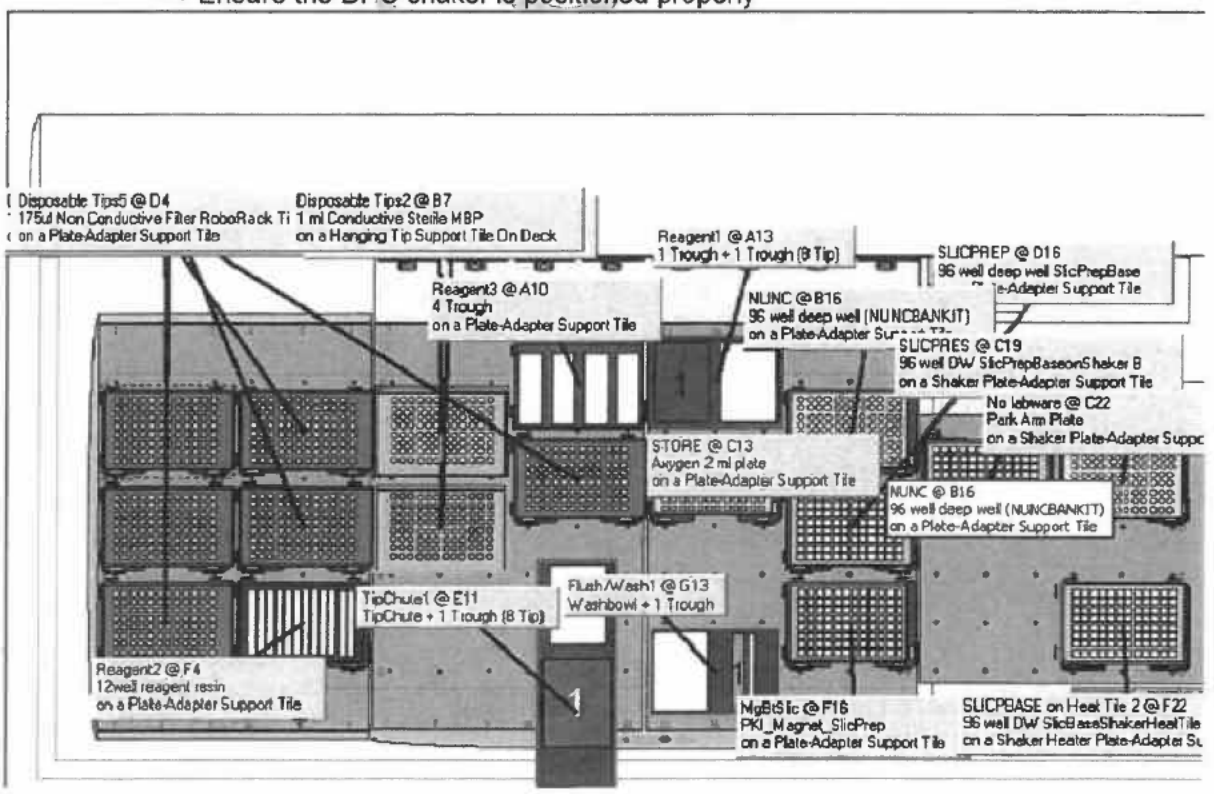

Figure 2. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

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

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

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

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

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

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

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

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

22. Message will appear (Figure 3 below):

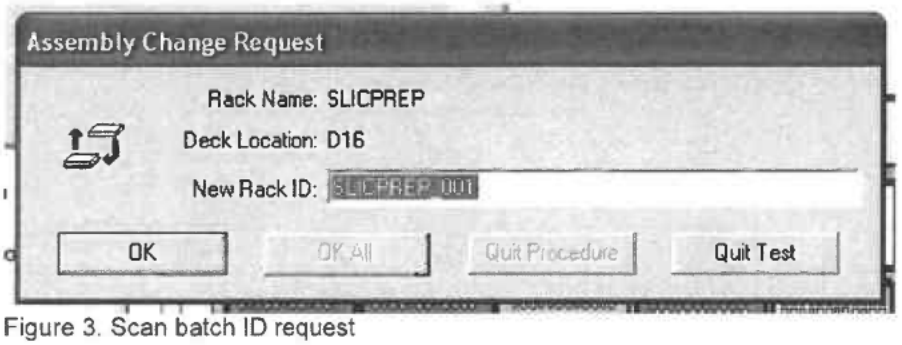

Figure 3. Scan batch ID request

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

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

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

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

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

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

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

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

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

31. After the first elution step when the plate is moved to the PKI Magnet, a User message will appear requesting:
"**Push down the plate on the PKI Magnet, Check Nunc™ Bank-It™ tubes are uncapped at position B16, then press OK.**"
Note: Allow the plate to cool slightly before pushing down on all four corners of the plate to lock it into place on the PKI Magnet.
Once this is done, do not open the cabinet doors unless absolutely necessary as this will increase the speed of cooling and DNA may re-bind to the resin.

32. After the second elution step, the above prompt will appear again. **Note:** Allow the plate to cool slightly before pushing down on all four corners of the plate to lock it into place on the PKI Magnet
Once this is done, do not open the cabinet doors unless absolutely necessary as this will increase the speed of cooling and DNA may re-bind to the resin.

33. Once the program is completed, a final User Message prompt appears asking to:
"**Remove plates and cover them with aluminium sealing film. Remove Nunc rack and recap Nunc tubes.**"

Once all plates are removed from the deck and sealed, place into small clipseal plastic bags. Click "**OK**" to proceed to the Amphyl wash step to decontaminate the system tubing. **Note:** review the supernatant storage plate for the transfer of beads.

Finalising the MP II run

34. Remove the resin-Lysis-DTT solution from the 12 channel plate in the glass Lysis-DTT bottle used. Discard the plate in the biohazard waste bin.

35. Remove Lysis Buffer with DTT (wear face shield) and dispose of left over reagent into the glass bottle used previously. Bring this bottle to the room 6122 and dispose in the brown Winchester bottle located in the fume hood.

36. Empty the contents of the Amphyl wash station into the sink and rinse the troughs with nanopure H_2O upon completion of the run.

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

38. Remove the tip chute and funnel, rinse with warm tap water to help loosen and remove any residue inside the chute before cleaning with 5% TriGene and 70% ethanol. Hang the chute to dry on the rack beside the sink. If running another extraction straight after, replace the tip chute with another spare, clean chute.

39. Move the platemap to **C:\PACKARD\EXT PLATE MAPS** to the "**Completed Extractions**" folder.

Recording Reagent Details and other information in AUSLAB

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

Importing the MP II log file into AUSLAB

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

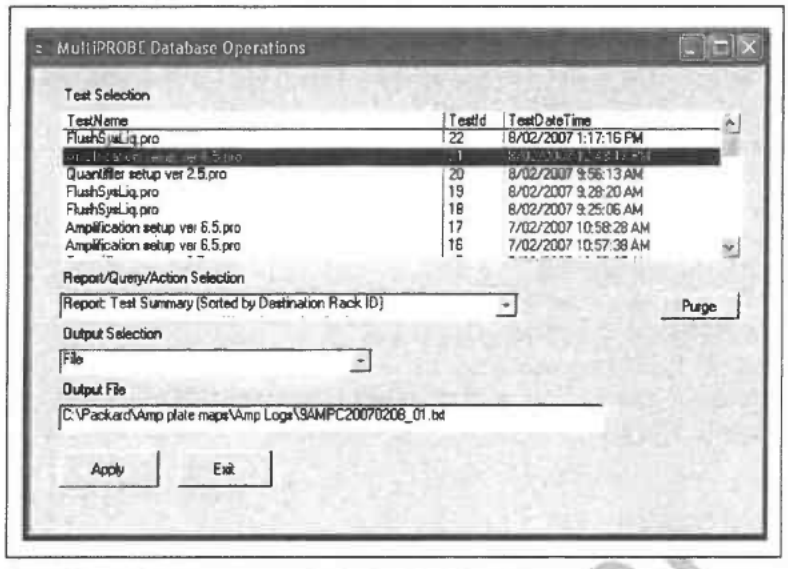

Figure 4. The MultiPROBE log database for collecting MP II run information

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

Importing Extraction "Results" into AUSLAB

61. Log into the **AUSLAB Main Menu**.
62. Select **5. Workflow Management.**
63. Select **2. DNA Batch Details.**
64. Scan the Extraction batch ID barcode located on the worksheet.
65. Press **[SF6]** ***Files***.
66. Press **[SF6]** ***Import Files***.
67. AUSLAB prompts "***Enter filename***"; enter batch name and extension and press **[Enter]**.(e.g. CWIQEXT20071115_01.txt)
68. AUSLAB prompts "***Is this a results file y/n?***" enter "**y**" and press **[Enter]**.
69. The file will be imported into AUSLAB and appear in the DNA file table.
70. Highlight entry and press **[Enter]**, for access to the DNA results table.
71. Page down through the table and check that **all** sample results have been imported.
72. Press **[SF8]** ***Table Sort Order***, this sorts the table so any samples that have failed Autovalidation are sorted to the top of the table.
73. For all samples that have failed check the **Processing Comments,** by entering into the sample.
74. **a)** If processing comments state sample is to be sent to another batch type **other** than quant. Request the appropriate rework test code via the SF7 results history table and

the SF8 request rework functions (e.g. samples requiring Microcon, NucleoSpin and pooling)
b) Press **[Esc]** to exit back to the DNA results table.
c) Do not toggle accept.
d) add the extraction batch ID into the 9PLEX or 9FTAR completed date fields in AUSLAB

