COMMISSION OF INQUIRY INTO DNA PROJECT 13

Commissions of Inquiry Act 1950

STATEMENT OF VANESSA KATE IENTILE

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

1. On 19 October 2023, I was requested to provide a statement answering the questions set out in Notice No. 4.001 issued by Commissioner Bennett AC SC under section 5(1)(d) of the *Commissions of Inquiry Act 1950* (Qld) (the **Notice**).

2. My responses are set out below.

3. I understand that this Commission of Inquiry is concerned with the report titled "Project 13: Report on the Verification of an Automated DNA IQ™ Protocol using the MultiPROBE II PLUS HT EX with Gripper Integration Platform". The report has been defined in the Notice as the '2008 Report'. For the reasons outlined in paragraph 67 below, in my opinion the 2008 Report appears to be an incomplete working draft. As such, in this statement I refer to the 2008 Report as the 'Incomplete Project 13 Report'. A copy of that Report is exhibited to this statement as "**VI-1**".

4. On 13 October 2023, my legal representatives requested a number of documents from Queensland Health (**QH**) to assist in my recollection of events, processes and documents. I am yet to receive those documents as at the time of signing this statement.

5. There are some preliminary matters I wish to draw to the Commission's attention:

   (a) In the absence of the records that I have specifically requested, this statement has been prepared based solely on my limited recollection of events, processes and documents over the period I worked at Queensland Health Forensic and Scientific Services (**QHFSS**), the limited documentary evidence that QH has supplied to me to date and what is publicly available.

   (b) My recollection of events, processes and records is limited because I have not performed work with QHFSS, or in DNA, since about July 2008, being a period of more than 15 years.

   (c) I understand that some of the events that this Inquiry is interested in extend back to as early as 2005, being approximately 18 years ago.

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

(d) As I am no longer a QH employee, I have no access to any laboratory records. I do not hold any personal notes or records from the relevant time.

(e) I do not have any independent recollection of any specific details in relation to the projects that I understand are the subject of this Inquiry, including:

(i) Project 9: Report on the Evaluation of Commercial DNA Extraction Chemistries, June 2007 (**Project 9**) (a copy of that Report is exhibited to this statement as "**VI-2**");

(ii) Project 11: Report on the Validation of a manual method for Extracting DNA using the DNA IQ™ System, August 2008 (**Project 11**) (a copy of that Report is exhibited to this statement as "**VI-3**"); and

(iii) Incomplete Project 13 Report.

(f) I do not recall performing any of the methods that underpinned Project 9, Project 11 and Project 13, and nor would I have expected to perform any of these methods in the management roles I held during 2007 and 2008. I also have no recollection of observing any of these methods being performed.

(g) While I have been listed in the Project 9 and 11 Reports and the Incomplete Project 13 Report as an author of the reports, consistent with the standard practice DNA Analysis Laboratory (**Laboratory**) at the time, I believe I was named as an author in acknowledgement of my role as Managing Scientist.

(h) In 2007 and 2008, when I understand the methods that are the subject of this Inquiry were introduced, there were periods of time when I was not performing the role of Managing Scientist in the Laboratory. That is because I performed higher duties in the role of Forensic Manager for a period in 2007 (but in the absence of the records requested I am unable to provide the precise dates) and travelled overseas in 2008 on three separate occasions (but again, in the absence of the records requested I am unable to provide the dates).

(i) I have no independent recollection of ever seeing the Incomplete Project 13 Report while I was employed at QHFSS. Indeed, I have no independent recollection of the Incomplete Project 13 Report at all.

(j) I have no independent recollection whether the Incomplete Project 13 Report was at some point ever finalised and published.

(k) The Incomplete Project 13 Report is dated August 2008, and to the best of my recollection I ceased work in the Laboratory in the end of July 2008.

(l) There is another version of a Project 13 report that has been referenced in the documents that have been made available to me, which does not list me as an author, and has a different title and date (as discussed further in paragraphs 71 to 77 below).

Identification

Question 1: State:

(a) your full name;

6. My full name is Vanessa Kate Ientile.

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

7. I hold a:

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

(b) Graduate Diploma in Biotechnology.

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

8. I have been employed in the following roles at QHFSS:

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

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

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

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

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

(f) Managing Scientist at QHFSS, from 2004 to about July 2008 (during that period I did act in the role of Forensic Manager at QHSS, for a period in 2007).

9. Throughout my time at QHSS and QHFSS, I worked in almost all areas of the Laboratory, including:

(a) in the analytical division, where DNA was extracted and processed;

(b) in the major crime division, where I examined crime scene exhibits for DNA testing, analysed DNA profile results and prepared reports; and

(c) as a quality scientist, managing the Laboratory's National Association of Testing Authorities accreditation, quality management systems and processes and staff records and training.

10. After I left my role at QHFSS in about July 2008, I continued to be employed by QH as a Project Manager for the Statewide AUSCARE Implementation Project. In that role, I did not work in DNA. My responsibilities in the role of Project Manager for the Statewide AUSCARE Implementation Project included:

(a) implementation of the AUSCARE Electronic Pathology Results Acknowledgement system in all QH facilities with an estimated user base of 44,000 users;

(b) establishment and maintenance of effective working relationships with QH District Executives and Clinical Staff;

(c) provision of change management support to clinical staff during implementation of AUSCARE, including the development of Statewide results acknowledgement guidelines to support clinical practice;

(d) provision of effective leadership and project management including direct responsibility for a total project budget of $7.6 million over three years and 14 temporary project staff;

(e) implementing an image storage solution in AUSCARE managed by the Telehealth team to support diabetic foot/wound management within the Diabetes Network;

(f) implementation of the new AUSCARE distributed architecture designed to deliver new high-availability distributed database architecture for results delivery to clients; and

(g) liaison with the eHealth integrated Electronic Medical Record team, including participation in planning workshops, provision of expert advice in relation to pathology business requirements for orders entry and results acknowledgement.

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

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

12. After ceasing employment with QH, I took some time off paid work to raise a family and then pursued a new career in teaching.

Manual and Automated DNA Extraction Methods

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

1. Abstract

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

2. Introduction

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

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

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

Manual Method

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

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

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

13. I have no independent recollection of the Manual Method.

14. Based on my review of the documents available to me at the time of preparing this statement, I believe the Manual Method refers to the extraction method described in Section 5.8 of the Project 11 Report (exhibited as **"VI-3"**).

15. I believe that the Manual Method may have been based on a validated and published automated protocol from the Centre of Forensic Sciences in Toronto. I have formed this belief because:

(a) on page 6 of the Project 11 Report, section 5.8 states "the manual method used was based on an automated protocol developed by the Centre of Forensic Sciences (CFS) in Toronto, Ontario (PerkinElmer, 2004)."

(b) on page 20 of the Project 11 Report, one of the sources mentioned, in reference to the IQ™ system is 'PerkinElmer, 2004' as follows:

> PerkinElmer (2004). MultiPROBE II Liquid Handling Forensic Workstation Application Guide: Automated DNA IQ™ System for Mixed Casework Sample DNA Isolation [PN 8842157]. PerkinElmer Life and Analytical Sciences: Downers Grove. IL. USA.

(c) there are two references listed in the Quality Information System (**QIS**) procedure[1] 24897V1 titled "Automated DNA IQ™ Method for Extracting DNA from Blood and Cell Substrates" (in exhibit TN-02 to Thomas Nurthen's Statement dated 17 October 2022 that was tendered into the Commission of Inquiry into Forensic DNA Testing in Queensland (**COI-1**)) that refers to automated DNA extraction protocols:

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

6. Komonski, D.I., Marignani, A., Richard, M.L., Frappier, J.R.H., & Newman, J.C., Validation of the DNA IQ™ System for use in the DNA extraction of high volume forensic casework. Can.Soc.Forensic Sci.J., 2004. 37(2): p. 103-109.

11. PerkinElmer, Automated DNA IQ™ System for Mixed Casework Sample DNA Isolation. MultiPROBE II Liquid Handling - Forensic Workstation Application Guide. 2004: p 1-25.

A copy of that QIS is exhibited to this statement as "**VI-4**".

16. I do not have access to these referenced papers, nor do I recall having read them previously. I am therefore not in a position to confirm whether the Manual Method is an adapted version of these protocols.

17. In response to whether the Manual Method was devised within the Laboratory or was otherwise a modification of an existing manual method, due to the passage of time, I cannot answer this question based on my independent recollection. The limited documents that are available to me suggest that the Manual Method was a manual adaptation of an automated method published by PerkinElmer (2004).

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

18. I have no independent recollection of the method by which the Manual Method's "routine use" in DNA Analysis (FSS) was validated. My understanding of how the Manual Method was validated is based on my review of the Project 11 Report (exhibited as **"VI-3"**). I do not have access to any other Laboratory records to assist with my recollection.

19. The Project 11 Report describes the steps taken to validate the Manual Method in sections 5.1 to 5.11. Based on my reading of sections 5.1 to 5.11 of the Project 11 Report, my simplified summary of the validation method is as follows:

(a) Collect and prepare known samples of blood and buccal (cheek) cells to use for the validation trials;

(b) Perform a cell count to estimate the number of cells in subsequent sample dilutions;

(c) Prepare dilutions of blood and cell samples of undiluted, 1/10, 1/100 and 1/1000 dilutions;

(d) Prepare test samples in replicate using undiluted and diluted samples on cotton and rayon swabs. This is likely intended to be reflective of casework samples;

(e) Prepare test samples in replicate using samples with known DNA inhibitors to assess the effectiveness of the DNA extraction procedure;

(f) Prepare replicate test samples of substrates typically seen in casework such as tape lifts, different fabric types, leather, chewing gum, cigarette butts and FTA cards (used for reference samples);

(g) Prepare replicate test samples of mixed DNA from a known male and female donor in a range of ratios from 1:1 to 1:100;

(h) Prepare a range of fabric test samples to assess the best substrate size for samples for DNA extraction effectiveness; and

(i) Extract the prepared samples using the DNA IQ™ protocol described in section 5.8 of Project 11. The amount of DNA in the extract was estimated using Quantifiler, the DNA extracts were amplified using Profiler Plus followed by capillary electrophoresis and DNA fragment analysis. These steps were performed using the documented Laboratory processes and analysis thresholds in place at that time.

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

20. I have no independent recollection or access to records that allows me to be in a position to comment on whether, and if so how, the Manual Method differed from or otherwise modified the DNA IQ™ protocol that was "verified or validated by various laboratories for use on the Multiprobe® II PLUS platform" (as stated in the second line of the second paragraph of the Introduction to the Incomplete Project 13 Report).

(d) state when the Manual Method was so devised;

21. I have no independent recollection of when the Manual Method was devised. Based on my review of the documents available to me, it appears that the Manual Method was devised during 2007. The following documents that have been made available to me suggest that the testing performed to produce the data analysed and reported on in the Project 9 and 11 Reports and the Incomplete Project 13 Report was undertaken between January and October 2007:

(a) Exhibit JH-3 to Justin Howe's Statement dated 6 October 2022 that was tendered into COI-1, being a copy of a list of the Forensic DNA analysis projects (a copy of that document is exhibited to this statement as "**VI-5**");

(b) Exhibit CA-94 to Cathie Allen's Statement dated 11 October 2022 that was tendered into COI-1, being a copy of a PowerPoint presentation titled "Update on DNA Analysis Issues" dated 31 October 2008 (a copy of that document is exhibited to this statement as "**VI-6**");

(c) Exhibit ARM-104 to Allan McNevin's Statement dated 13 October 2022 that was tendered into COI-1, being a copy of the Change Register – Minor changes and emerging or novel practices as at 20-09-2022 (a copy of that document from pages WIT.0040.0077.1410 to WIT.0040.0077.1424 is exhibited to this statement as "**VI-7**");

(d) Exhibit TN-02 to Thomas Nurthen's Statement dated 17 October 2022 that was tendered into COI-1 (exhibited at **"VI-4"**); and

(e) Section 9 Validation of QIS 24897V1 (exhibited at "**VI-4**").

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

22. I have no independent recollection of those within the Laboratory who were responsible for devising the Manual Method.

23. Based on the limited documents available to me, I believe that the persons who could have been responsible for devising the Manual Method were Nurthen T., Hlinka V., Muhuram I., Gallagher B., Lundie G., Iannuzzi C., as the authors of the Project 11 Report (exhibited as **"VI-3"**).

24. My belief that some or all of the authors of Project 11 could have been responsible for devising the Manual Method is consistent with the evidence of Thomas Nurthen in his Statement for the COI-1, where he stated in paragraph 10 that validation reports were prepared by his direct reports and provided to him for review, approval and publication.

25. While I have been listed in the Project 11 Report as an author of the report, I believe I have only been named as an author in acknowledgement of my role as Managing Scientist as this was the standard practice at the time.

26. I do not recall being involved in the Laboratory work that underpinned the Project 11 Report or writing of the Report. I do not believe I would have had such involvement given the managerial nature of my work as Managing Scientist.

27. I also note that in 2007, when the Manual Method appears to have been devised, the position of Managing Scientist reported to the Forensic Manager of the Laboratory, who was Robyn Kelly, who then reported to the Director of QHFSS, Greg Shaw. Ms Kelly left the position of Forensic Manager sometime in 2007.

28. At some stage during 2007, I acted in the Forensic Manager role and one of the Team Leaders (who reported to me in my role of Forensic Manager) would have acted in my Managing Scientist position. I do not recall when or for how long I acted in that position or who acted in my role at the time. I have requested documents to confirm this but I have not received those documents as at the date of this statement.

29. It is possible that Allan McNevin as the Senior Scientist of the Analytical Section may also have had input into the development of the testing performed as part of the validation projects.

CFS Automated Protocol

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

30. I have no independent recollection of the Incomplete Project 13 Report and I have not been provided access to any information related to this reference.

31. I have no independent recollection of this protocol.

Manual DNA IQ™ Protocol

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

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

(ii) was devised within the Laboratory; or

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

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

(j) state whether, and if so how, the Manual DNA IQ™ Protocol differed from or otherwise modified the DNA IQ™ protocol that was "verified or validated by

various laboratories for use on the Multiprobe® II PLUS platform" (as stated in the second line of the second paragraph of the Introduction to the 2008 Report);

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

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

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

32. I have no independent recollection of the matters outlined in Questions 1(h) to (m) of the Notice. Based on my review of the documents available to me, it appears that the reference to the "manual DNA IQ™ protocol" is the same protocol that is referred to as the "Manual Method" described in the Project 11 Report (exhibited as **"VI-3"**) and detailed in my responses above for Questions 1(a) to (f).

Automated DNA IQ™ Protocol

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

33. I have no independent recollection of whether the Automated DNA IQ™ Protocol is the same as the automated protocol the subject of the Incomplete Project 13 Report.

34. Based on my review of the documents available to me, it appears that the "automated DNA IQ™ protocol" referred to in the first line of the third paragraph of the Introduction is the same as the automated protocol, the subject of the Incomplete Project 13 Report.