75. **a)** If processing comment does not state next step for sample the sample will be processed as normal.
b) Press [Esc] to exit back to the DNA results table.
c) Highlight any entries to be changed and press [SF7] Toggle Accept
76. Press **[F7]** ***Complete Batch***, all of the samples with a ***Yes*** in the Accept column will be transferred to the outstanding Quant Casework or Quant Reference lists.
77. File the Extraction worksheet into the relevant folder in Room 6117.

10 SAMPLE STORAGE

Please refer to "Analytical Sample Storage" (QIS 24255) for how to store the old original 5 mL sample tubes, the DNA extract Nunc™ Bank-It™ tubes, ABgene 96-deep well and Axygen store plates.

11 TROUBLESHOOTING

1. If the barcode reader is not reading the barcodes of the Nunc™ Bank-It™ tube rack, or the Slicprep Plate or the Store plate, manually scan the appropriate barcodes.
2. When reading the Nunc™ Bank-It™ tube rack barcode, if the Gripper is not picking up or placing the Nunc™ Bank-It™ tube rack properly on the deck, just manually place the rack properly on the plate adapter support tile.
3. When reading the Store plate barcode, if the Gripper is not picking up or placing the Store plate properly on the deck, just manually place the plate properly on the plate adapter support tile.
4. When reading the Slicprep plate barcode if the Gripper is not picking up the plate properly :
    a. if the plate was not properly placed on the plate adapter support tile with the Wallac Isoplate, just manually place the plate properly.
    b. if the plate was properly placed on the plate adapter support tile with the Wallac Isoplate on it: it means that the gripper needs to be initialised. Abort the run, Initialise the instrument and restart the run. If problem persists, shutdown the MPII and PC, restart and then initialise the whole instrument. Otherwise, contact your line manager.
    c. Calibrate relevant labware using the SlicPrep Calibration plate. This has preset standardised positions that need to be the same on all labware where the Slicprep plate is being moved. The same plate is used on both extraction platforms A and B.
    d. Check the calibrations against the run program DNAIQGripperTest.pro. This program moves the Slicprep across all the labware the gripper moves across. Start with the Slicprep at D16.

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

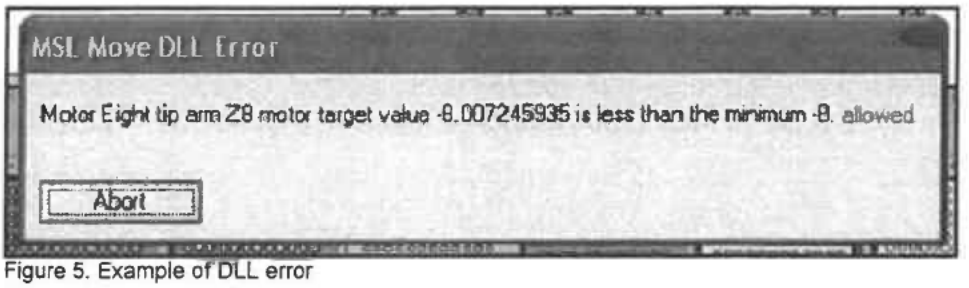

Figure 5. Example of DLL error

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

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

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

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

9. If the message Figure 6 below has appeared:

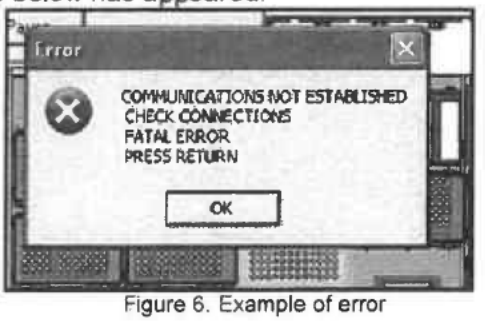

Figure 6. Example of error

press OK and the program will be aborted automatically. Check that all the connections to the instrument (DPC shaker, heater and computer) are properly plugged in. If

everything is OK, you need to close WinPrep, shut down the instrument, DPC shaker, heater and PC. After 2 min restart everything. Once Winprep has been opened, reinitialise the instrument and start the program (check version number according to which step the message has came up). Please read troubleshooting 5 for barcode reading of plates.

12 VALIDATION

- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 9. Automation: Report on the Evaluation of DNA Extraction Chemistries." June 2007.
- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 11: Report on the Validation of the Automated Extraction Chemistry Kit using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Casework Platform." 2007.
- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 13: Report on the Verification of the Automated Extraction Chemistry Kit using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Casework Platform." 2007.
- Muharam I., Hlinka V., Gallagher, B., Lundie, G., Iannuzzi, C., Nurthen T., McNevin A., Ientile V. "Project 21: A Modified DNA IQ™ Method Consisting of Off-Deck Lysis to Allow Supernatant Retention for Presumptive Identification of α-Amylase" 2008
- Muharam I., Hlinka V., Gallagher, B., Lundie, G., Iannuzzi, C., Nurthen T., McNevin A., Ientile V. "Project 22: A Modified DNA IQ™ Method for Off-Deck Lysis Prior to Performing Automated DNA Extraction" 2008

13 QUALITY ASSURANCE/ACCEPTANCE CRITERIA

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

14 REFERENCES

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3. Cowan, C., The DNA IQ™ System on the Tecan Freedom EVO® 100 Profiles in DNA. Profiles in DNA, 2006: p. 8-10.
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8. Mandrekar, P.V., Kreneke, B. E., & Tereba, A., DNA IQ™: The Intelligent Way to Purify DNA. Profiles in DNA, 2001: p. 16.

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

15 STORAGE OF DOCUMENTS

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

16 ASSOCIATED DOCUMENTS

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

17 AMENDMENT HISTORY

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

18 APPENDIX

18.1 Reagents Calculation Tables

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

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

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

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

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

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

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

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

Lysis batch:

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

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

Extraction batch:

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

MultiPROBE Platform:	Operator:
Date and Start time:	

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

18.3 Fully automated method for extraction using DNA IQ™

18.3.1 Sampling and Sample Preparation

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

18.3.2 Procedure

Preparation of Reagents prior to extraction

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

Setting up the EP-A or EP-B MPIIs

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

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

Figure 7 The WinPrep® icon.