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

(i) the Manual Method;

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

(iii) the CFS Automated Protocol; and

(iv) the Manual DNA IQ™ Protocol;

35. I have no independent recollection of the methodology/protocols referred to in this question to be able to answer the question.

36. From my review of the protocols described in the Project 11 Report (exhibited as **"VI-3"**) for the Manual Method and the Incomplete Project 13 Report for the Automated DNA IQ™ Protocol (and noting my view, based on my review of the records, that the Manual DNA IQ™ Protocol is the same as the Manual Method that is the subject of the Project 11 Report and the "Automated DNA IQ™ Protocol" is the same as the automated protocol that is the subject of the Incomplete Project 13 Report), I have identified the following differences:

(a) the Extraction Buffer volume is 500uL in the automated protocol compared to 300uL for the Manual Method described in the Project 11 Report; and

(b) the lysis buffer volume is 957uL in the automated protocol compared to 500uL for the Manual Method described in the Project 11 Report.

37. In the absence of being provided with further records, I cannot comment on how the Automated DNA IQ™ protocol differed to the:

(a) DNA IQ™ protocol that was "verified or validated by various laboratories for use on the Multiprobe® II PLUS platform" (as stated in the second line of the second paragraph of the Introduction to the Incomplete Project 13 Report); and

(b) CFS Automated Protocol.

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

38. I have no independent recollection of when the Automated DNA IQ™ Protocol was devised.

39. Based on my review of the documents available to me, it appears that the Automated DNA IQ™ Protocol was devised in 2007. The documents available to me suggest that the testing performed to produce the data analysed and reported on in Projects 9, 11 and the Project 13 was undertaken between January and October 2007 (see exhibit CA-94 to Cathie Allen's Statement dated 11 October 2022).

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

40. I have no independent recollection of those within the Laboratory who were responsible for devising the Automated DNA IQ™ Protocol.

41. Based on my review of the documents available to me, it would appear that the persons responsible for devising the Automated DNA IQ™ Protocol were Nurthen T., Hlinka V., Muhuram I., Gallagher B., Lundie G., Iannuzzi C, as the authors of the Project 11 Report (exhibited as **"VI-3"**).

42. As above, I have been listed as an author of the Project 11 Report. However, I do not recall being involved in the Laboratory work relating to the Automated DNA IQ™ Protocol or the creation of the Report.

43. I repeat and rely on my response to Question 2(e) above.

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

44. I have no independent recollection of the reason why the Laboratory chose to devise the Automated DNA IQ™ Protocol rather than the manufacturer method.

45. It is recorded in the QIS procedure 24897V1 Automated DNA IQ™ Method of Extracting DNA from Blood and Cell Substrates (exhibited at **"VI-4"**) that the protocol was modified to incorporate work practices used in the Laboratory.

46. These included:

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

(b) The increase of the extraction buffer volume to 500uL;

(c) The increase of lysis buffer volume to 957uL proportional to the increase of extraction buffer volume, according to manufacturer's instructions;

(d) Double elution step with an elution buffer volume of 60uL for a final volume of 100uL; and

(e) The use of specific tubes for storage of the final DNA extract.

Multiprobe II PLUS HT EX with Gripper Integration Platform (Multiprobe II Device)

Question 3: State when the Laboratory received the Multiprobe II Device.

47. I have no independent recollection of when the Multiprobe II device was received by the Laboratory. However, it appears from exhibit CA-94 to Cathie Allen's Statement dated 11 October 2022 that the device was received in January 2006.

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

48. I have no independent recollection of what modifications were made to the device (if any).

49. Further, I do not believe that in my role of Managing Scientist I would have been made aware of any modifications or changes to factory settings at the time of validation.

50. The Project 11 Report (exhibited as **"VI-3"**) and the Incomplete Project 13 Report detail reagent usage. As previously stated, the Project 11 Report detailed usage of 300uL of extraction buffer, however in the Incomplete Project 13 Report it was stated that 500uL of extraction buffer was used.

51. The QIS Procedure 24897V3 (in exhibit TN-03 to Thomas Nurthen's Statement dated 17 October 2022 that was tendered into the COI-1) details the reagents, temperature settings and volumes that were implemented for the Automated and Manual Methods. A copy of that QIS is exhibited to this statement as "**VI-8**".

52. As I do not have access to any manufacturer's manual or factory settings, I cannot comment on how the SOP compares.

Question 5: State when the modifications were made.

53. I have no independent recollection nor information available to me about why the modifications were made (if any).

Question 6: Identify those within the Laboratory responsible for the modifications.

54. I have no independent recollection of who was responsible for any modifications (if any).

55. Based on my recollection of the practice at the time, I would expect that the automation team, along with engineers from the manufacturer may have been involved if any modifications were made.

56. Due to the passage of time, I have no independent recollection of who worked in the automation team or the engineers from the manufacturer who may have been involved in any modifications made.

Question 7: State the reasons why the modifications were made.

57. I have no independent recollection nor information available to me about why the modifications were made (if any).

2008 Report

8. Describe your role in the preparation of the 2008 Report

58. In answering this question, I have set out below:

(a) my recent review of the Incomplete Project 13 Report;

(b) my role as Managing Scientist around the time the Incomplete Project 13 Report appears to have been created and worked on;

(c) my comments on the SOPs annexed to Thomas Nurthen's Statement dated 17 October 2023 to the COI-1, which refers to a Project 13 report, with a different title and date;

(d) my comments on the authors listed in the version of the Incomplete Project 13 Report that has been made available to me; and

(e) the leave I took in 2008, and my eventual departure from the Laboratory in about July 2008.

My review of the Incomplete Project 13 Report since re-opening of the COI-1

59. Since becoming aware that the COI-1 was being re-opened to investigate Project 13, I have been provided with, and reviewed, the Incomplete Project 13 Report.

60. I have no independent recollection of the Incomplete Project 13 Report.

61. I have no independent recollection of ever seeing the Incomplete Project 13 Report while I was employed at QHFSS.

62. I do not believe I would have been involved in preparing or drafting the Incomplete Project 13 Report, given my role as Managing Scientist was managerial.

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

64. While I do not believe I was involved in preparing or drafting the Incomplete Project 13 Report, I do not have access to the records to confirm that.

65. To the best of my recollection and belief, the progress of new projects such as Project 13 would have been discussed in weekly team management meetings. In 2008, I believe the team management meetings would have been attended by the Managing Scientist, Team Leaders and Senior Scientists.

66. The method or project output would only be used in the Laboratory once it had been approved at the team management meetings, and there would be some form of records showing that approval process. However, at the time of signing this statement, I do not have access to the records regarding the implementation of the automation process, or the Incomplete Project 13 Report to confirm that.

67. The Incomplete Project 13 Report appears to be an incomplete work in progress having regard to the following:

(a) there are sections in the document that have question marks, highlighted text and incomplete sentences, as extracted below;

5.6.4. Modifying extraction volumes

The performance of the automated DNA IQ™ protocol was assessed for varying volumes of extraction buffer at 300, 350, 400, 450 and 500µL. In each case, the volume of DNA IQ™ Lysis Buffer was kept at 2x the volume of extraction buffer. Samples extracted were blood swabs, prepared as per ???.

5.6.5. Sensitivity of the automated DNA IQ™ protocol

The sensitivity of the automated DNA IQ™ protocol was assessed using dilutions of whole blood at neat, 1:10, 1:50, 1:100 and 1:1000.

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

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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

Deponent [redacted] .....

Checkerboard 1
Position E3 (Sample Cells 6) was known to have been contaminated prior to the start of the extraction during the STORstar process (???). The result showed a mixed DNA profile, with contributing alleles originating from the expected wells (Table 5). In addition to this contamination event, eight of the designated blank samples (positions D3, A10, F1, H5, C4, E4, B7 and E6), two of the cell samples (A1 and B10) and two of the blood samples (F4

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(b) there is no explanation or data to explain how the yields were determined;

(c) section 6.7 with the heading "Sensitivity of the automated DNA IQ™ protocol" has only one graph and no explanation of the results, as extracted below;

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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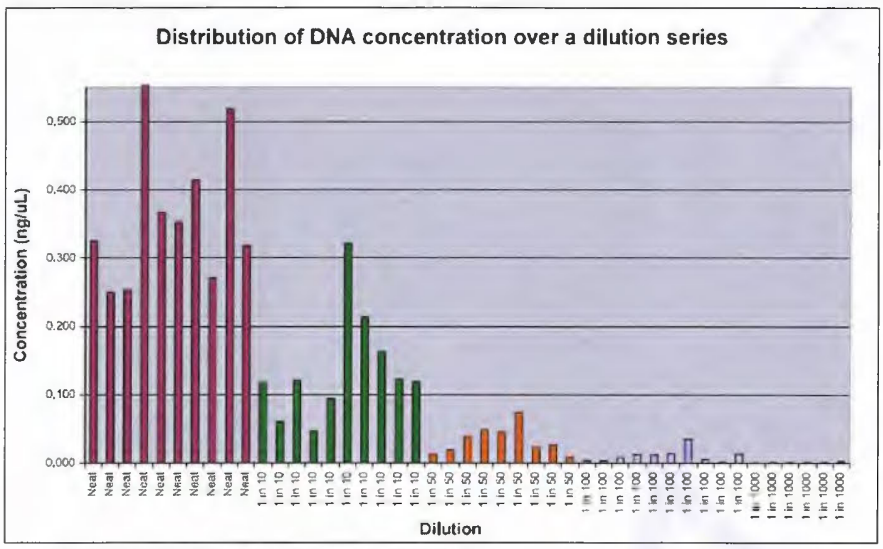
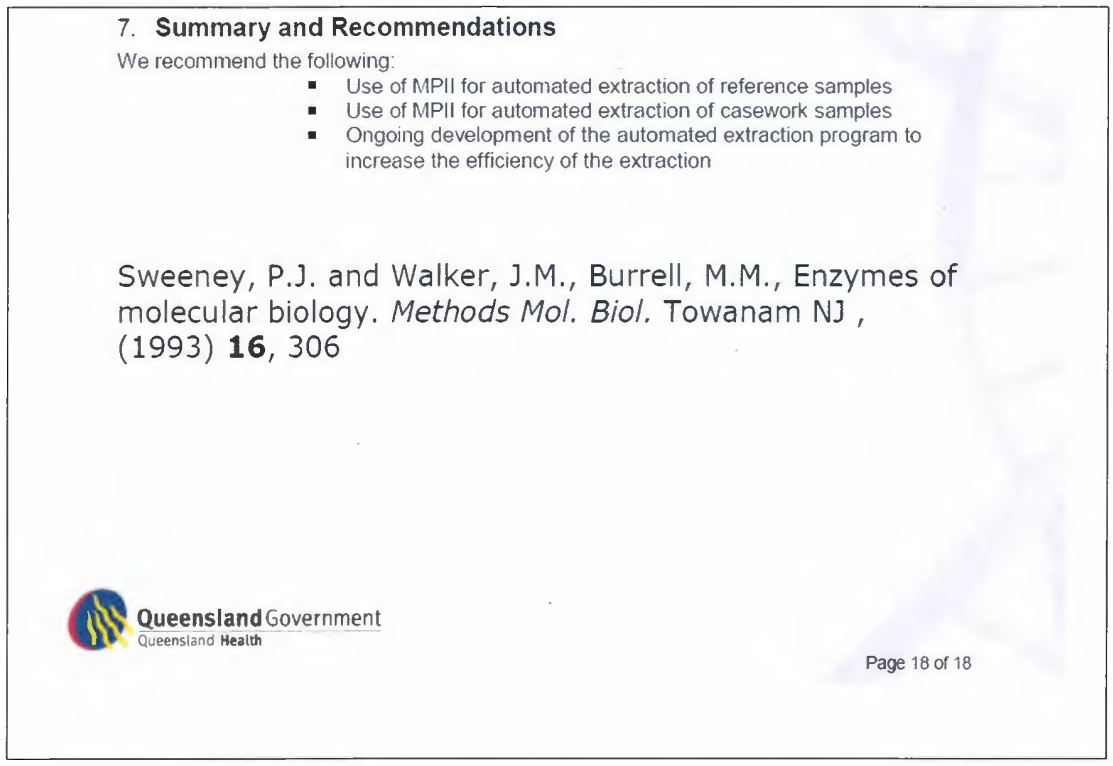


Figure 13. DNA IQ™ sensitivity across various dilutions

as

(d) there is no information about whether any of the samples tested as part of the Incomplete Project 13 Report were amplified and any DNA profiles obtained; and

(e) the references section has one reference listed only. Having read the content of the Incomplete Project 13 Report, I believe that there would have been multiple references that would have needed to have been referred to in any finalised report, as extracted below.



7. **Summary and Recommendations**

We recommend the following:

- Use of MPII for automated extraction of reference samples
- Use of MPII for automated extraction of casework samples
- Ongoing development of the automated extraction program to increase the efficiency of the extraction

Sweeney, P.J. and Walker, J.M., Burrell, M.M., Enzymes of molecular biology. *Methods Mol. Biol.* Towanam NJ , (1993) **16**, 306

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My role as Managing Scientist

68. As Managing Scientist, it was not my usual practice to be directly involved in preparing validation reports, and I do not believe that I was involved in preparing the Incomplete Project 13 Report.

69. While I am yet to receive a copy of the role description for the Managing Scientist position that applied at the time I held the role, to the best of my recollection of my work in this role 15 to 20 years ago, my key responsibilities included:

(a) Providing effective leadership, management and innovation of Forensic Biology (approximately 100 staff) and an annual operating budget of $10 million;

(b) Implementing strategic planning, monitoring, evaluating and reporting systems;

(c) Establishing and maintaining effective working relationships with relevant agencies, such as the Queensland Police Service, Office of the Director of Public Prosecutions and Department of Justice;

(d) Providing a quality client focused Forensic Biology Service;

(e) Investigating innovation and supporting the changing environment of Forensic Services; and

(f) Promoting values and interests of Queensland Health Scientific Services.

70. My direct reports were the two Team Leaders, the Senior Scientists in charge of the analytical section, the automation project and the quality systems, and the Administration Manager. These positions were responsible for the day-to-day management of their own teams and staff.

Other Project 13 report

71. I have been provided with a copy of exhibit "TN-02" to Thomas Nurthen's Statement dated 17 October 2022 to the COI-1 (exhibited at **"VI-4"**), which is a SOP titled "Automated DNA IQ™ Method of Extracting DNA from Blood and Cell Substrates" (**Automated DNA IQ™ Method SOP 24897V1**).

72. The method described in the Automated DNA IQ™ Method SOP 24897V1 related to 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. The method that was provided for in the Automated DNA IQ™ Method SOP 24897V1 applied to all Forensic Biology staff who were required to extract cell and blood samples.

73. Relevantly, at page WIT.0050.0002.0044 of exhibit TN-02, there is a list of validation reports that underpinned the Automated DNA IQ™ Method SOP 24897V1 . Included in this list is a report titled "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."

74. The authors of the Project 13 report listed in the Automated DNA IQ™ Method SOP 24897V1 are Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. I have not been listed in the Automated DNA IQ™ Method SOP 24897V1 as an author to the Project 13 report listed in the SOP.

75. I approved a number of SOPs during my time as Managing Scientist. I do not recall the exact details of this SOP, why there is another Project 13 report listed with a slightly different name and date, and why I am not listed as an author (given I had been listed as an author of the other Incomplete Project 13 Report the subject of this Commission of Inquiry).

76. I also note that the Project 13 report that is listed in the Automated DNA IQ™ Method SOP 24897V1 is dated 2007. This date differs to the date of the Incomplete Project 13 Report that is dated August 2008.

77. This other Project 13 report is also referenced at:

(a) Exhibit TN-03 at page WIT.0050.0002.0065 (exhibited at **"VI-8"**); and

(b) Exhibit TN-37 to Thomas Nurthen's Statement dated 17 October 2022 that was tendered into the COI-1 at page WIT.0050.0002.0328 (a copy of that QIS is exhibited to this statement as "**VI-9**").

Authors listed on Incomplete Project 13 Report

78. Whilst I have no independent recollection, based on my review of the listed authors of the Incomplete Project 13 Report, I believe that at least some of the authors listed in the Incomplete Project 13 Report were responsible for the testing. This appears to be confirmed by Thomas Nurthen's Statement to the COI-1 dated 17 October 2022, where he states at paragraph 10 that validation reports were prepared by his direct reports and provided to him for review, approval and publication. It is possible that Allan McNevin, as the Senior Scientist of the Analytical Section, may also have had input into the development of the testing performed as part of the validation projects.

Leave and departure from QHFSS in 2008

79. I ceased working as Managing Scientist at QHFSS in or around late July 2008.

80. Prior to my departure, to the best of my recollection and belief and noting that I am yet to receive the records I have requested to verify, I attended:

(a) a two week holiday in New Zealand in February 2008;

(b) site visits and conferences in Europe, Canada and USA (commencing 20 April 2008 for approximately 3 weeks); and

(c) a Biology Specialist Advisory Group meeting in Auckland (in July 2008 for approximately 1 week).

81. It is possible that the Incomplete Project 13 Report could have been created after I had ceased employment with QHFSS, but I do not have access to the records to confirm that.

82. I decided to leave my position at QHFSS because, at that time, I believed the Laboratory had implemented systems and workflow processes to manage the workload more effectively, including the backlog of samples.

83. I also wanted to try transferring my skills into a new area and to gain new knowledge. It was a completely personal decision to leave my role. It was not a sudden announcement.

84. I did not resign from my position straight away. I moved to another project with QH at AUSCARE, and Cathie Allen (who was a Team Leader at the time) acted in my position as Managing Scientist. After some time at AUSCARE, and on a date I cannot recall, I

decided to relinquish my position with QHFSS. Ms Allen was subsequently permanently appointed to the role of Managing Scientist.

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

85. To the best of my recollection and belief, I do not believe that I received directions in relation to the preparation of the Incomplete Project 13 Report, but I do not have access to the records to confirm that.

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

86. I have no independent recollection of any communications between myself, and the other authors listed concerning the purpose or intended purpose of the Incomplete Project 13 Report.

87. It is possible that communications occurred between myself, other people named as authors of the Incomplete Project 13 Report and any other person in a position of management concerning the purpose or intended purpose of the Project 13 Report, but I do not have access to the records to confirm that and I do not have any independent recollection of it.

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

88. I have no independent recollection of who the Incomplete Project 13 Report was distributed to, or if it was even finalised and distributed.

89. It is possible the Incomplete Project 13 Report was distributed internally, externally or both, but I do not have access to the records to confirm that.

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

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

90. I have no independent recollection of how the conclusions on page 1 of the Incomplete Project 13 Report were reached because I have no independent recollection of being involved in the Incomplete Project 13 Report.

91. It is possible the conclusion on page 1 of the Incomplete Project 13 Report was discussed within QHFSS, but I do not have access to records to confirm that.

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

92. I have no independent recollection of any discussions or communications with the other named authors of the Incomplete Project 13 Report in relation to the conclusions on page 1 or the referenced data.

93. It is possible that the conclusion on page 1 of the Incomplete Project 13 Report was discussed within QHFSS, but I do not have access to records to confirm that.

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

94. I am unaware how the conclusion on page 1 can be reconciled with the data and figures outlined in part 6.4 of the Incomplete Project 13 Report.

95. From my review, the abstract and part 6.4 do contradict each other.

96. Further, section 6.7 contains a single statement and one graph. There is no explanation or summary of the results observed by the testing outlined in the Methods section. This is in contrast to the Results and Discussion sections of the Project 9 and 11 Reports and appears to me to support the conclusion that the Incomplete Project 13 Report is a work in progress.

97. I have no independent recollection as to why the Incomplete Project 13 Report was written that way.

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

(i) your role in the decision;

98. I have no independent recollection of how the recommendations summarised on page 18 of the Incomplete Project 13 Report were decided. Given that the Incomplete Project 13 Report appears to be a draft, work in progress document, it may be that the recommendations had not been "decided", but I have no independent recollection of whether that is so or not.

99. I do not believe that I was involved in any decision (if any was formally made) about the recommendations summarised on page 18 of the Incomplete Project 13 Report, but I do not have access to records to confirm that.

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

100. I have no independent recollection of any discussions regarding the recommendations summarised on page 18 of the Incomplete Project 13 Report.

101. It is possible the recommendations summarised on page 18 of the Incomplete Project 13 Report were discussed within QHFSS, but I do not have access to records to confirm that.

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

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

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

DECLARED by

Vanessa Kate Ientile
at Brisbane

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

24 October 2024

In the presence of:

[redacted]

.....
Thomas Daniel Robert Goodwin
[signature of witness]

Australian Legal Practitioner

24 October 2023

Holding Redlich

Schedule of Exhibits

Exhibit	Document title	Page
VI-1	Project 13: Report on the Verification of an Automated DNA IQ™ Protocol using the MultiPROBE II PLUS HT EX with Gripper Integration Platform, August 2008	1-18
VI-2	Project 9: Report on the Evaluation of Commercial DNA Extraction Chemistries, June 2007	19-54
VI-3	Project 11: Report on the Validation of a manual method for Extracting DNA using the DNA IQ™ System, August 2008	55-76
VI-4	Exhibit TN-02 to the statement of Thomas Nurthen's dated 17 October 2022, being Standard Operating Procedure titled: "Automated DNA IQ™ Method of Extracting DNA from Blood and Cell Substrates 24897V1"	77-90
VI-5	Exhibit JH-3 to the statement of Justin Howes dated 6 October 2022, being a list of the Forensic DNA analysis projects	91-95
VI-6	Exhibit CA-94 to the statement of Cathie Allen dated 11 October 2022, being a PowerPoint presentation titled "Update on DNA Analysis Issues" dated 31 October 2008	96-101
VI-7	Exhibit ARM-104 to the statement of Allan McNevin dated 13 October 2022, being a copy of the Change Register – Minor changes and emerging or novel practices as at 20-09-2022 from pages WIT.0040.0077.1410 to WIT.0040.0077.1424	102-116
VI-8	Exhibit TN-03 to the statement of Thomas Nurthen dated 17 October 2022, being Standard Operating Procedure titled: "DNA IQ™ Method of Extracting DNA from Casework and Reference Samples 24897V3"	117-149
VI-9	Exhibit TN-37 to the Statement of Thomas Nurthen dated 17 October 2022, being Standard Operating Procedure titled: "DNA IQ™ Method of Extracting DNA from Casework and Reference Samples 24897V6"	150-174

[redacted] .....
Vanessa Kate Ientile
Declarant

[redacted] .....
Thomas Daniel Robert Goodwin
Australian Legal Practitioner

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Project 13. Report on the Verification of an Automated DNA IQ™ Protocol using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform

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

1. Abstract

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

2. Introduction

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

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

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

3. Aim

To verify an automated DNA IQ™ protocol for use on the MultiPROBE® II PLUS HT EX platforms to allow extraction of DNA from various sample types.

4. Equipment and Materials

- MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (PerkinElmer, Downers Grove, IL, USA)
- Gravimetric Performance Evaluation Option with Mettler SAG285/L balance (Mettler-Toledo, Greifensee, Switzerland)
- DNA IQ™ System (Promega Corp., Madison, WI, USA)
- Extraction Buffer (10mM Tris, 1mM EDTA, 100mM NaCl, 20% SDS)
- SlicPrep™ 96 Device (Promega Corp., Madison, WI, USA)
- Nunc™ Bank-It tubes (Nunc A/S, Roskilde, Denmark)
- 175µL non-conductive sterile filter RoboRack tips (PerkinElmer, Downers Grove, IL, USA)
- 1000µL Conductive sterile filter Robotix tips (Molecular BioProducts, San Diego, CA, USA)
- ABI Prism® 7000 SDS (Applied Biosystems, Foster City, CA, USA)
- Quantifiler™ Human DNA Quantification kits (Applied Biosystems, Foster City, CA, USA)
- AmpFℓSTR® Profiler Plus Amplification kits (Applied Biosystems, Foster City, CA, USA)
- GeneAmp® 9700 thermalcycers (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 96-well optical reaction plates (Applied Biosystems, Foster City, CA, USA)
- ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA)
- GeneScan™ 500 ROX™ Size Standard (Applied Biosystems, Foster City, CA, USA)
- Hi-Di™ Formamide (Applied Biosystems, Foster City, CA, USA)
- 3100 POP-4™ Polymer (Applied Biosystems, Foster City, CA, USA)
- Cytobrush® Plus Cell Collector (Cooper Surgical, Inc., Trumbull, CT, USA)
- 0.9% saline solution (Baxter Healthcare, Old Toongabbie, NSW, Australia)
- Stem digital tilting head thermometer
- For mock samples:
  - FTA™® Classic Card (Whatman Inc., Florham Park, NJ, USA)
  - Sterile cotton swabs (Medical Wire & Equipment, Corsham, Wiltshire, England)
  - Sterile rayon swabs (Copan Italia SPA, Brescia, Italy)

5. Methods

5.1 Gravimetric Evaluation of Pipetting Accuracy and Precision

Gravimetric analysis was performed by placing the SAG285/L balance on the platform deck and instructing the MP II to repeatedly pipette certain volumes of system liquid onto the balance pan. Readings were taken automatically by the software and compiled into a results table, which was then used to automatically generate an Excel-based results chart containing mean, %CV and %inaccuracy values. The mean values obtained were used to calculate R^2, slope and Y-intercept (offset) values to calibrate the system's pipetting.

Pipetting performance was assessed for various volumes using three different tips in order to calculate appropriate R^2, slope and Y-intercept (offset) values which were then added to the performance file. Values were calculated for both Blowout (single-liquid transfer) and Waste (multidispense) modes for the 1000µL conductive tips, and Blowout mode only for the 175µL non-conductive tips and fixed tips.

For the addition of resin, a specialised performance file was created based on the performance file for 175µL tips in blowout mode, except the "Blowout Volume" column values were set to 0 to allow pipetting performance that is similar to waste mode. Retesting was performed to confirm accurate and precise pipetting with these settings.

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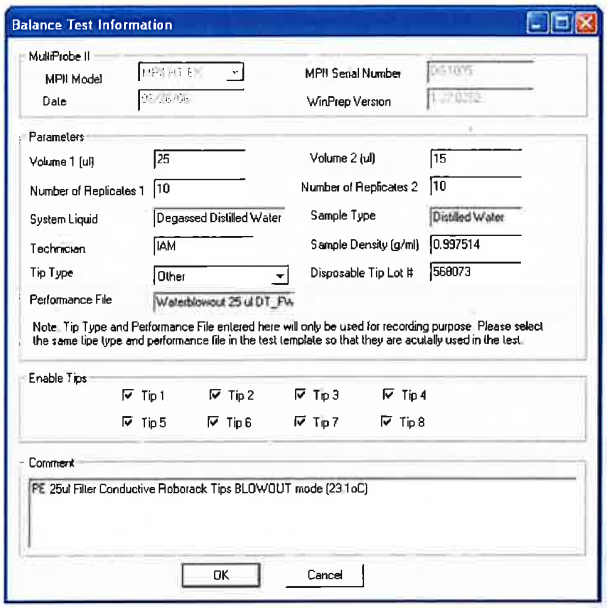

Figure { SEQ Figure * ARABIC }. The Balance Test Information Window as present within the Balance Test DT program. All pipetting parameters are entered here and are subsequently transferred to the result output file.

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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350-1 swab	350	OK
350-2 swab		OK
350-3 swab		OK
350-4 swab		OK
350-5 swab		OK
350-6 swab		OK
350-7 swab		OK
350-8 swab		OK
400-1 swab	400	OK
400-2 swab		OK
400-3 swab		OK
400-4 swab		OK
400-5 swab		OK
400-6 swab		OK
400-7 swab		OK
400-8 swab		OK
450-1 swab	450	OK
450-2 swab		OK
450-3 swab		OK
450-4 swab		OK
450-5 swab		OK
450-6 swab		OK
450-7 swab		AI vWA, D18
450-8 swab		OK
500-1 swab	500	OK
500-2 swab		OK
500-3 swab		OK
500-4 swab		OK
500-5 swab		OK
500-6 swab		OK
500-7 swab		OK
500-8 swab		OK

6.7 Sensitivity of the automated DNA IQ™ protocol

DNA was detected from samples that were diluted down to 1:1000 (Figure 13).