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

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

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

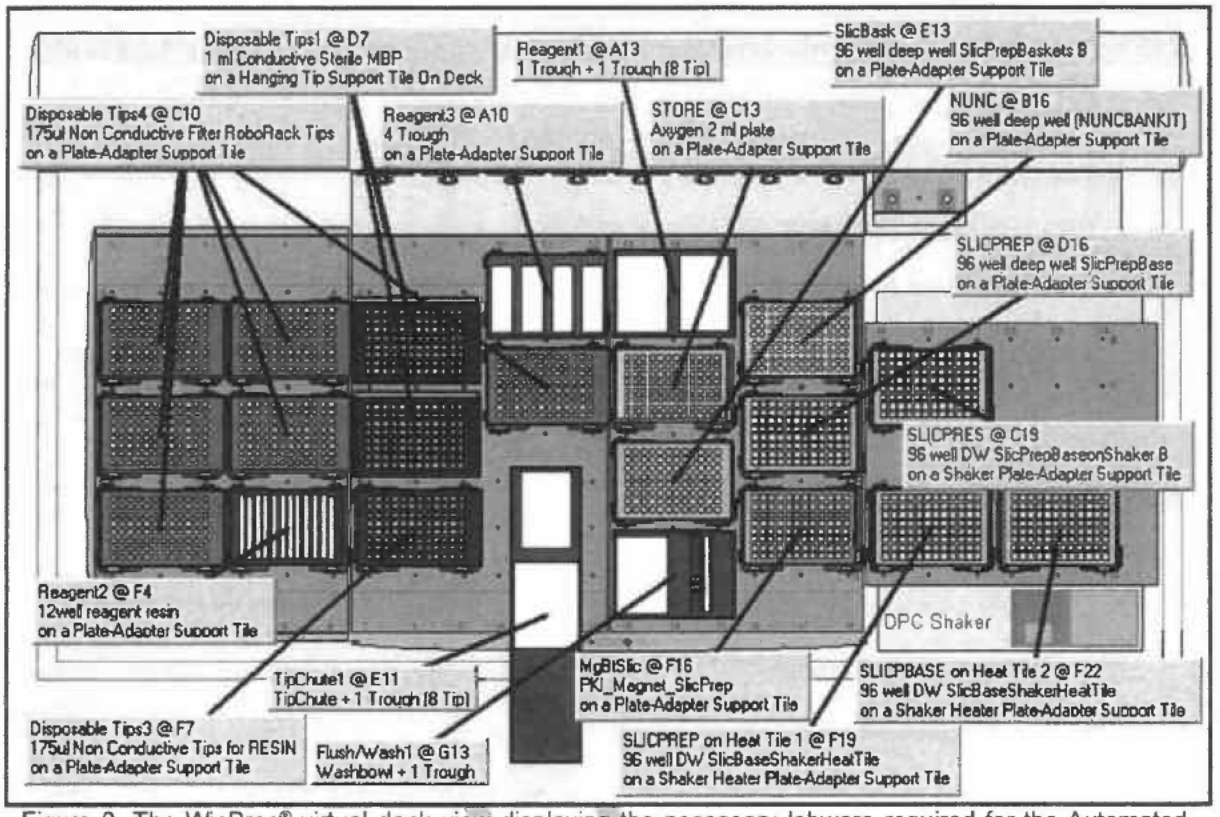

Figure 8. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Finalising the MP II run

35. Discard the 12-channel plate used for Resin and Elution Buffer in the biohazard waste bin.
36. Remove Lysis Buffer with DTT 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)

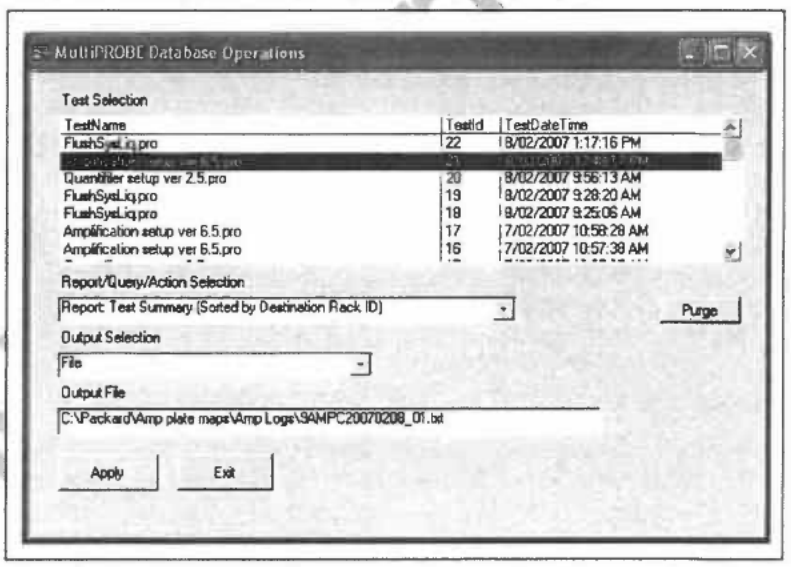

Figure 9. The MultiPROBE log database for collecting MP II run information

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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Project 13. Report on the Verification of an Automated DNA IQ™ Protocol using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform

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

1. Abstract

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

2. Introduction

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

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

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

3. Aim

To verify an automated DNA IQ™ protocol for use on the MultiPROBE® II PLUS HT EX platforms to allow extraction of DNA from various sample types.

4. Equipment and Materials

- MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (PerkinElmer, Downers Grove, IL, USA)
- Gravimetric Performance Evaluation Option with Mettler SAG285/L balance (Mettler-Toledo, Greifensee, Switzerland)
- DNA IQ™ System (Promega Corp., Madison, WI, USA)
- Extraction Buffer (10mM Tris, 1mM EDTA, 100mM NaCl, 20% SDS)
- SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA)
- Nunc™ Bank-It tubes (Nunc A/S, Roskilde, Denmark)
- 175µL non-conductive sterile filter RoboRack tips (PerkinElmer, Downers Grove, IL, USA)
- 1000µL Conductive sterile filter Robotix tips (Molecular BioProducts, San Diego, CA, USA)
- ABI Prism® 7000 SDS (Applied Biosystems, Foster City, CA, USA)
- Quantifiler™ Human DNA Quantification kits (Applied Biosystems, Foster City, CA, USA)
- AmpFℓSTR® Profiler Plus Amplification kits (Applied Biosystems, Foster City, CA, USA)
- GeneAmp® 9700 thermalcycers (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 96-well optical reaction plates (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA)
- GeneScan™ 500 ROX™ Size Standard (Applied Biosystems, Foster City, CA, USA)
- Hi-Di™ Formamide (Applied Biosystems, Foster City, CA, USA)
- 3100 POP-4™ Polymer (Applied Biosystems, Foster City, CA, USA)
- Cytobrush® Plus Cell Collector (Cooper Surgical, Inc., Trumbull, CT, USA)
- 0.9% saline solution (Baxter Healthcare, Old Toongabbie, NSW, Australia)
- Stem digital tilting head thermometer
- For mock samples:
  - FTA™® Classic Card (Whatman Inc., Florham Park, NJ, USA)
  - Sterile cotton swabs (Medical Wire & Equipment, Corsham, Wiltshire, England)
  - Sterile rayon swabs (Copan Italia SPA, Brescia, Italy)

5. Methods

5.1 Gravimetric Evaluation of Pipetting Accuracy and Precision

Gravimetric analysis was performed by placing the SAG285/L balance on the platform deck and instructing the MP II to repeatedly pipette certain volumes of system liquid onto the balance pan. Readings were taken automatically by the software and compiled into a results table, which was then used to automatically generate an Excel-based results chart containing mean, %CV and %inaccuracy values. The mean values obtained were used to calculate R^2, slope and Y-intercept (offset) values to calibrate the system's pipetting.

Pipetting performance was assessed for various volumes using three different tips in order to calculate appropriate R^2, slope and Y-intercept (offset) values which were then added to the performance file. Values were calculated for both Blowout (single-liquid transfer) and Waste (multidispense) modes for the 1000µL conductive tips, and Blowout mode only for the 175µL non-conductive tips and fixed tips.

For the addition of resin, a specialised performance file was created based on the performance file for 175µL tips in blowout mode, except the "Blowout Volume" column values were set to 0 to allow pipetting performance that is similar to waste mode. Retesting was performed to confirm accurate and precise pipetting with these settings.

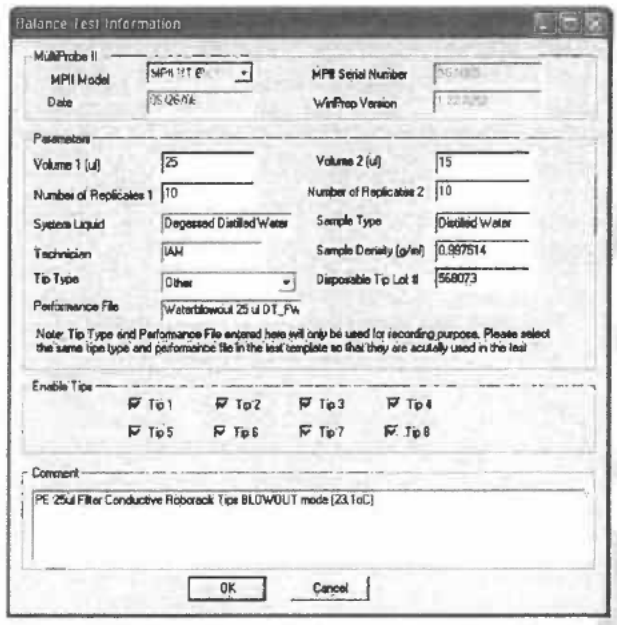

Figure { SEQ Figure * ARABIC }. The Balance Test Information Window as present within the Balance Test DT program. All pipetting parameters are entered here and are subsequently transferred to the result output file.

All gravimetric testing was performed using the Balance Test DT test program within WinPrep®. Parameter values that needed to be entered into the Balance Test Information Window (Figure 1) included those as outlined in Table 1.

Table { SEQ Table * ARABIC }. Input values that are required for the various Balance Test Information parameters.