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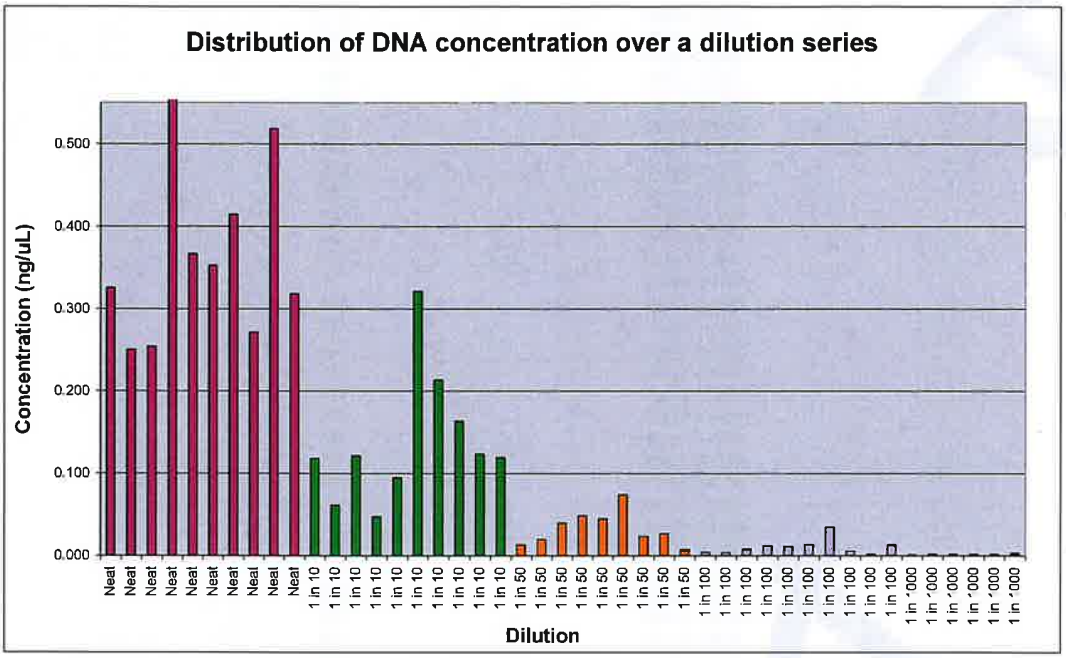

Figure 13. DNA IQ™ sensitivity across various dilutions

as

7. Summary and Recommendations

We recommend the following:

- Use of MPII for automated extraction of reference samples
- Use of MPII for automated extraction of casework samples
- Ongoing development of the automated extraction program to increase the efficiency of the extraction

Sweeney, P.J. and Walker, J.M., Burrell, M.M., Enzymes of molecular biology. *Methods Mol. Biol.* Towanam NJ , (1993) **16**, 306

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Project 9. Report on the Evaluation of Commercial DNA Extraction Chemistries

2007

Automation and LIMS Implementation Project Team,

DNA Analysis

Forensic And Scientific Services

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Project 9. Report on the Evaluation of Commercial DNA Extraction Chemistries

Breanna Gallagher^, Vojtech Hlinka^, Cecilia Iannuzzi^, Generosa Lundie^, Iman Muharam^, Thomas Nurthen^, Vanessa Ientile
^ These authors contributed equally.
Automation/LIMS Implementation Project, DNA Analysis FSS (June 2007)

1. Abstract

DNA Analysis FSS performed an evaluation of various commercial DNA extraction chemistries in order to compare their overall performance (quality, yield, user-friendliness and the ability to automate) against the current in-house Chelex® protocol. Out of five commercial kits evaluated, the DNA IQ™ system from Promega Corporation (Madison, WI, USA) was found to be the best out-of-the-box method for DNA extraction of blood and cell samples and will be validated for routine in-house use. This document presents data from the evaluation and provides a discussion of the results observed. For the manual DNA IQ™ validation report, see Project 11. Verification of an automated DNA IQ™ method is reported in Project 13.

2. Introduction

There have been many DNA extraction methods published since DNA was first isolated in 1953 (Butler, 2005). As technology developed and the demand for DNA testing increased, the methods for extracting and purifying DNA have improved. The Chelex® extraction procedure (Walsh *et al.*, 1991) became a quick and easy alternative to the more technically-demanding phenol/chloroform protocol and was more compatible for extracting samples from forensic exhibits, although the resulting DNA extract is still crude and unpurified because inhibitors are not removed from the solution. As the demand for extracting trace DNA samples has increased within the last 10 years to allow interrogation of low copy number forensic samples, coupled with the increase in the need to analyse difficult samples such as touched objects and degraded bone material, new DNA extraction technologies that are designed specifically for forensic samples have increased in availability.

The new DNA extraction chemistries on the market aim to overcome problems encountered in forensic DNA samples as they are designed to:

- Improve removal of inhibitors present in the sample that can affect DNA extraction (e.g. hemoglobin, textile dyes) or prevent successful PCR amplification (e.g. hematin, melanin, polysaccharides, bile salts, humic compounds);
- Maximise recovery of DNA in trace (low copy number) samples by using special buffers that promote cell lysis and integrating a DNA capture system that allows efficient binding and elution of sample DNA, therefore increasing total yields;
- Increase the overall quality and purity of recovered DNA by using special elution or storage buffers, therefore enhancing DNA stability for long-term storage, ensuring reliability and consistency in the sample DNA for reworks and future use.

DNA Analysis FSS obtained various commercial forensic DNA extraction kits (Table 1) in order to evaluate their performance against the in-house Chelex® protocol (see QIS 17171 for detailed information and literature on the Chelex® system).

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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.

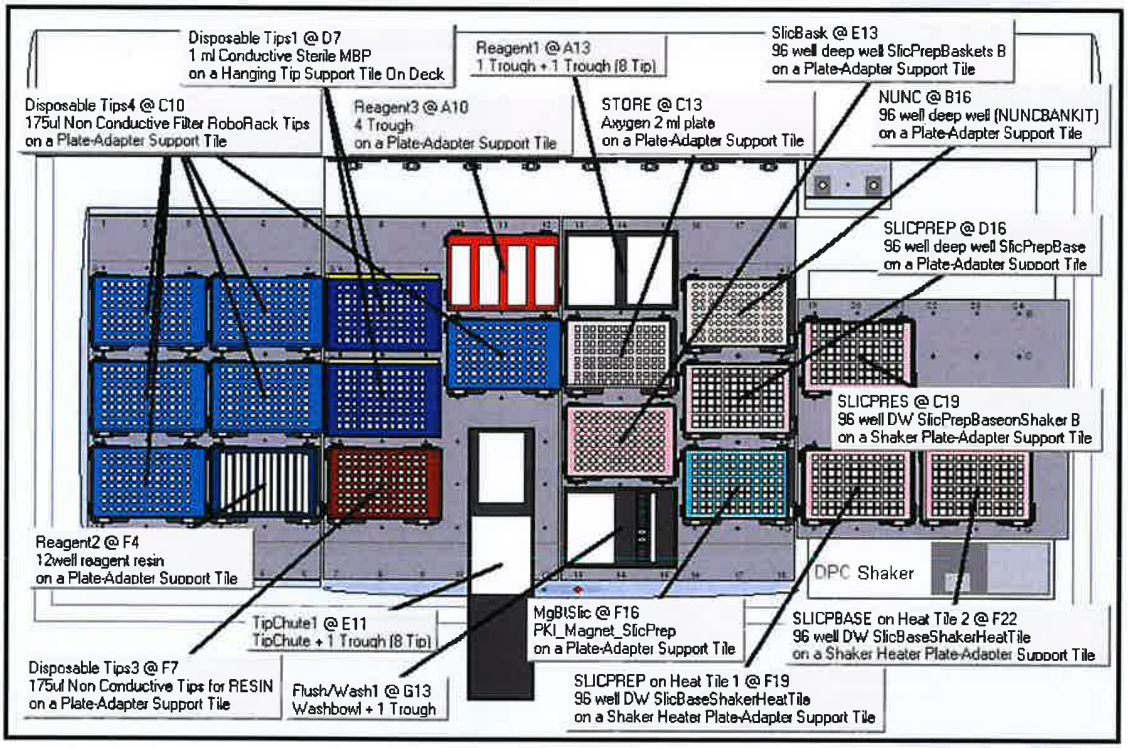

Figure 3. The deck layout for DNA IQ Extraction_Ver1.1.mpt, displaying the required labware on the platform deck.

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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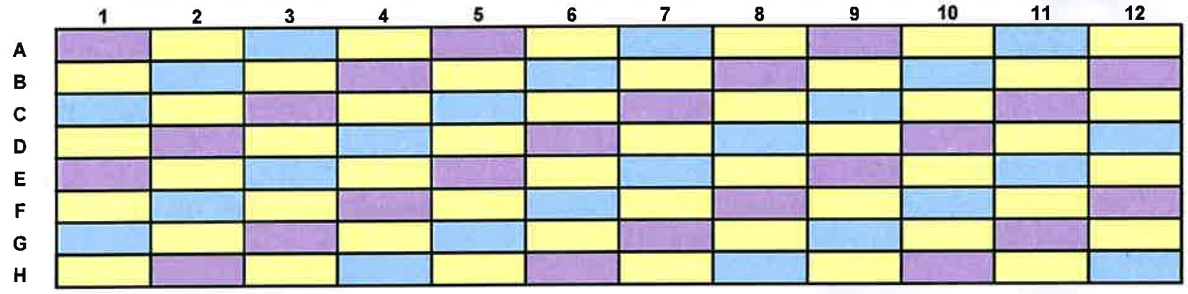
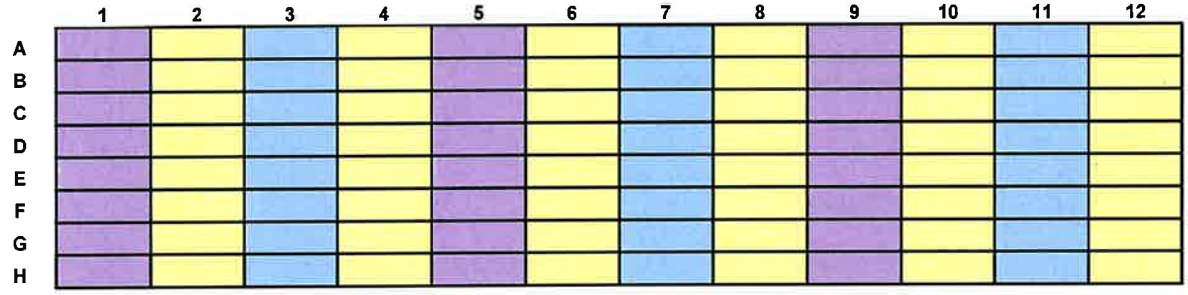


(a) Checkerboard Pattern

b) Zebra Stripe Pattern



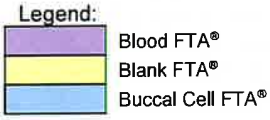


Figure 4. Checkerboard and zebra-stripe patterns utilised in the contamination check.

5.6.2. Comparisons with the manual DNA IQ™ method

Comparisons were made between results generated by the automated and manual methods to verify the performance of the automated DNA IQ™ protocol.

Verification samples consisted of different dilutions of blood and cells spotted in 30µL aliquots onto quartered cotton and rayon swabs. Four blood dilutions of neat, 1/10, 1/100 and 1/1000 and four cell dilutions of neat, 1/5.2, 1/52.2 and 1/522 were used to test the sensitivity of both the manual and automated methods. Dilutions were created using 0.9% saline solution for both sample types. Four replicates of each dilution were made up for each substrate and sample type.

The blood was collected using the same method as in 5.2. Four separate extractions were performed for the manual set based on the combination of sample type and swab type: Blood Rayon, Blood Cotton, Cell Rayon and Cell Cotton. For the automated verification, all sample types were extracted together after being transferred to a SlicPrep™ 96 Device to allow automated processing.

5.6.3. Resin volume

The performance of the automated DNA IQ™ protocol was assessed when either 7µL or 14µL of DNA IQ™ resin was used in the protocol to extract blood samples.

5.6.4. Modifying extraction volumes

The performance of the automated DNA IQ™ protocol was assessed for varying volumes of extraction buffer at 300, 350, 400, 450 and 500µL. In each case, the volume of DNA IQ™ Lysis Buffer was kept at 2x the volume of extraction buffer. Samples extracted were blood swabs, prepared as per ???.

5.6.5. Sensitivity of the automated DNA IQ™ protocol

The sensitivity of the automated DNA IQ™ protocol was assessed using dilutions of whole blood at neat, 1:10, 1:50, 1:100 and 1:1000.

6. Results and Discussion

6.1 Gravimetric Evaluation of Pipetting Accuracy and Precision

Pipetting on both automated platforms was assessed gravimetrically as per laboratory practice. Gravimetric results indicate that pipetting performance for five different pipetting behaviours using 500µL syringes on the instruments is accurate and precise to within the established threshold of ±5% (Table 2). The maximum CV at the maximum volume was 0.78%, whereas the maximum CV at the minimum volume was 1.1%. The CV for pipetting at lower volumes is expected to be slightly higher than the CV at higher volumes using 500µL syringes, because accuracy at small volumes is harder to achieve with larger syringe sizes. Nevertheless, pipetting on the extraction platforms is limited to a minimum of 50µL, which exhibited a CV of 0.36%.

Table 2. Gravimetric evaluation results for various performance files used on either MP II EXTN A or MP II EXTN B.

Performance File	Max. Vol. µL	Min. Vol. µL	Max. Vol. µL Mean	Max. Vol. %CV	Max. Vol. %Inac.	Min. Vol. µL Mean	Min. Vol. %CV	Min. Vol. %Inac.
EXTN A								
Water Blowout 175µL DT_FW _13112007RESIN.prf	50µL	N/A	49.98	0.36	0.0	N/A	N/A	N/A
Water Blowout 175µL DT_FW QHSS _13112007.prf	175µL	15µL	172.26	0.21	1.6	12.47	3.38	16.19
WaterWaste 1mL_FW_QHSS 12112007.prf	1000µL	100µL	999.11	0.24	0.1	99.22.	0.71	0.8
Water Blowout 1mL DT_QHSS _09112007.prf	1000µL	100µL	1001.02	0.27	0.1	100.65	0.63	0.7
Water Blowout Fixed Tips_08112007.prf	1000µL	100µL	995.97	0.31	0.4	99.6	0.71	0.4
EXTN B								
Water Blowout 175µL DT_FW_ 25102007RESIN.prf	50µL	N/A	50.12	0.36	0.2	N/A	N/A	N/A
Water Blowout 175µL DT_FW_ 25102007.prf	175µL	15µL	175.58	0.14	0.3	15.23	1.1	1.5
WaterWaste 1mLDT_FW_QHSS 24102007.prf	1000µL	100µL	1002.39	0.78	0.2	99.56	0.89	0.4
Water Blowout 1mL DT_QHSS 23102007.prf	1000µL	100µL	998.2	0.44	0.2	99.44	0.68	0.6

Water Blowout Fixed Tips_FW 26102007.prf	1000µL	100µL	998.87	0.68	0.1	100.37	0.74	0.4

6.2 Heater tile temperature verification

Two heater tiles on each MP II platform was verified to reach either 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 (???). 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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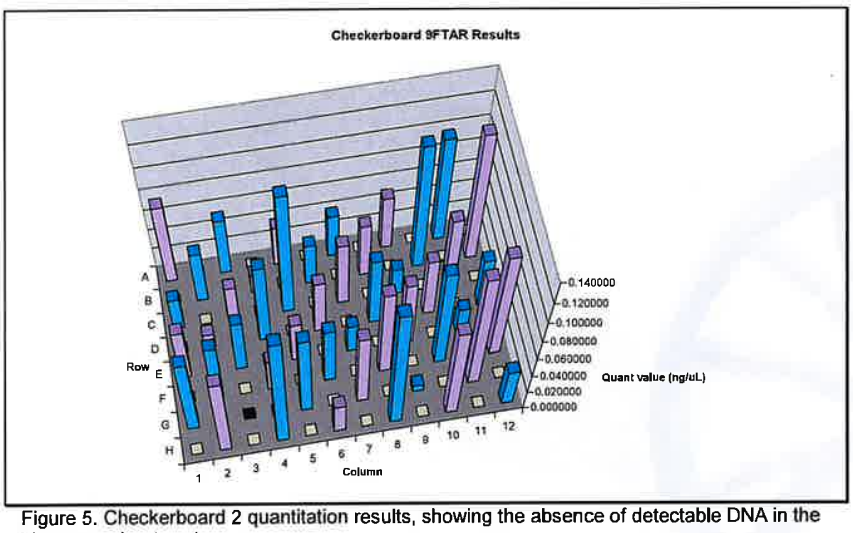

Figure 5. Checkerboard 2 quantitation results, showing the absence of detectable DNA in the blank samples (grey).

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.

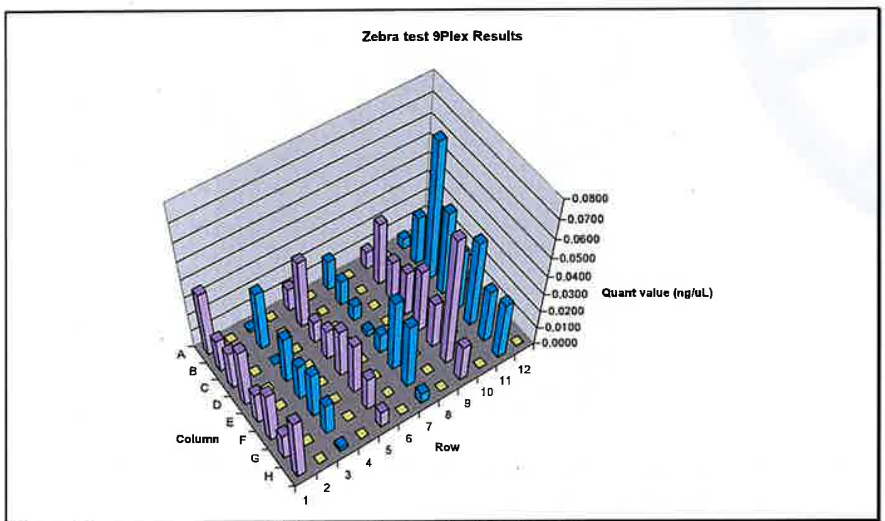

Figure 6. Zebra-Stripe 1 quantitation results, showing the absence of detectable DNA in the blank samples (grey).

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.

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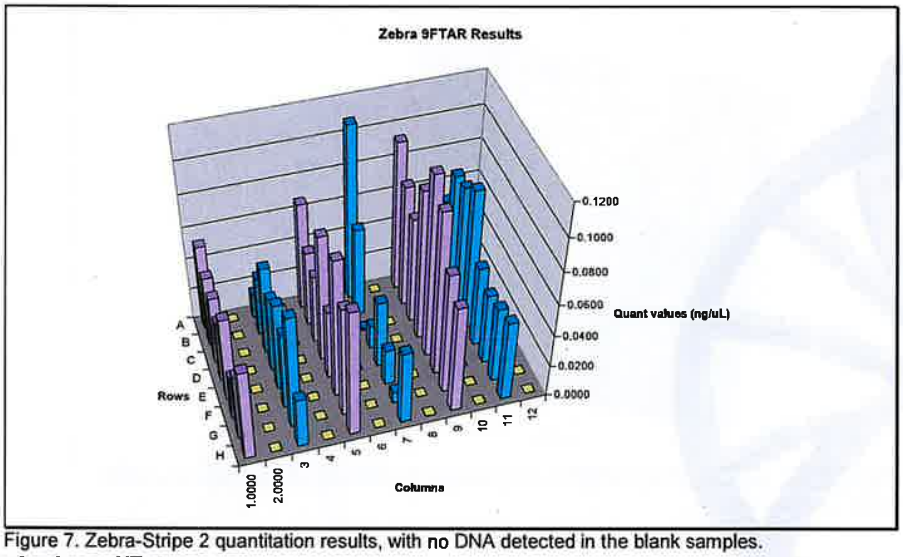

Figure 7. Zebra-Stripe 2 quantitation results, with no DNA detected in the blank samples.

Checkerboard/Zebra

None of the blank samples yielded DNA profiles, all of the positive cell and positive blood samples yielded the correct DNA profile. DNA was undetected in the blank samples (Figure 8).

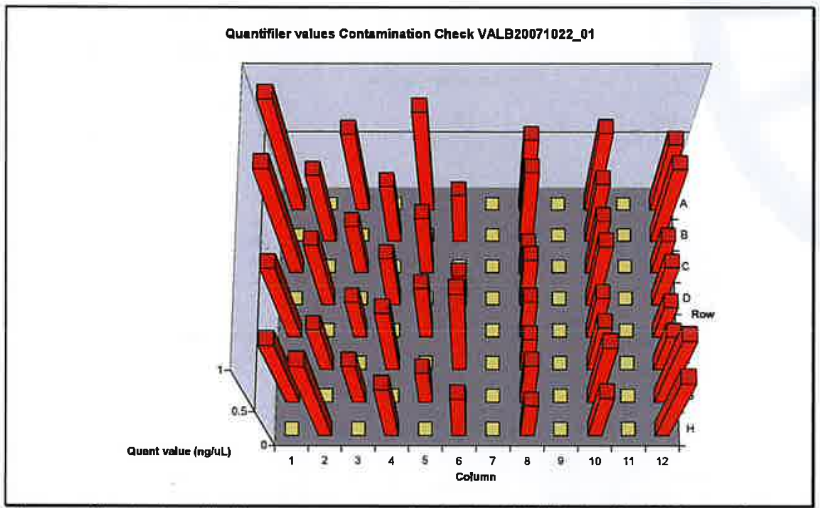

Figure 8. Checkerboard/zebra plate that was extracted on MP II Extraction Platform A because the previous plate was invalidated. DNA was not detected in the blank samples (grey).

6.4 Comparisons with the manual DNA IQ™ method

When dilutions of either blood or cells were applied on to either rayon or cotton swabs, followed by extraction using the DNA IQ™ method, the results of the automated method were always lower in yield compared to the manual method. For blood samples on rayon

swabs, the automated method generated yields that were on average around 8% (SD 8.45%) of the automated method. For blood on cotton swabs, the yield from the automated method was also around 8% (SD 3.62%). The yields for cell samples were higher at around 33% (SD 16.29%) and 25% (10.32%) for cells on rayon and cotton swabs respectively.

The manual method was found to be more sensitive than the automated method. Out of five replicates at the 1/100 and 1/1000 dilutions for blood on rayon swabs that were processed using the manual method, five and three replicates respectively were detected (and none from the automated method) (see Figure 9). The trend is repeated for blood on cotton swabs (Figure 10). For cell samples on either rayon or cotton swabs, the automated method was found to be more sensitive as evidenced by detection of DNA at the 1/522 dilutions (Figure 11 and 12).

Cell clumping may have occurred with the cell dilutions, therefore causing inaccurate dilutions as can be observed in the ratios between each dilution.

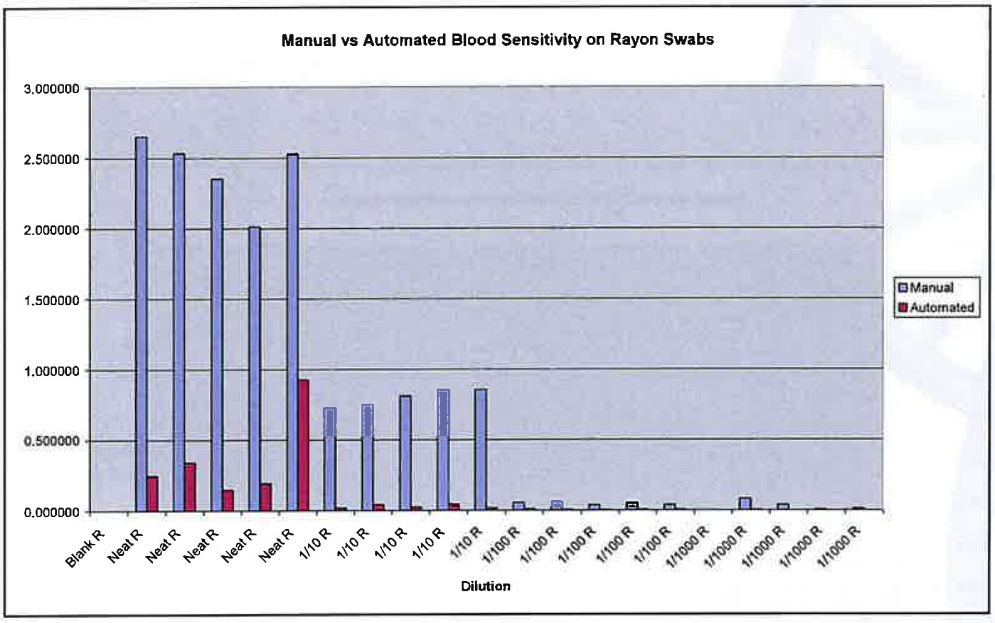

Figure 9. Comparison of sensitivity between the manual and automated DNA IQ™ methods for blood samples on rayon swabs.

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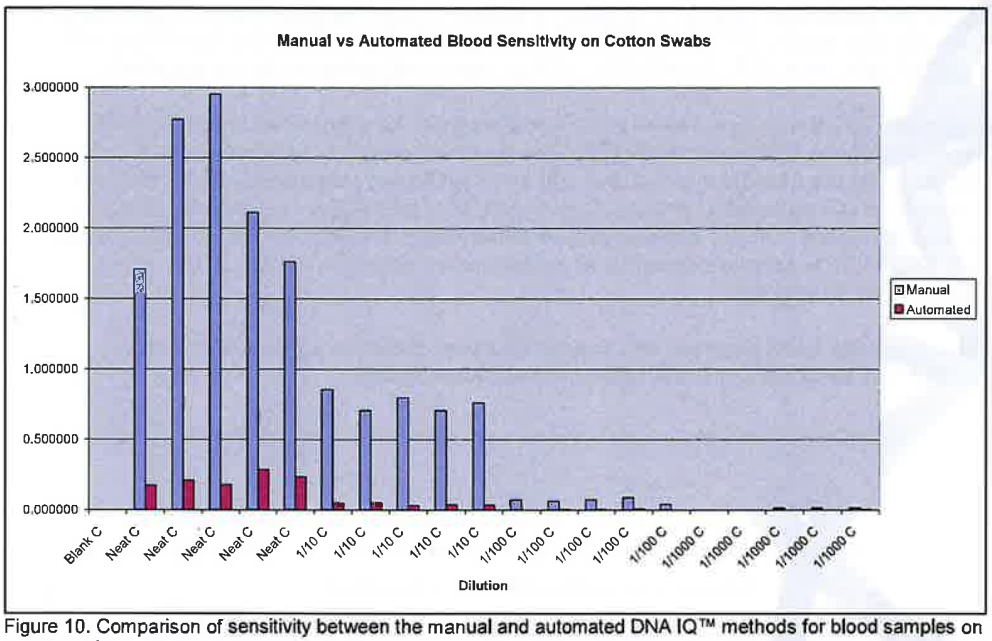

Figure 10. Comparison of sensitivity between the manual and automated DNA IQ™ methods for blood samples on cotton swabs.

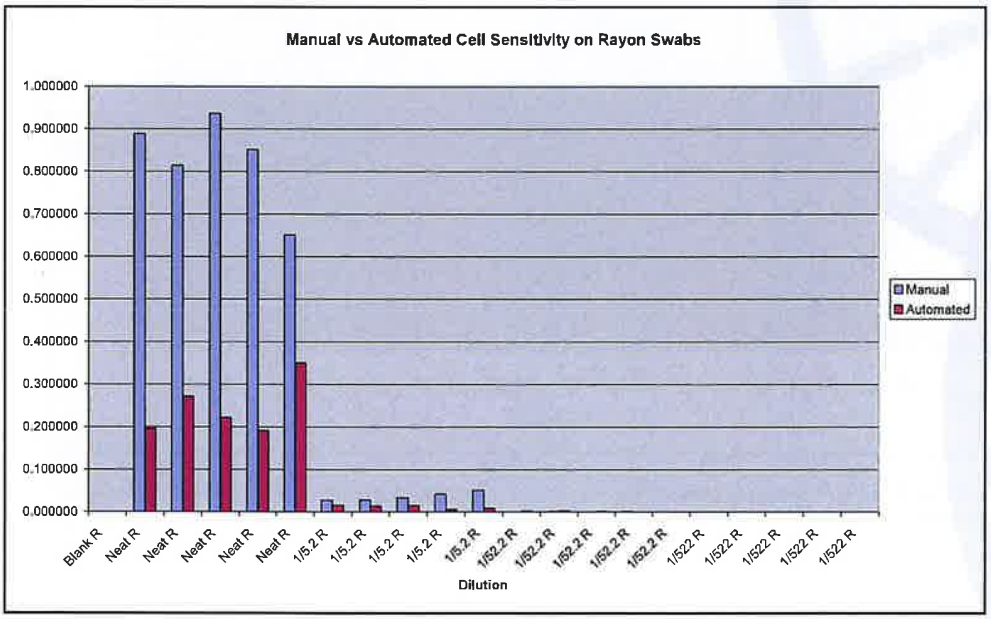

Figure 11. Comparison of sensitivity between the manual and automated DNA IQ™ methods for cells samples on rayon swabs.

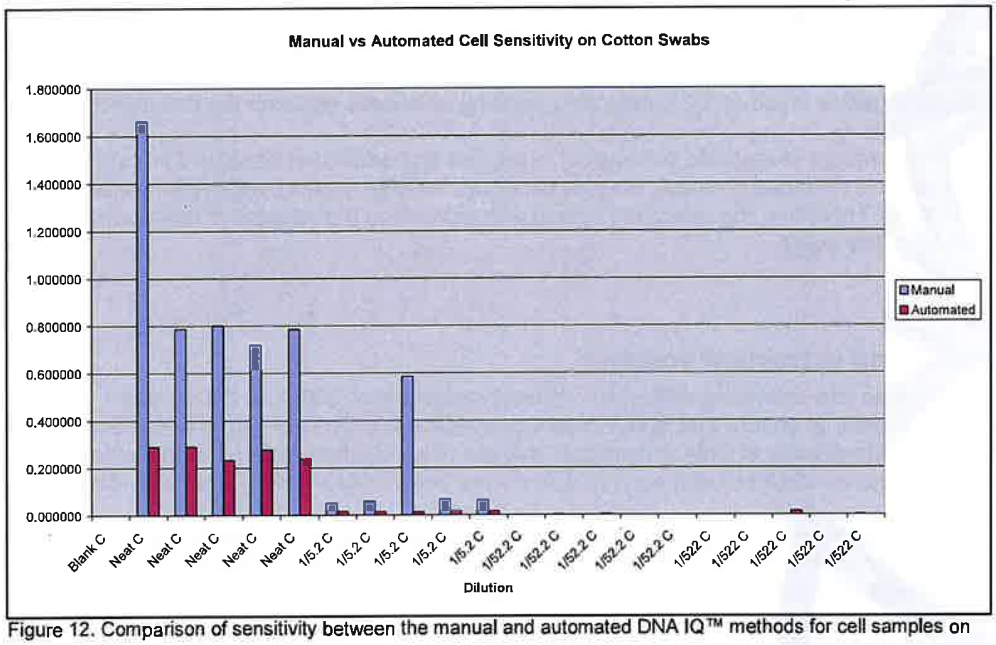

Figure 12. Comparison of sensitivity between the manual and automated DNA IQ™ methods for cell samples on cotton swabs.