Parameter(s)	Value
Volume 1 and Volume 2	For 175µL tips: 175, 100, 50, 15µL For 1000µL tips: 1000, 700, 400, 100µL For fixed tips: 1000, 700, 400, 100µL
Number of Replicates	10
System Liquid	Degassed Nanopure Water
Sample Type	Nanopure Water
Technician	Initials of the operator performing the test
Sample Density (g/ml)	The density of water at environmental temperature*
Tip Type	Other
Disposable Tip Lot #	The lot number of the particular tips in use
Performance File	The appropriate Performance File for the tip (175µL, 1000µL or fixed tips) and pipetting mode (Blowout or Waste) in use
Enable Tips (checkboxes)	Select the actual tips (1 to 8) to be tested
Comments	Free text box to add additional information (eg. Tip type, mode, current environmental room temperature, etc).

* Water density values were obtained from http://www.simetric.co.uk/si_water.htm

Pipetting accuracy and precision were examined at four different volumes for each tip size: 175, 100, 50, 15µL for 175µL tips and 1000, 700, 400, 100µL for the 1000µL and fixed tips.

In order to calculate unbiased values for each set of volumes, the slope and offset values in the relevant Performance File were changed to the default 1 and 0 respectively prior to testing. The mean volumes that were pipetted by each tip (10 replicates per tip) at the four designated volumes were used to generate a standard curve. The slope and offset calculated from this curve was used to calibrate the relevant Performance File. The final Performance File settings were then tested at the highest and lowest volumes (as per Table 1) to confirm accurate and precise pipetting.

5.2 Blood Collection

Blood samples were collected from 2 staff donors (DJC/VKI) by a phlebotomist as per normal in three 4mL EDTA vials. Blood samples were stored at 4°C.

5.3 Cell Collection

Buccal cells were collected using a modified Cytobrush® protocol (Mulot *et al.*, 2005; Satia-Abouta *et al.*, 2002). The donor was instructed to brush the inside of one cheek for one minute using a Cytobrush®. Then, with another Cytobrush®, the other cheek was also sampled. Once each cheek was swabbed, the cells on the brush were suspended in 2mL of 0.9% saline solution. Buccal cell samples were stored at 4°C.

5.4 FTA cell Collection

Cells were collected from two staff donors (VKI/CJA) by using a "lolly-pop" swab to sample the inside of the donor's cheek for 15 seconds before pressing the swab onto the FTA™ paper to transfer the DNA. FTA™ cards were stored at room temperature.

5.5 Heater tile temperature verification

Heat tiles supplied with the MultiPROBE® II PLUS HT EX platforms were modified to accept the SlicPrep™ 96 Device. For testing, 1mL of nanopure water (at room temperature) was added to each well. The plate was then placed on a heater tile (controlled by the MP II heater controller) and allowed to reach temperature. The temperatures tested were 37°C and 65°C. Temperature readings for specific outer and inner wells (i.e. A1, A6, A12, D1, D6, D12, H1, H6, H12) were taken at regular intervals up to and including 45 minutes, using calibrated stem digital tilted head thermometer probes. The data were collated and means calculated to determine the distribution of heat over the tile.

5.6 Verification of automated DNA IQ™ Protocol

The automated DNA IQ™ protocol, based on the validated manual method (refer to Project 11), was programmed in WinPrep™ software. The final, optimised protocol was named "DNA IQ Extraction_Ver1.1.mpt". A screenshot of the Test Outline window for this protocol is depicted below in Figure 2. The deck layout is illustrated in Figure 3.

The automated DNA IQ™ protocol was designed to mimic the validated manual method, with minor modifications. Briefly, the changes include:

- Increasing the volume of Extraction Buffer to 500µL;
- A SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA) was used for sample lysis;
- Incubation steps and any shaking steps were performed on the integrated DPC shaker;

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- CRS toroid magnet (P/N 5083175) was used for isolating the DNA IQ™ resin.
- Instead of a single elution of 100µL, a double elution method (2 x 50µL) is used.

Reagents used in the automated protocol were as per the manual method.

Figure 2. The Test Outline window displaying individual nodes within the DNA IQ Extraction_Ver1.1.mpt program test file.

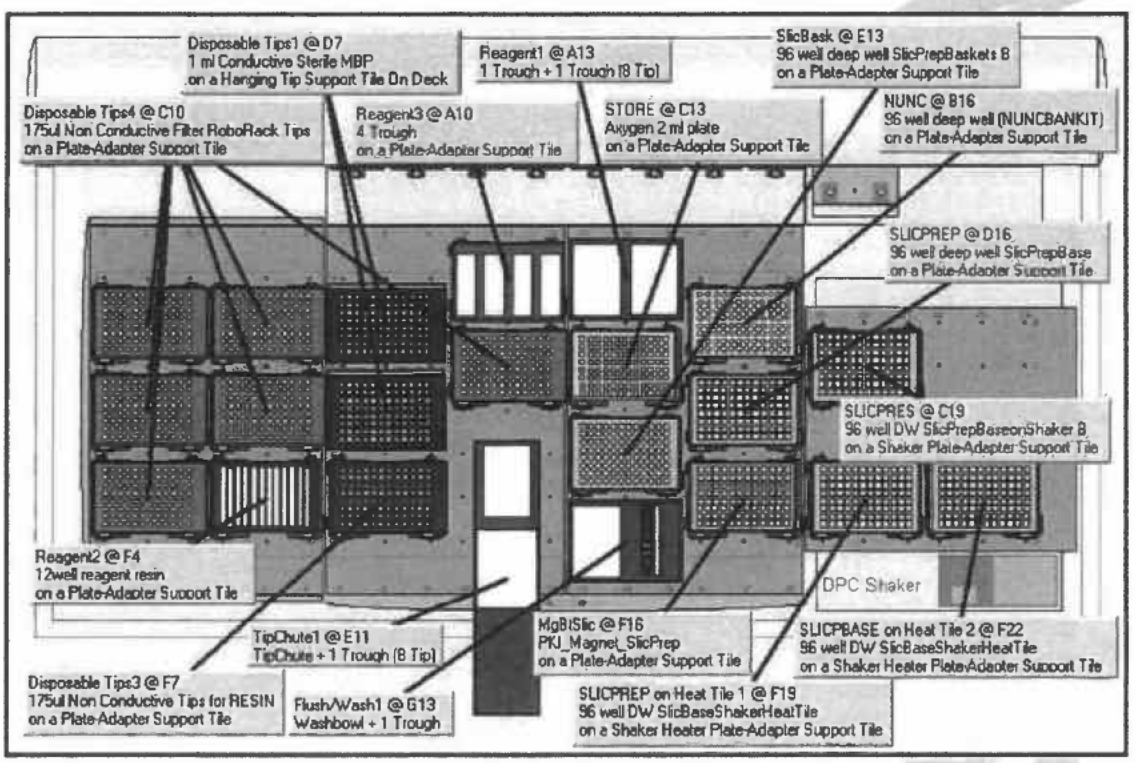

Figure 3. The deck layout for DNA IQ Extraction_Ver1.1.mpt, displaying the required labware on the platform deck.

The automated DNA IQ™ protocol was used to perform the following tests.

5.6.1. Contamination Check via Checkerboard and Zebra-stripe Patterns

Samples consisting of two 3.2mm FTA® discs (containing blood, buccal cells, or blank cards) were arranged in a checkerboard and zebra-stripe pattern (Figure 4) in SlicPrep™ plates using the BSD Duet 600 instrument (BSD Robotics, Brisbane, QLD, Australia) and extracted on the MultiPROBE® II PLUS HT EX platforms using the automated DNA IQ™ protocol. One checkerboard and one zebra-stripe plate was processed on each platform.