6.5 Investigating resin volume

Promega recommends the use of 7µL of DNA IQ™ resin with their protocol. We investigated the performance of the protocol with double the amount of resin (14µL) in order to assess any benefits that may be gained in terms of the resulting yield and quality of the STR profile.

It was observed that doubling the resin resulted in a proportional doubling of the yield. On average, doubling the resin increased the yield by an additional 77.28% (*n*=4). The average yield from an extraction using 7µL of resin was 64.725ng (SD 32.21ng, *n*=4), whereas 14µL resin generated 114.75ng (SD 10.72ng, *n*=4) (Table 6). At the higher resin concentration, the amount of DNA isolated appears to be capped at around 100ng, indicating no change in the ability of the reaction to isolate more DNA due to saturation of resin.

Table 6. Comparison of the effects of doubling the amount of recommended DNA IQ™ resin.

Sample ID	Resin volume	[DNA] ng/µL	Reportable alleles
33383-4216	7µL	0.701	18/18
33383-4225		1.070	18/18
33383-4239		0.319	18/18
33383-4248		0.499	18/18
33383-4252	14µL	1.140	18/18
33383-4261		1.270	18/18
33383-4270		1.010	18/18
33383-4284		1.170	18/18

Samples extracted using either amount of resin generated concordant full DNA profiles (18/18 alleles). Samples processed using the 14µL method produced peaks that were slightly higher. The difference in peak heights between alleles within the same loci ranged from 59-86%, with a mean of 71%, indicating minimal difference between the two methods.

Doubling the amount of resin did not appear to provide any additional benefits compared to the original recommended protocol. More importantly, full DNA profiles were resolved using either method. Therefore, the costs associated with increasing the amount of resin cannot be justified at this stage.

6.6 Modifying extraction volumes

An investigation into optimising extraction volumes ranging from 300µL to 500µL was performed in order to ensure that buffer coverage over the samples was sufficient to enable optimal lysis and release of DNA. In addition, the use of an optimum volume of extraction reagents increases efficiency and economy, therefore potentially lowering laboratory costs.

Although the higher extraction volume generated higher yields when processed using the automated DNA IQ™ protocol (Table 7), DNA profile results were comparable across the various extraction volumes tested for eight replicates each (Table 8). Three instances of allelic imbalance were encountered in two samples from the 300µL and 450µL tests. In all instances, allelic imbalance was greater than 69%.

Table 7. DNA profile results for samples extracted using various volumes of Extraction Buffer, for 8 replicates.

Extraction Buffer Volume (µL)	Mean [DNA] (ng/µL)	SD
300	2.04	0.07
350	2.16	0.09
400	1.69	0.10
450	3.14	0.13
500	3.64	0.17

Table 8. DNA profile results for samples extracted using various volumes of Extraction Buffer, for 8 replicates.

Sample	Extraction Buffer Volume (µL)	DNA Profile Result
300-1 swab	300	OK
300-2 swab		OK
300-3 swab		OK
300-4 swab		OK
300-5 swab		OK
300-6 swab		OK
300-7 swab		AI D13
300-8 swab		OK

Table 1. Extraction kits that were evaluated by Forensic Biology FSS.

DNA extraction kit and manufacturer	Technology type
DNA IQ™ (Promega Corp., Madison, WI, USA)	Novel paramagnetic beads
QIAamp® DNA Micro (Qiagen GmbH, Hilden, Germany)	Silica-based membrane
ChargeSwitch® (Invitrogen, Carlsbad, CA, USA)	Magnetic beads
*forensic*GEM™ (ZyGEM, Hamilton, NZ)	Thermophilic proteinase incubation
NucleoSpin® 8 Trace (Macherey-Nagel, Düren, Germany)	Silica-based membrane

Magnetic bead technology is based on the use of magnetic resin that has the capability to bind DNA when subjected to a particular environmental pH or ionic strength. Therefore, by using buffers with different pH values or different ionic components, the binding and elution of DNA can be controlled. Furthermore, whilst the DNA is bound to the resin, the resin-DNA complex can be washed using an alcohol-containing buffer in order to remove inhibitors and residual proteins. A magnetic force is applied during the washing procedure to immobilise the resin-DNA complex and ensure no DNA is lost during washing. Membrane technology is based on a similar principle, except the DNA is immobilised in a thin silica-based membrane within the column.

*forensic*GEM™, the recently-released one-tube proteinase incubation system, uses a thermostable enzyme to digest nucleases in order to yield a crude DNA extract. The enzyme digest method does not incorporate any washing steps, however, and therefore inhibitors are not removed from solution.

3. Aim

To evaluate several commercial DNA extraction kits (as per Table 1) that were specifically designed for forensic DNA samples, using the manufacturer's recommended manual protocols, and compare against the current in-house Chelex® protocol, in order to select a suitable kit for manual validation and automated verification.

4. Equipment and Materials

- Chelex®-100, P/N 143-2832 (Biorad, Hercules, CA, USA)
- DNA IQ™ System, P/N DC6701 (Promega Corp., Madison, WI, USA)
- QIAamp® DNA Micro Kit, P/N 56304 (Qiagen GmbH, Hilden, Germany)
- ChargeSwitch® Forensic DNA Purification Kit, P/N CS11200 (Invitrogen, Carlsbad, CA, USA)
- *forensic*GEM™ (ZyGEM, Hamilton, NZ)
- NucleoSpin® 8 Trace, P/N 740 722.1 (Macherey-Nagel, Düren, Germany)

For preparation of buffers and reagents specific for each kit, see the Methods section that is relevant for that kit.

5. Methods

5.1 Mock sample creation

Refer to document "Mock sample creation for cell and blood samples" (Gallagher *et al.*, 2007) for the detailed protocol.

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5.2 DNA extraction kit protocols

The following section provides the principle and protocol for each DNA extraction kit as recommended by the manufacturer. The Chelex® method was as per QIS 17171.

5.2.1. *Chelex®-100 (BioRad)*

Principle

Chelex® is a chelating resin composed of styrene divinylbenzene copolymers, which have a high affinity for polyvalent metal ions. The copolymers contain paired iminodiacetate ions acting as chelating groups which chelate metal ions, including some that degrade DNA while boiling the sample to obtain eluted DNA. Chelex® is the current Forensic Biology FSS standard in-house extraction protocol.

Equipment and Materials

- 20% Chelex® solution (w/v)
- Waterbath
- Magnetic stirrer plate
- 1.5mL sterile tubes
- Spin baskets
- Autoclaved nanopure water
- Vortex
- Centrifuge
- Twirling sticks
- Proteinase K (10mg/mL)
- FTA® Classic Card, P/N WB120205 (Whatman Plc)

Preparation of reagents

- *20% Chelex®-100*
  On balance, to a beaker containing a magnetic stirrer bar, add 2 grams of Chelex®-100 resin. To this, add 10mL of autoclaved nanopure water to make a 20% w/v solution and cover with parafilm. To ensure that the Chelex® is evenly dispersed, place beaker onto a magnetic stirrer plate before pipetting.

Methods (see QIS 17171R9)

1. Label sterile 1.5mL screw-capped tubes which contain sample as well as new elution tubes including extraction controls.
2. Pipette 1mL of autoclaved nanopure water into each tube, vortex gently.
3. Incubate at room temperature for 30 minutes.

The following steps are determined by sample type.

<u>For Cells</u>

4. For buccal FTA® punches, place tubes on multitube vortex for 5min at 12,000rpm.
5. For cell and/or fabric samples, twirl the substrate with a sterile twirling stick for 2min.

Note: Vortex FTA® punches samples then go to "For all sample types."

6. Transfer swab/fabric into spin baskets.
7. Spin tubes with spin basket for 30s at maximum speed (~15,800g or the applicable centrifuge's maximum setting). Discard spin basket with swab.

8. Vortex supernatant, then pour back into original extract tube.

For all sample types

9. Vortex, then spin in centrifuge for 3min at maximum speed (~15,800g or the applicable centrifuge's maximum setting).
10. Carefully remove all but 50µL of supernatant. Leave substrate in tube with pellet.
11. Add 150µL of 20% Chelex® to each tube and vortex.

Note: When pipetting Chelex, the resin beads must be distributed evenly in the solution. Use magnetic stirrer in beaker of Chelex and wide bore pipette tips.

12. Add 4µL of Proteinase K (10mg/mL) to cells and mix gently by vortexing.
13. Incubate in 56°C water bath for 30min for blood and cell samples.
14. Vortex until mixed, then incubate in boiling water bath for 8min.
15. Vortex until mixed, then centrifuge for 3min at maximum speed (~15,800g or the applicable centrifuge's maximum setting).
16. Transfer supernatant to new labelled 1.5mL screw-capped tube leaving Chelex® beads behind.
17. Samples are stored at -20°C.

5.2.2. *DNA IQ™ System (Promega Corp.)*

Principle

The Promega DNA IQ™ system for small casework samples incorporates two distinct steps. The first step provides an easy, rapid, efficient and almost universal cell lysis method to extract biological materials off stains on solid supports. The second step utilised a specific paramagnetic resin that purifies DNA without extensive washing to remove the lysis reagent. The DNA IQ™ system is designed to purify DNA samples approximately 100ng or less, and is more efficient with samples containing less than 10ng of DNA.

Equipment and Materials

- DNA IQ™ System (100 samples, Cat.# DC6701) containing:
  - 0.9mL Resin
  - 40mL Lysis Buffer
  - 30mL 2X Wash Buffer
  - 15mL Elution Buffer
- MagneSphere® Magnetic Separation Stand, 12-position (Cat.# Z5342)
- DNA IQ™ Spin Baskets (Cat.# V1221)
- Microtube 1.5mL (Cat.# V1231)
- 95-100% ethanol
- Isopropyl alcohol
- 1M DTT
- 65°C heat block
- 70°C heat block
- Vortex mixer

Preparation of Buffers

- *Preparing 1X Wash Buffer*
  i. For DC6701 (100 samples), add 15mL of 95-100% ethanol and 15mL of isopropyl alcohol to 2X Wash Buffer.
  ii. Replace cap and thoroughly mix by inversion.

iii. Mark label to record addition of alcohols.
iv. Label bottle as "1X Wash Buffer".
v. Store bottle at room temperature with lid closed tightly to prevent evaporation.

- *Preparing Lysis Buffer*
  i. Determine the total amount of Lysis Buffer to be used (Table 2) and add 1µL of 1M DTT for every 100µL of Lysis Solution.

Table 2. Total amount of Lysis Buffer required for different sample material types.

Material	Lysis Buffer[1]	Lysis Buffer[2]	Total Buffer
Liquid blood	100µL	100µL	200µL
Cotton swab	250µL	100µL	350µL
1/4th CEP swab	250µL	100µL	350µL
15-50mm² S&S 903 paper	150µL	100µL	250µL
3-30mm² FTA® paper	150µL	100µL	250µL
Cloth up to 25mm²	150µL	100µL	250µL

[1] For use in Step 2; [2] For use in Step 9.

ii. Mix by inversion.
iii. Mark and date label to record addition of DTT.
iv. Seal tube and store solution at room temperature for up to one month if required.

Method

DNA isolation from stains on solid material (non-liquid samples)

1. Place sample in a 1.5mL Microtube. The recommended amount of resin can capture a maximum of ~100ng DNA, therefore consider this when determining amount of sample to add.
2. Add 250µL of prepared Lysis Buffer (Table 2). Close lid and place on a 70°C heat block for 30min.
3. Remove tube from heat block and transfer the Lysis Buffer and sample to a DNA IQ™ Spin Basket.
4. Centrifuge at room temperature for 2min at maximum speed. Remove spin basket.
5. Vortex the stock Resin for 10s until it is thoroughly mixed. Add 7µL Resin to the sample. Keep the Resin resuspended while dispensing to obtain uniform results.
6. Vortex sample / Lysis Buffer / Resin mix for 3s. Incubate at room temperature for 5min.
7. Vortex for 2s and place tube in the MagneSphere® Magnetic Separation Stand. Separation will occur instantly.
8. Carefully remove and discard all of the solution without disturbing the Resin on the side of the tube.
9. Add 100µL of prepared Lysis Buffer. Remove the tube from the MagneSphere® Magnetic Separation Stand and vortex for 2 seconds.
10. Return tube to the MagneSphere® Magnetic Separation Stand and discard all Lysis Buffer, without disturbing the resin on the side of the tube.
11. Add 100µL prepared 1X Wash Buffer. Remove tube from the MagneSphere® Magnetic Separation Stand and vortex for 2s.
12. Return tube to the MagneSphere® Magnetic Separation Stand and discard all Wash Buffer, without disturbing the resin on the side of the tube.
13. Repeat steps 11 and 12 once for a total of 2 washes. Make sure that all of the solution has been removed after the last wash.

14. With lid open, air-dry the Resin in the MagneSphere® Magnetic Separation Stand for 5min to 15min.
15. Add 25-100µL Elution Buffer, depending on how much biological material was used. A lower elution volume ensures a higher final concentration of DNA.
16. Close the lid, vortex the tube for 2s and incubate at 65°C for 5min.
17. Remove the tube from the heat block and vortex for 2s. Immediately place on the MagneSphere® Magnetic Separation Stand.
18. Transfer the solution to a fresh tube.
19. Store the DNA extract at 4°C for short-term storage or at -20 or -70°C for long term storage.

5.2.3. *QIAamp® DNA Micro (Qiagen)*

Principle

The QIAamp® DNA Micro kit combines selective binding properties of a silica-based membrane with flexible elution volumes that is suitable for a wide range of sample materials such as small volumes of blood, blood cards, small tissue samples and forensic samples. The basic procedure consists of 4 steps:

- Lysis: the sample is lysed;
- Bind: the DNA in the lysate binds to the membrane of the QIAamp® MinElute column;
- Wash: the membrane is washed;
- Elute: DNA is eluted from the membrane.

Equipment and Materials

- QIAamp® DNA Micro kit containing:
  - QIAamp® MinElute Columns;
  - collection tubes (2mL);
  - Buffer ATL;
  - Buffer AL;
  - Buffer AW1 (concentrate);
  - Buffer AW2 (concentrate);
  - Buffer AE;
  - carrier RNA (red cap);
  - Proteinase K.
- Ethanol (96-100%)
- 1.5mL or 2mL microcentrifuge tubes (for lysis steps)
- 1.5mL microcentrifuge tubes (for elution steps)
- Pipette tips
- Thermomixer
- Microcentrifuge with rotor for 2mL tubes
- Scissors
- Blood collection cards or FTA® card
- Sterile cotton swabs
- DTT

<u>Important points before starting</u>

- Perform all centrifugation steps at room temperature (15-25°C).
- Check whether carrier RNA is required; for purification of DNA from very small amounts of sample, such as low volumes of blood (<10µL) or forensic samples, it is recommended to add

carrier RNA to Buffer AL. For samples containing larger amounts of DNA, addition of carrier RNA is optional.

Steps to perform before starting

- Equilibrate Buffer AE or distilled water for elution to room temperature (15-25°C).
- Set a thermomixer or heated orbital incubator to 56°C for use in step 2, and a second thermomixer or heated orbital incubator to 70°C for use in step 5. If thermomixer or heated orbital incubators are not available, heating blocks or water baths can be used instead.
- If Buffer AL or Buffer ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.
- If processing semen stains, hair, or nail clippings, prepare an aqueous 1M DTT (dithiothreitol) stock solution. Store aliquots at -20°C. Thaw immediately before use.
- Ensure that Buffers AW1 and AW2 have been prepared according to the instructions.

Preparation of Buffers

- *Preparing Buffer ATL*
  Before starting the procedure, check whether precipitate has formed in Buffer ATL. If necessary, dissolve by heating to 70°C with gentle agitation.
- *Preparing Buffer AL*
  Before starting the procedure, check whether precipitate has formed in Buffer AL. If necessary, dissolve by heating to 70°C with gentle agitation.
- *Preparing Buffer AW1*
  Add 25mL ethanol (96-100%) to the bottle containing 19mL Buffer AW1 concentrate. Tick the check box on the bottle label to indicate that ethanol has been added. Reconstituted Buffer AW1 can be stored at room temperature (15-25°C) for up to 1 year. Note: before starting the procedure, mix the reconstituted Buffer AW1 by shaking.
- *Preparing Buffer AW2*
  Add 30mL ethanol (96-100%) to the bottle containing 13mL Buffer AW2 concentrate. Reconstituted Buffer AW2 can be stored at room temperature (15-25%) for up to 1 year. Note: before starting the procedure, mix the reconstituted Buffer AW2 by shaking.

Method

1. Lysing material stained with blood or saliva: cut out up to 0.5cm^2 of stained material and then cut into smaller pieces. Transfer the pieces to a 2mL microcentrifuge tube. Add 300µL buffer ATL, and 20µL Proteinase K. Close the lid and mix by pulse-vortexing for 10s. Continue this procedure from step 2.
2. Place the tube in a thermomixer or heated orbital incubator, and incubate at 56°C with shaking at 900rpm for at least 1hr. In general, hair is lysed in 1hr. If necessary, increase the incubation time to ensure complete lysis.
3. Briefly centrifuge the tube to remove droplets from the inside of the lid.
4. Add 300µL Buffer AL, close the lid, and mix by pulse vortexing for 10s. To ensure efficient lysis, it is essential that the sample and buffer AL are

thoroughly mixed to yield a homogeneous solution. A white precipitate may form when Buffer AL is added to buffer ATL. The precipitate does not interfere with the QIAamp® procedure and will dissolve during incubation in step 5. Note: if carrier RNA is required, add 1µg dissolved carrier RNA to 300µL buffer AL.

5. Place the tube in the thermomixer or heated orbital incubator, and incubate at 70°C with shaking at 900rpm for 10min. If using a heating block or water bath, vortex the tube for 10s every 3min to improve lysis
6. Centrifuge the tube at full speed on a bench top centrifuge (20,000g; 14,000rpm) for 1min.
7. Carefully transfer the supernatant from step 6 to the QIAamp® MinElute column without wetting the rim. Close the lid, and centrifuge at 6,000g (8,000rpm) for 1min. Place the QIAamp® MinElute column in a clean 2mL collection tube, and discard the collection tube containing the flow-through.
8. If lysate has not completely passed through the membrane after centrifugation, centrifuge again at a higher speed until QIAamp® MinElute column is empty.
9. Carefully open the QIAamp® MinElute column and add 500µL Buffer WA1 without wetting the rim. Close the lid and centrifuge 6,000g (8,000rpm) for 1min. Place the QIAamp® MinElute column in a clean 2mL collection tube and discard the collection tube containing the flow-through.
10. Carefully open the QIAamp® MinElute column and add 500µL Buffer AW2 without wetting the rim. Close the lid and centrifuge at 6,000g (8,000rpm) for 1min. Place the QIAamp® MinElute column in a clean 2mL collection tube, and discard the collection tube containing the flow-through. Contact between the QIAamp® MinElute column and the flow-through should be avoided. Some centrifuge rotors may vibrate upon deceleration, resulting in the flow through, which contains ethanol, coming into contact with the QIAamp® MinElute column. Take care when removing the QIAamp® MinElute column and collection tube from the rotor, so that flow-through does not come into contact with the QIAamp® MinElute column.
11. Centrifuge at full speed (20,000g; 14,000rpm) for 3min to dry the membrane completely. This step is necessary, since ethanol carryover into the eluate may interfere with some downstream applications.
12. Place the QIAamp® MinElute column in a clean 1.5mL microcentrifuge tube and discard the collection tube containing the flow through. Carefully open the lid of the QIAamp® MinElute column and apply 45µL Buffer AE (equilibrated to room temperature) to the centre of the membrane to ensure complete elution of bound DNA. QIAamp® MinElute columns provide flexibility in the choice of elution volume.
13. Close the lid and incubate at room temperature (15-25°C) for 1min. Centrifuge at full speed (20,000g; 14,000rpm) for 1min. Incubating the QIAamp® MinElute columns loaded with Buffer AE or water for 5min at room temperature before centrifugation generally increases DNA yield.

5.2.4. *ChargeSwitch® (Invitrogen)*

Principle

ChargeSwitch® uses a novel magnetic bead-based technology known as ChargeSwitch Technology® (CST®). CST® provides a switchable surface charge, which is switched on and off by changing the pH. With a low pH buffer, the negatively charged DNA backbone binds to the positively charged beads and with a high pH buffer, DNA is eluted by neutralising the charge on the beads.

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ChargeSwitch® uses a universal lysis step for all forensic sample types and has been designed to elute DNA from small sample volumes.

ChargeSwitch® uses a basic 4 step principle:

1. Lyse sample;
2. Negatively charged DNA binds to positively charged beads in a buffer with a pH ≤ 6 so charge is switched on;
3. At a pH of 7, charge is still on while beads and bound DNA is washed, removing any contaminants;
4. In a buffer with a pH of 8.5, charge is switched off and DNA is eluted from the beads.

Equipment and Materials

- ChargeSwitch® Forensic DNA Purification kit (stored at room temperature) includes (for 100 preps):
  - ChargeSwitch® Lysis Buffer (L13) – 100mL
  - ChargeSwitch® Magnetic Beads (storage buffer: 10mM MES, pH 5.0, 10mM NaCl, 0.1% Tween 20) – 2 x 1mL
  - Proteinase K (20mg/ml in 50mM Tris-HCl, pH 8.5, 5mM $CaCl^2$ 50% glycerol stored at 4°C) – 1mL
  - ChargeSwitch® Purification Buffer (N5) – 20mL
  - ChargeSwitch® Wash Buffer (W12) – 100mL
  - ChargeSwitch® Elution Buffer (E5; 10mM Tris-HCl, pH 8.5) – 15mL
- MagnaRack™, P/N CS15000 (Invitrogen)
- Sterile, 1.5mL microcentrifuge tubes
- Vortex mixer
- Waterbath set at 55°C

Method

1. Set water bath at 55°C and prepare Lysis master mix in appropriate sized tube using the following formula: *n* x (1mL ChargeSwitch® Lysis buffer + 10µL Proteinase K) where *n* is the number of samples.
2. To tube add 1mL of ChargeSwitch® Lysis Buffer (L13) and immerse forensic sample in mix.
3. Vortex/invert samples for 10-15s to mix then incubate in 55°C water bath for 1hr. Incubation can be shortened to 30min if sample is vortexed or inverted during this step.
4. Remove sample or transfer lysate to clean tube using 1mL pipette tips and pipette.
5. Vortex ChargeSwitch® Magnetic Beads to resuspend evenly in storage buffer.
6. Add 200µL of ChargeSwitch® Purification Buffer (N5) to lysate and mix gently by pipetting up and down.
7. Add 20µL of ChargeSwitch® Magnetic Beads to sample. Pipette-mix to ensure that no bubbles form.
8. Incubate for 1-5min at room temperature to allow the DNA to bind and then place sample tube in MagnaRack™ until a tight pellet has formed. Once this has occurred, aspirate supernatant from tube whilst still in rack and discard, ensuring that the pellet is not disturbed.
9. When supernatant has been completely discarded, remove tube from rack and add 500µL ChargeSwitch® Wash Buffer (W12). Mix gently by pipetting up and down to resuspend the pellet.

10. Allow beads to form a tight pellet by placing tube in MagnaRack™ and remove supernatant completely, without removing from rack or disturbing the pellet and discard.
11. Repeat steps 9 and 10 again.
12. Remove tube from rack, ensuring that supernatant has been completely removed and add 150µL ChargeSwitch® Elution Buffer (E5). Mix by pipetting up and down 10 times.
13. At room temperature, incubate for 1-5min then resuspend pellet and mix like in step 12.
14. Place tube in MagnaRack™ for 1min or until a tight pellet forms. Without removing tube from rack, aspirate DNA supernatant and place in a clean, sterile 1.5mL microcentrifuge tube, ensuring that the pellet is not disturbed. If elution is discoloured repeat steps 12 to 14 again.
15. Discard beads once extraction process is finished and either quantify immediately or store at -20°C.

5.2.5. *forensicGEM™ (ZyGEM)*

Principle

*forensic*GEM™ is a novel thermophilic proteinase developed as a rapid, cheap and effective DNA extraction solution for forensic laboratories that was recently released. It is a simple closed tube forensic DNA extraction method using a thermostable proteinase.

Protocols are available for blood and cell samples.

Equipment and Materials

- *forensic*GEM™ buffer
- *forensic*GEM™
- Heat block or water bath set at 75°C and 95°C
- 20µL sterile Aerosol Resistant Tips
- 0.5-10µL pipettor
- 300µL sterile Aerosol Resistant Tips
- 20-200µL pipettor
- 1mL sterile Aerosol Resistant Tips
- 50µL-1mL pipettor

Method

DNA extraction from buccal swabs using *forensic*GEM™

1. Add buccal swab to tube.
   Note: 1/4 head of swab specified but can utilise up to whole swab.
2. Add 200µL of *forensic*GEM™ buffer.
   Note: if more than 1/4 head of buccal swab is used need to add more *forensic*GEM™ buffer. Moss *et al.* (2003) added 200µL more of the *forensic*GEM™ buffer for trace samples.
3. Add 2µL of *forensic*GEM™.
   Note: *forensic*GEM™ buffer and *forensic*GEM™ can be added as a mastermix.
4. Incubate at 75°C for 15min.
5. Incubate at 95°C for 5min.
6. Remove supernatant to a new tube for storage.

DNA extraction from FTA® containing blood or salive using *forensic*GEM™

1. UV irradiate plasticware for 5min.

2. Add FTA® punches to each well of a 96-well plate.
   Note: Larger punches can be added but not scalable SOP. PCR tubes can also be used for processing.
3. Add 100µL H_2O and leave at room temperature for 15min.
4. Decant water (remove by pipetting).
5. Add 100µL *forensic*GEM™ buffer and 2µL of *forensic*GEM™.
   Note: The method is not listed as scalable.
6. Incubate at 75°C for 15min.
7. Incubate at 95°C for 5min.
8. Remove supernatant to a new tube for storage.

5.2.6. *NucleoSpin® 8 Trace (Macherey-Nagel)*

Principle

With the NucleoSpin® 8 Trace method, genomic DNA is prepared from forensic samples. Lysis is achieved by incubation of samples in a solution containing chaotropic ions in the presence of proteinase K at room temperature. Appropriate conditions for binding of DNA to the silica membrane in the NucleoSpin® Trace Binding Strips are created by addition of isopropanol to the lysate. The binding process is reversible and specific to nucleic acids. Inhibitors are removed by two washing steps with ethanolic buffer. Pure genomic DNA is finally eluted under low ionic strength conditions in a slightly alkaline elution buffer.

Equipment and Materials

- NucleoSpin® 8 Trace kit, containing:
  - Buffer FLB
  - Buffer B5 (concentrate)
  - Proteinase K (lyophilised)
  - Proteinase Buffer
  - Buffer BE
  - NucleoSpin® Trace Binding Strips
  - MN Wash Plate
  - MN Square-well Blocks
  - MN Tube Strips
  - Cap Strips
  - Self-adhering PE Foil
- NucleoSpin® 8 Trace Starter Set A containing Column Holders A and Dummy Strips
- PVM vacuum manifold (from MultiPROBE® II PLUS HT EX platform)

Preparation of Buffers

- *Proteinase K*
  Add 3mL Proteinase Buffer per vial to dissolve the lyophylised proteinase K and store at -20°C.

- *Buffer B5*
  Add 160mL ethanol to 40mL Buffer B5.
- Store all other components of the kit at room temperature. Storage at lower temperatures may cause precipitation of salts. If a salt precipitate is observed, incubate the bottle at 30-40°C for a few minutes and mix well until all precipitation is redissolved.

Method

1. Premix 25µL Proteinase K and at least 125µL buffer FLB and add to sample. Incubate the sample at room temperature for 3 hours.
2. Insert spacers "MTP/Multi 96 plate" into the vacuum manifold. Place the waste container inside the vacuum manifold and insert a MN Wash Plate into the notches of the spacers. Close the manifold with the lid.
3. Place a NucleoSpin® Trace Binding Strips inserted in Column Holder A into the rubber seal of the vacuum manifold's lid and apply the samples to the wells of the plate.
4. Add 1 volume isopropanol to 2 volumes of lysate, mix three times and transfer to NucleoSpin® Trace Binding Strips.
5. Bind genomic DNA by applying vacuum until all lysates have passed through the columns (-200mbar 2min; -600mbar 10s). Ventilate the vacuum manifold.
6. Wash silica membrane by adding 900µL Buffer B5 to each well of the NucleoSpin® Trace Binding Strips. Apply vacuum (-200mbar 1min) until all buffer has passed through the columns. Ventilate the vacuum manifold.
7. Repeat the wash procedure once.
8. After the final washing step, close the valve, ventilate the vacuum manifold and remove the wash plate and waste container from the vacuum manifold.
9. Remove any residual washing buffer from the NucleoSpin® Trace Binding Strips. If necessary, tap the outlets of the NucleoSpin® Trace Binding Strips onto a clean paper sheet (supplied with the MN Wash Plate) or soft tissue until no drops come out. Insert the column holder with NucleoSpin® Trace Binding Strips into the lid and close the manifold. Apply maximum vacuum (-600mbar) for at least 10min to dry the membrane completely. This step is necessary to eliminate traces of ethanol. Close the valve and ventilate the vacuum manifold.
10. For elution, insert spacers "Microtube Rack" into manifold and rest rack with MN Tube Strips on spacers. Insert Column Holder A with NucleoSpin® Trace Binding Strips into manifold lid. Pipette 100µL Buffer BE directly to the bottom of each well and incubate for 5min at room temperature. Apply vacuum (-400mbar 2min).