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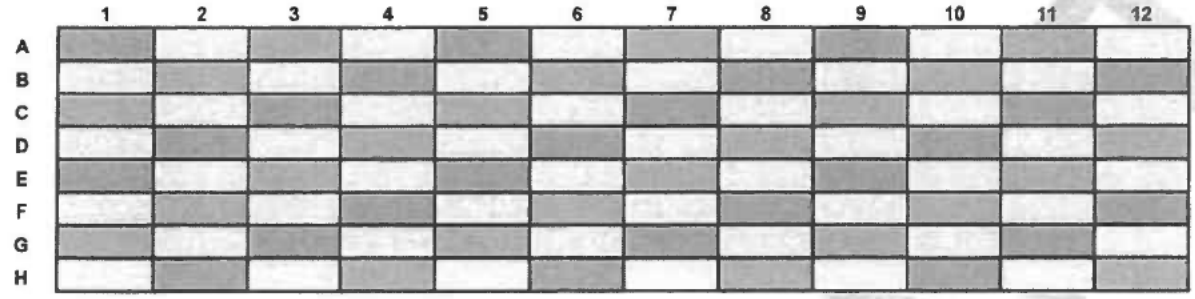
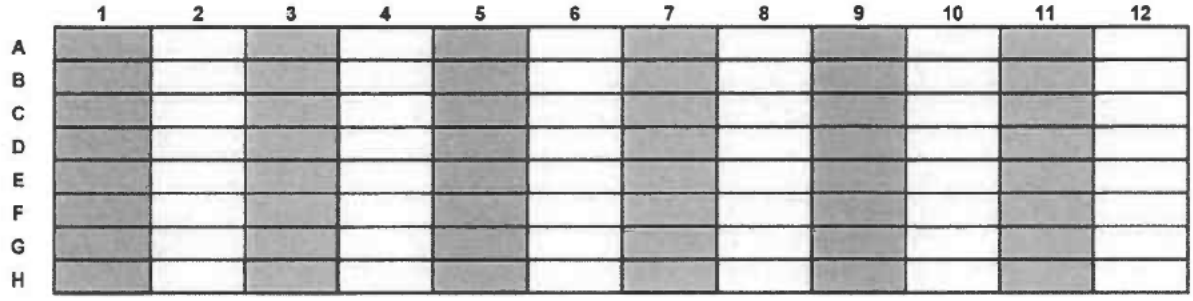


(a) Checkerboard Pattern

b) Zebra Stripe Pattern



Legend:

Blood FTA®

Blank FTA®

Buccal Cell FTA®

Figure 4. Checkerboard and zebra-stripe patterns utilised in the contamination check.

5.6.2. Comparisons with the manual DNA IQ™ method

Comparisons were made between results generated by the automated and manual methods to verify the performance of the automated DNA IQ™ protocol.

Verification samples consisted of different dilutions of blood and cells spotted in 30µL aliquots onto quartered cotton and rayon swabs. Four blood dilutions of neat, 1/10, 1/100 and 1/1000 and four cell dilutions of neat, 1/5.2, 1/52.2 and 1/522 were used to test the sensitivity of both the manual and automated methods. Dilutions were created using 0.9% saline solution for both sample types. Four replicates of each dilution were made up for each substrate and sample type.

The blood was collected using the same method as in 5.2. Four separate extractions were performed for the manual set based on the combination of sample type and swab type: Blood Rayon, Blood Cotton, Cell Rayon and Cell Cotton. For the automated verification, all sample types were extracted together after being transferred to a SlicPrep™ 96 Device to allow automated processing.

5.6.3. Resin volume

The performance of the automated DNA IQ™ protocol was assessed when either 7µL or 14µL of DNA IQ™ resin was used in the protocol to extract blood samples.

5.6.4. Modifying extraction volumes

The performance of the automated DNA IQ™ protocol was assessed for varying volumes of extraction buffer at 300, 350, 400, 450 and 500µL. In each case, the volume of DNA IQ™ Lysis Buffer was kept at 2x the volume of extraction buffer. Samples extracted were blood swabs, prepared as [redacted].

5.6.5. Sensitivity of the automated DNA IQ™ protocol

The sensitivity of the automated DNA IQ™ protocol was assessed using dilutions of whole blood at neat, 1:10, 1:50, 1:100 and 1:1000.

6. Results and Discussion

6.1 Gravimetric Evaluation of Pipetting Accuracy and Precision

Pipetting on both automated platforms was assessed gravimetrically as per laboratory practice. Gravimetric results indicate that pipetting performance for five different pipetting behaviours using 500µL syringes on the instruments is accurate and precise to within the established threshold of ±5% (Table 2). The maximum CV at the maximum volume was 0.78%, whereas the maximum CV at the minimum volume was 1.1%. The CV for pipetting at lower volumes is expected to be slightly higher than the CV at higher volumes using 500µL syringes, because accuracy at small volumes is harder to achieve with larger syringe sizes. Nevertheless, pipetting on the extraction platforms is limited to a minimum of 50µL, which exhibited a CV of 0.36%.

Table 2. Gravimetric evaluation results for various performance files used on either MP II EXTN A or MP II EXTN B.

Performance File	Max. Vol. µL	Min. Vol. µL	Max. Vol. µL Mean	Max. Vol. %CV	Max. Vol. %Inac.	Min. Vol. µL Mean	Min. Vol. %CV	Min. Vol. %Inac.
EXTN A								
Water Blowout 175µL DT_FW _13112007RESIN.prf	50µL	N/A	49.98	0.36	0.0	N/A	N/A	N/A
Water Blowout 175µL DT_FW QHSS _13112007.prf	175µL	15µL	172.26	0.21	1.6	[redacted]	[redacted]	[redacted]
WaterWaste 1mL_FW_QHSS 12112007.prf	1000µL	100µL	999.11	0.24	0.1	99.22.	0.71	0.8
Water Blowout 1mL DT_QHSS _09112007.prf	1000µL	100µL	1001.02	0.27	0.1	100.65	0.63	0.7
Water Blowout Fixed Tips_08112007.prf	1000µL	100µL	995.97	0.31	0.4	99.6	0.71	0.4
EXTN B								
Water Blowout 175µL DT_FW_ 25102007RESIN.prf	50µL	N/A	50.12	0.36	0.2	N/A	N/A	N/A
Water Blowout 175µL DT_FW_ 25102007.prf	175µL	15µL	175.58	0.14	0.3	15.23	1.1	1.5
WaterWaste 1mLDT_FW_QHSS 24102007.prf	1000µL	100µL	1002.39	0.78	0.2	99.56	0.89	0.4
Water Blowout 1mL DT_QHSS 23102007.prf	1000µL	100µL	998.2	0.44	0.2	99.44	0.68	0.6

Water Blowout Fixed Tips_FW 26102007.prf	1000µL	100µL	998.87	0.68	0.1	100.37	0.74	0.4

6.2 Heater tile temperature verification

Two heater tiles on each MP II platform was verified to reach either 37°C or 65°C, the optimum incubation temperatures for sample lysis and DNA elution respectively (using the DNA IQ™ kit). Each tile, upon completion of the verification process, could only be used for a specific temperature, and as such was labelled appropriately to ensure use of the correct tile for specific incubation steps (Table 3).

Table 3. Verified heater tiles for use in the automated DNA IQ™ protocol.

Extraction platform	Tile number	Heater Controller Setting	Average °C reached	Verified temperature	Incubation Step
EXTN A	3 (45W)	50°C	37°C	37°C	Sample Lysis
EXTN A	1 (45W)	85°C	[redacted]	65°C	DNA Elution
EXTN B	1 (45W)	50°C		37°C	Sample Lysis
EXTN B	2 (45W)	85°C	65°C	65°C	DNA Elution

A slight variation in the incubation temperature to achieve sample lysis is acceptable, because Proteinase K exhibits stable activity and broad specificity over a wide range of temperatures between 20-60°C, at which the serine protease still retains greater than 80% of its activity (Sweeney & Walker, 1993).

The efficiency of the elution step is dependent on heating the sample to 65°C in the presence of DNA IQ™ Elution Buffer (Huston, 2002). If the sample is not sufficiently heated, the extraction yield may be lower than expected. Two heater tiles were able to be verified for this crucial incubation step, with both tiles exhibiting minimal variation.

6.3 Contamination Check via Checkerboard and Zebra-stripe Patterns

Table 4 below lists the Extraction Batch ID's of the contamination checks.

Table 4. Extraction Batch ID's for the various contamination check plates that were processed on the MP II platforms using the automated DNA IQ™ protocol.

Type of plate	Extraction batch Id	Extraction Platform	Check passed
Checkerboard 1	VALB20070817_02	Extraction A	Invalidated
Checkerboard 2	VALB20070803_02	Extraction B	Yes
Zebra-Stripe 1	VALB20070803_03	Extraction A	Yes
Zebra-Stripe 2	VALB20070817_03	Extraction B	Yes
Checkerboard/Zebra	VALB20071022_01	Extraction A	Yes

Checkerboard 1

Position E3 (Sample Cells 6) was known to have been contaminated prior to the start of the extraction during the STORstar process [727]. The result showed a mixed DNA profile, with contributing alleles originating from the expected wells (Table 5). In addition to this contamination event, eight of the designated blank samples (positions D3, A10, F1, H5, C4, E4, B7 and E6), two of the cell samples (A1 and B10) and two of the blood samples (F4

and G7) all exhibited a partial DNA profile that was previously unknown (Table 5). This profile did not match any of the positive control samples present on the batch. The DNA profile was searched against the Staff Database and no matches were found. The source of this contaminating DNA profile could not be identified.

None of the other blank samples yielded any DNA profile. The rest of the cell and blood samples yielded the correct DNA profile. Although there is no evidence of well-to-well contamination, the unknown DNA profile obtained has invalidated this plate. A further checkerboard/Zebra-Stripe combination plate was performed to ensure...

Table 5. The DNA profile of the unknown contaminant that was observed in Checkboard-1.

Sample description	D3	vWA	FGA	Amel	D8	D21	D18	D5	D13	D7
Blk23-E6	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	11,13
Blk25-B7	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	
Blk15-E4	14,17	14,17	22,24	X,Y	11,11	29,32.2	14,15	9,11	11,12	11,13
Blk14-C4		14		X	11	32.2		9		
Blk20-H5	14,14	17,17	20,21	X,X	13,16	29,30	14,16	11,13	11,12	11,11
Blk3-F1	14	17		X	13	29,30	14		12	11
Blk10-D3	14,17	14		X,Y	11	29,32.2	14	9,11		11,13
Blk37-A10	14,17	14	22,24	X,Y	11	29	14	9,11		
Cells19-B10	14,17	14,17	20,21,22,24	X,Y	11,13,16	29,30,	14,15,16	11,15	11,12	11,11
Cells13-A1	14,17	14,17	20,21,22,24	X,Y	11,13,16	29,30,32,32.2,33	14,15,16	9,11,13	11,12	11,13
Blood14-G7	NR,17,18	NR,16	20,21	X,Y	NR,13,14	29,30,31,NR	NR,14	NR,12	10,10	10,NR,12
Blood8-F4	NR,17,18	NR,16,17	20,21,NR,24	X,Y	11,13,14	29,30,NR,NR	14,14	9,11,12	10,NR	10,NR,12
Cells 6-E3	14,17,18	16,17	20,21	X,Y	13,14,16	29,30,31	NR,14,16	11,12,13	10,11,12	10,11,12

Checkerboard 2

None of the blank samples yielded DNA profiles; all of the positive cell and positive blood samples yielded the correct DNA profile. Figure 5 illustrates the DNA quantitation results from this plate. DNA was not detected in any of the blank samples.

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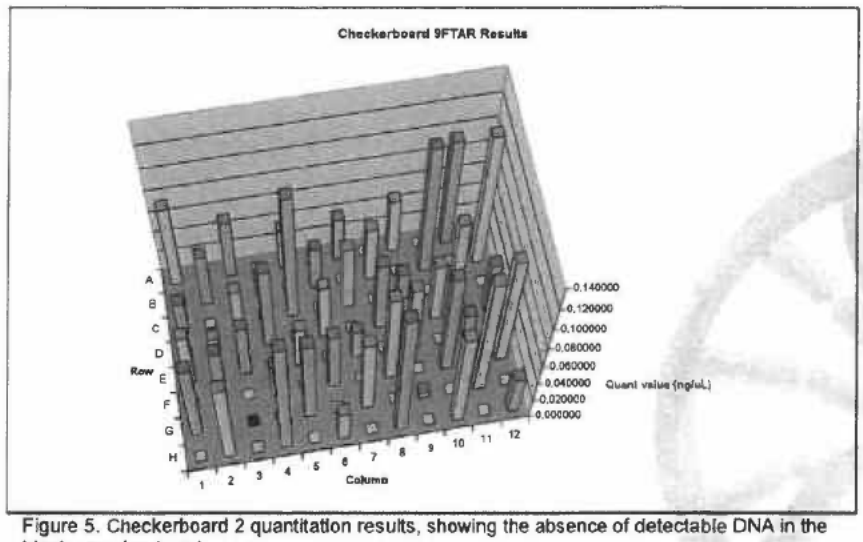

Figure 5. Checkerboard 2 quantitation results, showing the absence of detectable DNA in the blank samples (grey).

Zebra-Stripe 1

None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile. Figure 6 illustrates the absence of detectable DNA in the blank samples.

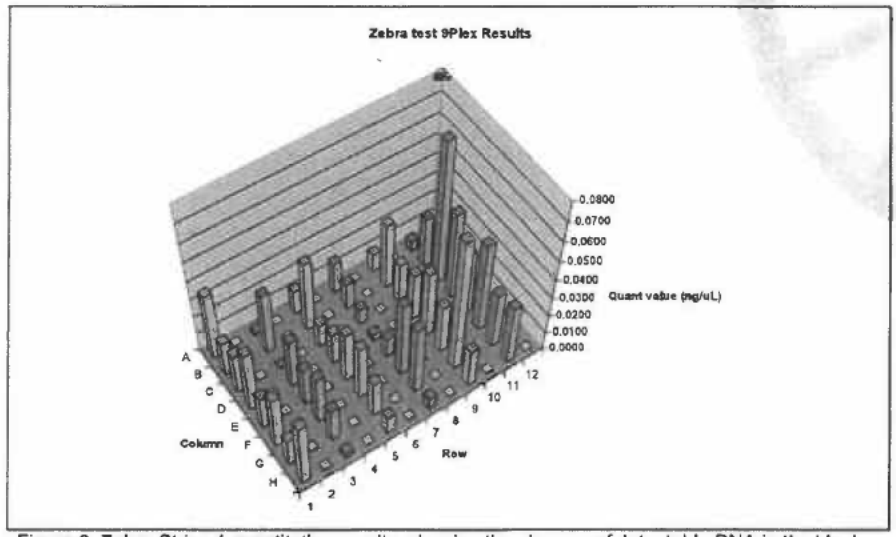

Figure 6. Zebra-Stripe 1 quantitation results, showing the absence of detectable DNA in the blank samples (grey).

Zebra-Stripe 2

None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile. Figure 7 shows the absence of detectable DNA in the blank samples.

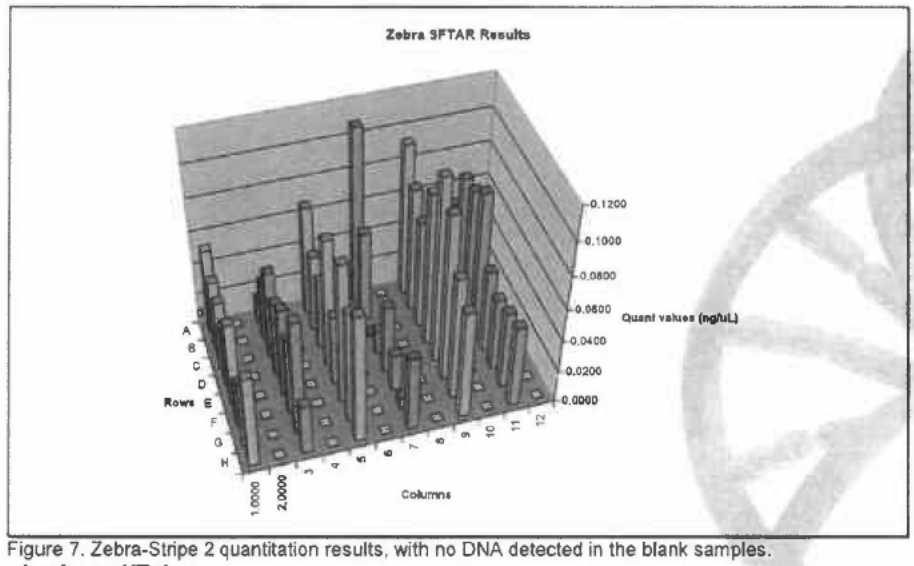

Figure 7. Zebra-Stripe 2 quantitation results, with no DNA detected in the blank samples.

Checkerboard/Zebra

None of the blank samples yielded DNA profiles, all of the positive cell and **positive** blood samples yielded the correct DNA profile. DNA was undetected in the blank **samples** (Figure 8).

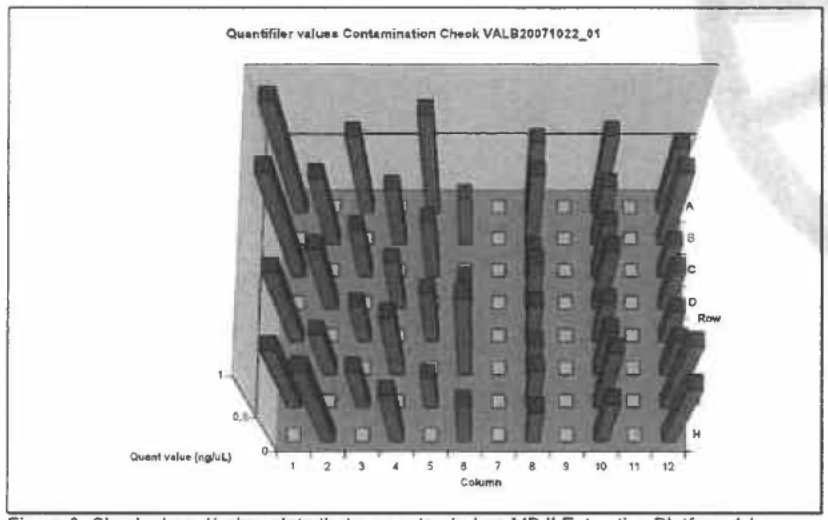

Figure 8. Checkerboard/zebra plate that was extracted on MP II Extraction Platform A because the previous plate was invalidated. DNA was not detected in the blank samples (grey).

6.4 Comparisons with the manual DNA IQ™ method

When dilutions of either blood or cells were applied on to either rayon or cotton swabs, followed by extraction using the DNA IQ™ method, the results of the automated method were always lower in yield compared to the manual method. For blood samples on rayon

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swabs, the automated method generated yields that were on average around 8% (SD 8.45%) of the automated method. For blood on cotton swabs, the yield from the automated method was also around 8% (SD 3.62%). The yields for cell samples were higher at around 33% (SD 16.29%) and 25% (10.32%) for cells on rayon and cotton swabs respectively.

The manual method was found to be more sensitive than the automated method. Out of five replicates at the 1/100 and 1/1000 dilutions for blood on rayon swabs that were processed using the manual method, five and three replicates respectively were detected (and none from the automated method) (see Figure 9). The trend is repeated for blood on cotton swabs (Figure 10). For cell samples on either rayon or cotton swabs, the automated method was found to be more sensitive as evidenced by detection of DNA at the 1/522 dilutions (Figure 11 and 12).

Cell clumping may have occurred with the cell dilutions, therefore causing inaccurate dilutions as can be observed in the ratios between each dilution.

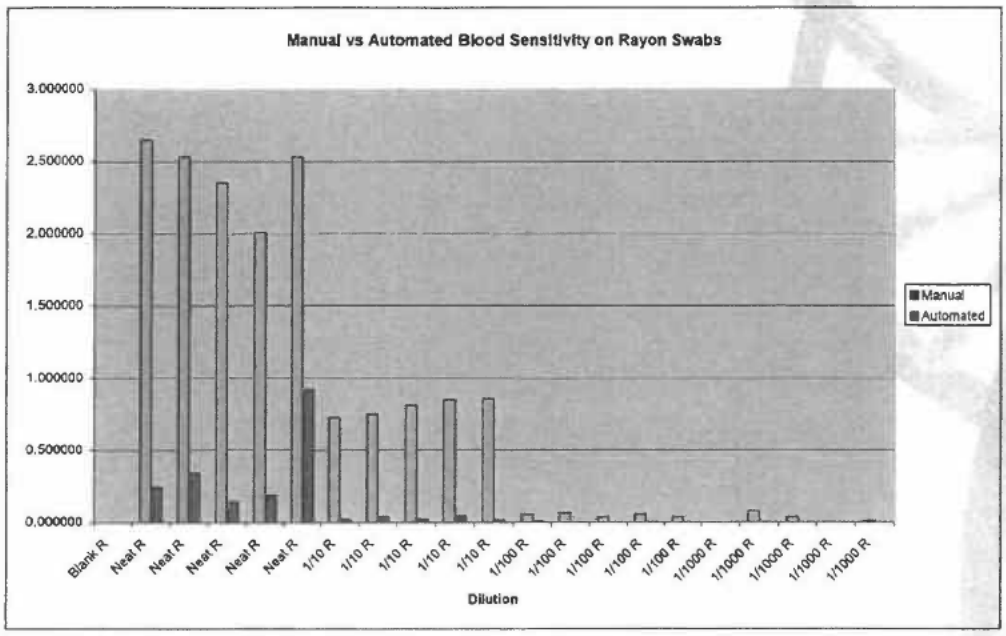

Figure 9. Comparison of sensitivity between the manual and automated DNA IQ™ methods for blood samples on rayon swabs.

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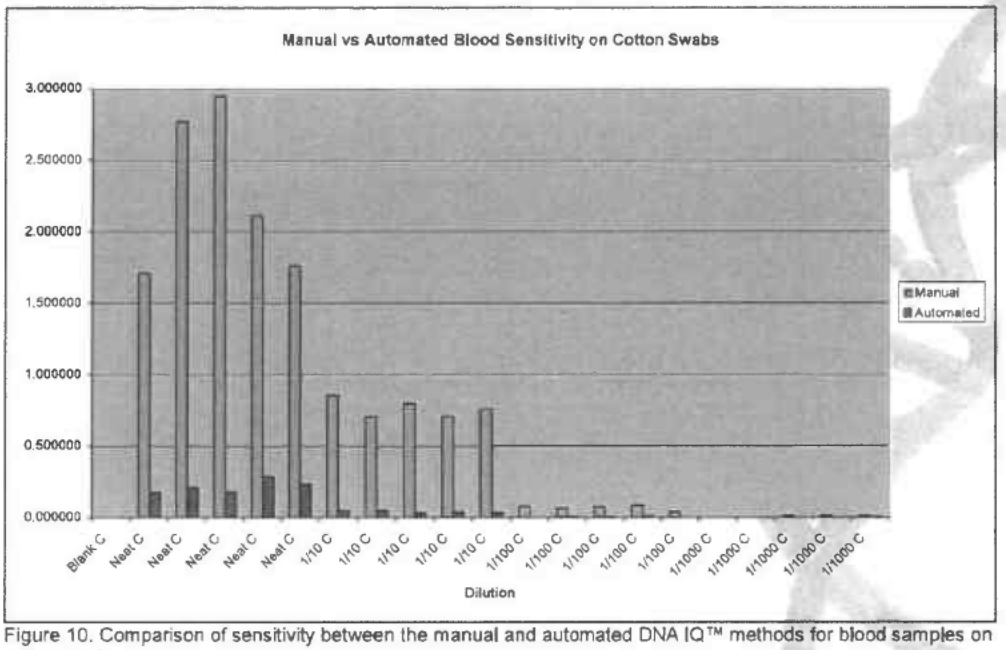

Figure 10. Comparison of sensitivity between the manual and automated DNA IQ™ methods for blood samples on cotton swabs.

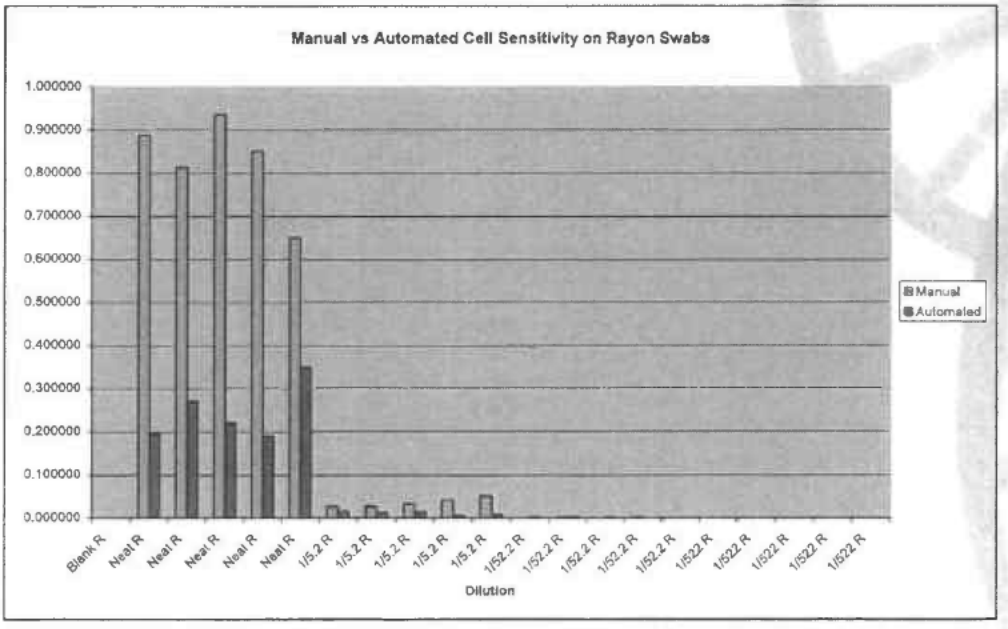

Figure 11. Comparison of sensitivity between the manual and automated DNA IQ™ methods for cells samples on rayon swabs.

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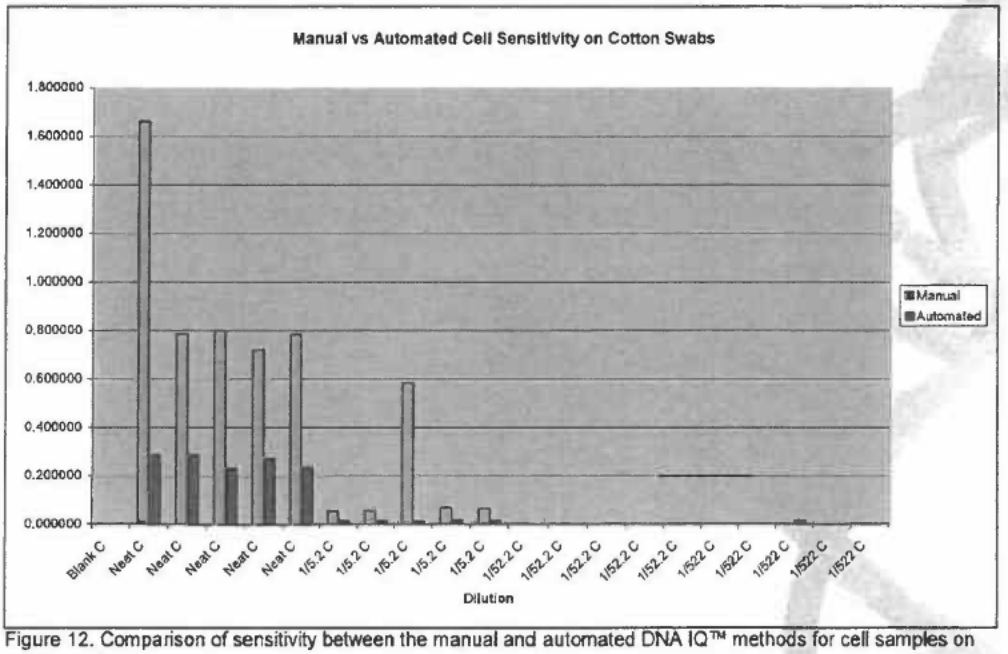

Figure 12. Comparison of sensitivity between the manual and automated DNA IQ™ methods for cell samples on cotton swabs.

6.5 Investigating resin volume

Promega recommends the use of 7µL of DNA IQ™ resin with their protocol. We investigated the performance of the protocol with double the amount of resin (14µL) in order to assess any benefits that may be gained in terms of the resulting yield and quality of the STR profile.

It was observed that doubling the resin resulted in a proportional doubling of the yield. On average, doubling the resin increased the yield by an additional 77.28% (*n*=4). The average yield from an extraction using 7µL of resin was 64.725ng (SD 32.21ng, *n*=4), whereas 14µL resin generated 114.75ng (SD 10.72ng, *n*=4) (Table 6). At the higher resin concentration, the amount of DNA isolated appears to be capped at around 100ng, indicating no change in the ability of the reaction to isolate more DNA due to saturation of resin.

Table 6. Comparison of the effects of doubling the amount of recommended DNA IQ™ resin.

Sample ID	Resin volume	[DNA] ng/µL	Reportable alleles
33383-4216	7µL	0.701	18/18
33383-4225		1.070	18/18
33383-4239		0.319	18/18
33383-4248		0.499	18/18
33383-4252	14µL	1.140	18/18
33383-4261		1.270	18/18
33383-4270		1.010	18/18
33383-4284		1.170	18/18

Samples extracted using either amount of resin generated concordant full DNA profiles (18/18 alleles). Samples processed using the 14µL method produced peaks that were slightly higher. The difference in peak heights between alleles within the same loci ranged from 59-86%, with a mean of 71%, indicating minimal difference between the two methods.

Doubling the amount of resin did not appear to provide any additional benefits compared to the original recommended protocol. More importantly, full DNA profiles were resolved using either method. Therefore, the costs associated with increasing the amount of resin cannot be justified at this stage.

6.6 Modifying extraction volumes

An investigation into optimising extraction volumes ranging from 300µL to 500µL was performed in order to ensure that buffer coverage over the samples was sufficient to enable optimal lysis and release of DNA. In addition, the use of an optimum volume of extraction reagents increases efficiency and economy, therefore potentially lowering laboratory costs.

Although the higher extraction volume generated higher yields when processed using the automated DNA IQ™ protocol (Table 7), DNA profile results were comparable across the various extraction volumes tested for eight replicates each (Table 8). Three instances of allelic imbalance were encountered in two samples from the 300µL and 450µL tests. In all instances, allelic imbalance was greater than 69%.

Table 7. DNA profile results for samples extracted using various volumes of Extraction Buffer, for 8 replicates.

Extraction Buffer Volume (µL)	Mean [DNA] (ng/µL)	SD
300	2.04	0.07
350	2.16	0.09
400	1.69	0.10
450	3.14	0.13
500	3.64	0.17

Table 8. DNA profile results for samples extracted using various volumes of Extraction Buffer, for 8 replicates.

Sample	Extraction Buffer Volume (µL)	DNA Profile Result
300-1 swab	300	OK
300-2 swab		OK
300-3 swab		OK
300-4 swab		OK
300-5 swab		OK
300-6 swab		OK
300-7 swab		AI D13
300-8 swab		OK

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350-1 swab	350	OK
350-2 swab		OK
350-3 swab		OK
350-4 swab		OK
350-5 swab		OK
350-6 swab		OK
350-7 swab		OK
350-8 swab		OK
400-1 swab	400	OK
400-2 swab		OK
400-3 swab		OK
400-4 swab		OK
400-5 swab		OK
400-6 swab		OK
400-7 swab		OK
400-8 swab		OK
450-1 swab	450	OK
450-2 swab		OK
450-3 swab		OK
450-4 swab		OK
450-5 swab		OK
450-6 swab		OK
450-7 swab		AI WA, D18
450-8 swab		OK
500-1 swab	500	OK
500-2 swab		OK
500-3 swab		OK
500-4 swab		OK
500-5 swab		OK
500-6 swab		OK
500-7 swab		OK
500-8 swab		OK

6.7 Sensitivity of the automated DNA IQ™ protocol

DNA was detected from samples that were diluted down to 1:1000 (Figure 13).

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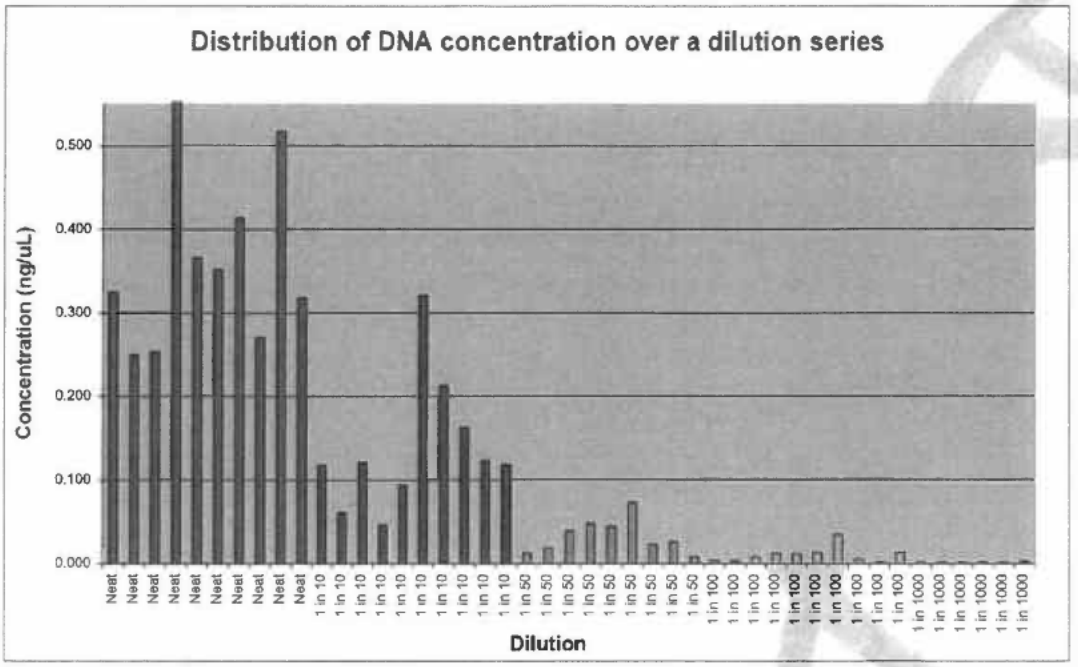

Figure 13. DNA IQ™ sensitivity across various dilutions

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7. Summary and Recommendations

We recommend the following:

- Use of MPII for automated extraction of reference samples
- Use of MPII for automated extraction of casework samples
- Ongoing development of the automated extraction program to increase the efficiency of the extraction

Sweeney, P.J. and Walker, J.M., Burrell, M.M., Enzymes of molecular biology. *Methods Mol. Biol.* Towanam NJ , (1993) **16**, 306