5.3 DNA quantitation

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

5.4 PCR amplification and fragment analysis

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

5.5 Capillary electrophoresis and fragment analysis

PCR product was prepared for capillary electrophoresis using the manual 9+1 protocol (refer to Project 15 and QIS 19978). Capillary electrophoresis was performed on an ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) under the following conditions: 3kV injection voltage,10 sec injection time, 15kV run voltage,100µA run current, and 45min run time. Data Collection Software version 1.1 was used to collect

raw data from the ABI Prism® 3100 Genetic Analyzer. Fragment size analysis was performed using GeneScan 3.7. Allele designation was performed using Genotyper 3.7, with thresholds for heterozygous and homozygous peaks at 150 and 300 RFU respectively. The allelic imbalance threshold is 70%.

6. Results and Discussion

6.1 Criteria for acceptance

Various commercial DNA extraction kits (as per Table 1) were evaluated in order to compare their performance against the current in-house Chelex® protocol. These kits were chosen because they were designed specifically for forensic samples and representative of the DNA capture technologies that were out on the market. Furthermore, these kits were manufactured by leaders in the field of DNA extraction technologies with a track record performance in supplying the forensic market with new and reliable products.

We assessed both magnetic bead and silica-based membrane technologies as the automated MultiPROBE® II platforms on which these systems will ultimately be operating on are fully compatible with both systems. The criteria against which the different kits were assessed on include:

1. *Total DNA yield*; the kit must yield sufficient DNA to perform multiple downstream tests such as DNA quantification and PCR amplification.
2. *Quality of the resulting DNA profiles*; the kit should be able to isolate DNA of a suitable quality for PCR amplification of STR loci, in order to generate DNA profiles that are suitable for forensic and human identification purposes.
3. *Ability to remove inhibitors*; the kit must be able to remove common inhibitors present in mock forensic samples (e.g. hemoglobin) using the basic manufacturer's procedure without the use of organic solvents.
4. *Usability*; the kit (and the manufacturer's recommended protocol) must be user-friendly. The necessary steps to prevent cross-contamination should also be described in the protocol. The extraction process should be able to be completed in a reasonable amount of time, comparable to the current procedure.
5. *Availability of validated forensic protocols*; the kit, including the manufacturer's protocol, must be validated for forensic use, either by the manufacturer or by a forensic laboratory, as determined from statements in the manufacturer's protocol or availability of publications in peer-reviewed journals.
6. *Availability of a validated MultiPROBE® II PLUS test file*; the kit should have a validated MPT file for use on the MultiPROBE® II PLUS HT EX platform.

Assessment of points 1, 2 and 3 was performed through experimentation. Point 4 was assessed based on operator feedback. This report provides results for points 1, 2, 3 and 4. A more extensive assessment of Point 3 was performed on the kit that was found to provide the best results for points 1, 2, 3 and 4 and is reported in Project 11. For points 5 and 6, the availability of validated protocols for all kits evaluated is outlined in Table 3.

The acceptance criteria were strictly adhered to in order to objectively evaluate the different systems. Out of all five DNA extraction technologies, there only existed a validated MultiPROBE® II PLUS test file for the DNA IQ™ system (Table 3). Although this was considered an advantage for DNA IQ™, we did not prematurely dismiss any of the other kits prior to evaluation. We decided that if a kit significantly outperformed the rest, and did not have a validated MPT file already created, that we would create a novel program file

with the kit manufacturer's assistance. This, however, would only be decided at the conclusion of the evaluation process.

Table 3. An assessment of available validated protocols for the various kits that were evaluated by Forensic Biology FSS.

Kit	Availability of validated forensic protocol	Availability of validated MPII test file
DNA IQ™	✓	✓
QIAamp® DNA Micro	✓	×
ChargeSwitch®	✓	×
*forensic*GEM™	✓	×
NucleoSpin® 8 Trace	✓	×

The results and discussion for each of the kits that were evaluated, in comparison to Chelex®, are provided in the following sections. Refer to Tables 4 and 5 for quantitation results for cell and blood samples respectively. Yield calculations for Chelex® samples assume a final elution volume of 150µL.

6.2 Evaluation of DNA IQ™

The DNA IQ™ system uses a novel paramagnetic resin for DNA isolation. It consist of two steps: (1) lysis of the biological material on solid support; (2) using the paramagnetic resin to bind DNA, which allows washing of the resin-DNA complex while the resin is immobilised by a magnetic force, in order to remove the lysis reagent and inhibitors in solution.

The manufacturer's method required the use of the MagneSphere® Magnetic Separation Stand. This magnetic stand is used for the separation of the magnetic pellet in 12 samples at a time. The time to process a batch of 12 samples using the DNA IQ™ system takes about 3 hours, including 30 minutes of incubation time.

Three controls were run with each extraction batch: (1) a negative extraction control (empty tube); (2) a positive extraction control (QC dot saliva or blood depending on the extraction); and (3) a substrate blank (the substrate with only saline).

Samples were extracted using the DNA IQ™ method as described in the Methods section, and eluted using 100µL Elution Buffer. Due to volume loss during pipetting, the final elution volume is actually around 95µL. The same set of samples was also extracted using the in-house Chelex® protocol for comparison. Tables 4 and 5 display the DNA concentration (ng/µL) and yield (ng) for all cell and blood samples, compared to the results generated by Chelex®.

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Table 4. Quantitation values for cell samples on different substrates after extraction by Chelex® and the evaluated DNA extraction kits.

Cells samples		Chelex		DNA IQ		QIAamp DNA Micro		ChargeSwitch		forensicGEM		NucleoSpin 8 Trace	
		Concentration	Yield*	Concentration	Yield	Concentration	Yield	Concentration	Yield	Concentration	Yield	Concentration	Yield
Sample ID	Substrate type	ng/uL	ng	ng/uL	ng	ng/uL	ng	ng/uL	ng	ng/uL	ng	ng/uL	ng
[redacted]	FTA	0.058800	11.877600	0.028700	2.870000	0.006030	0.271350	0.023900	3.585000	0.025700	2.621400	0.018200	1.820000
	Cotton swab	0.007410	1.111500	0.098000	9.800000	0.025800	1.161000	0.096700	14.505000	0.083300	16.826600	0.068900	6.890000
	Cotton cloth	0.001480	0.222000	0.050700	5.070000	0.004880	0.219600	0.014900	2.235000	0.037400	7.554800	0.071900	7.190000
	Denim cloth	0.002360	0.354000	0.028200	2.820000	0.002160	0.097200	0.003250	0.487500	0.041300	8.342600	0.043900	4.390000
	Rayon swab	0.001620	0.243000	0.010000	1.000000	0.000000	0.000000	0.011800	1.770000	0.024000	4.848000	0.031800	3.180000
	Rayon swab	0.001580	0.237000	0.019400	1.940000	0.005050	0.227250	0.018100	2.715000	0.019000	3.838000	0.115000	11.500000
	Rayon swab	0.000000	0.000000	0.015500	1.550000	0.006610	0.297450	0.027400	4.110000	0.011300	2.282600	0.057400	5.740000
	Rayon swab	0.000000	0.000000	0.011200	1.120000	0.007310	0.328950	0.005910	0.886500	0.019700	3.979400	0.029900	2.990000
		0.000800	*0.120000*	*0.014025*	*1.402500*	*0.004743*	*0.213413*	*0.015803*	*2.370375*	*0.018500*	*3.737000*	*0.058525*	*5.852500*
		0.000924	*0.138586*	*0.004291*	*0.429137*	*0.003300*	*0.148490*	*0.009195*	*1.379299*	*0.005285*	*1.067483*	*0.039683*	*3.968336*
	FTA	0.010300	2.060600	0.005790	0.579000	0.005270	0.237150	0.001260	0.189000	0.007510	0.766020	0.005710	0.571000
	Cotton swab	0.000756	0.113400	0.019000	1.900000	0.001480	0.066600	0.031600	4.740000	0.030900	6.241800	0.009500	0.950000
	Cotton cloth	0.000541	0.081150	0.015200	1.520000	0.040900	1.840500	0.000000	0.000000	0.011600	2.343200	0.018900	1.890000
	Denim cloth	0.000000	0.000000	0.045800	4.580000	0.041800	1.881000	0.001720	0.258000	0.013400	2.706800	0.017800	1.780000
	Rayon swab	0.000558	0.083700	0.005740	0.574000	0.001800	0.081000	0.002860	0.429000	0.002950	0.595900	0.006760	0.676000
	Rayon swab	0.000000	0.000000	0.002560	0.256000	0.001300	0.058500	0.006150	0.922500	0.002020	0.408040	0.001220	0.122000
	Rayon swab	0.000898	0.134700	0.009750	0.975000	0.005570	0.250650	0.006560	0.984000	0.002340	0.472680	0.010200	1.020000
	Rayon swab	0.000433	0.064950	0.000000	0.000000	0.001550	0.069750	0.001350	0.202500	0.004030	0.814060	0.016000	1.600000
		0.000472	*0.070838*	*0.004513*	*0.451250*	*0.002555*	*0.114975*	*0.004230*	*0.634500*	*0.002835*	*0.572670*	*0.008545*	*0.854500*
		0.000371	*0.055667*	*0.004208*	*0.420765*	*0.002020*	*0.090915*	*0.002536*	*0.380328*	*0.000885*	*0.178801*	*0.006196*	*0.619564*
	FTA	0.008170	1.650340	0.006410	0.641000	0.000000	0.000000	0.000000	0.000000	0.006310	0.643620	0.000000	0.000000
	Cotton swab	0.003710	0.556500	0.012100	1.210000	0.001680	0.075600	0.009130	1.369500	0.003970	0.801940	0.014900	1.490000
	Cotton cloth	0.002600	0.390000	0.010400	1.040000	0.000000	0.000000	0.000355	0.053250	0.005010	1.012020	0.006570	0.657000
	Denim cloth	0.000739	0.110850	0.007630	0.763000	0.015100	0.679500	0.000000	0.000000	0.007770	1.569540	0.000000	0.000000
	Rayon swab	0.000000	0.000000	0.001010	0.101000	0.000000	0.000000	0.000697	0.104550	0.003100	0.626200	0.007860	0.786000
	Rayon swab	0.000000	0.000000	0.000982	0.098200	0.000000	0.000000	0.000000	0.000000	0.003160	0.638320	0.013800	1.380000
	Rayon swab	0.000000	0.000000	0.001540	0.154000	0.000000	0.000000	0.003390	0.508500	0.000000	0.000000		
	Rayon swab	0.000739	0.110850	0.003050	0.305000	0.000000	0.000000	0.003360	0.504000	0.000000	0.000000		
		0.000185	*0.027713*	*0.001646*	*0.164550*	*0.000000*	*0.000000*	*0.001862*	*0.279263*	*0.001565*	*0.316130*	*0.010830*	*1.083000*
		0.000370	*0.055425*	*0.000971*	*0.097088*	*0.000000*	*0.000000*	*0.001770*	*0.265562*	*0.001807*	*0.365069*	*0.004200*	*0.420021*
	FTA	0.000000	0.000000	0.000935	0.093500	0.003940	0.177300	0.000000	0.000000	0.001840	0.187680	0.000000	0.000000
	Cotton swab	0.000000	0.000000	0.002900	0.290000	0.000000	0.000000	0.001520	0.228000	0.002280	0.460560	0.000000	0.000000
	Cotton cloth	0.000000	0.000000	0.005010	0.501000	0.001870	0.084150	0.000000	0.000000	0.000741	0.149682	0.000000	0.000000
	Denim cloth	0.000000	0.000000	0.002870	0.287000	0.000227	0.102150	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
	Rayon swab	0.000000	0.000000	0.000717	0.071700	0.000000	0.000000	0.000000	0.000000	0.001200	0.242400	0.000000	0.000000
	Rayon swab	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000632	0.127664	0.002280	0.228000
	Rayon swab	0.000720	0.108000	0.002230	0.223000	0.000000	0.000000	0.003640	0.546000	0.002590	0.523180	0.004480	0.448000
	Rayon swab	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000788	0.159176		
		0.000180	*0.027000*	*0.000737*	*0.073675*	*0.000000*	*0.000000*	*0.000910*	*0.136500*	*0.001303*	*0.263105*	*0.002253*	*0.225333*
		0.000360	*0.054000*	*0.001051*	*0.105131*	*0.000000*	*0.000000*	*0.001820*	*0.273000*	*0.000891*	*0.180012*	*0.002240*	*0.224012*

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Table 5. Quantitation values for blood samples on rayon swab substrates after extraction by Chelex® and the evaluated DNA extraction kits.

Blood samples	Chelex		DNA IQ		QIAamp DNA Micro		ChargeSwitch		forensicGEM		NucleoSpin 8 Trace	
	[redacted]tion	Yield*	Concentration	Yield	Concentration	Yield	Concentration	Yield	Concentration	Yield	Concentration	Yield
Sample ID		ng	ng/uL	ng	ng/uL	ng	ng/uL	ng	ng/uL	ng	ng/uL	ng
[redacted]		355.5	0.482	48.2	2.31	103.95	0.751	112.65	0.00833	1.68266	1.16	116
		213	0.078	7.8	3.58	161.1	0.754	113.1	0.0066	1.3332	2.61	261
		76.8	0.356	35.6	3.32	149.4	0.929	139.35	0.0046	0.9292	1.61	161
		140.1	0.467	46.7	2.46	110.7	0.916	137.4	0.00727	1.46854	2.18	218
		196.3500	*0.3458*	*34.5750*	*2.9175*	*131.2875*	*0.8375*	*125.6250*	*0.0067*	*1.3534*	*1.8900*	*189.0000*
		119.8085	*0.1871*	*18.7137*	*0.6270*	*28.2137*	*0.0983*	*14.7451*	*0.0016*	*0.3173*	*0.6361*	*63.6082*
		32.85	0.238	23.8	0.227	10.215	0.219	32.85	0.00211	0.42622	0.611	61.1
		12.675	0.198	19.8	1.72	77.4	0.101	15.15	0.000597	0.120594	0.3	30
		32.4	0.195	19.5	4.59	206.55	0.0673	10.095	0.00128	0.25856	0.251	25.1
		24.75	0.136	13.6	0.657	29.565	0.0787	11.805	0.00166	0.33532	0.227	22.7
		25.6688	*0.1918*	*19.1750*	*1.7985*	*80.9325*	*0.1165*	*17.4750*	*0.0014*	*0.2852*	*0.3473*	*34.7250*
		9.4262	*0.0420*	*4.2019*	*1.9639*	*88.3776*	*0.0698*	*10.4628*	*0.0006*	*0.1294*	*0.1784*	*17.8438*
		1032	0.0554	5.54	0.0936	4.212	0.094	14.1	0.0126	2.5452	0.154	15.4
		24.6	0.114	11.4	0.175	7.875	0.0735	11.025	0.00174	0.35148	0.148	14.8
		42.9	0.145	14.5	0.123	5.535	0.0521	7.815	0.00363	0.73326	0.178	17.8
		76.95	0.125	12.5	0.0151	0.6795	0.0939	14.085	0.00167	0.33734	0.0819	8.19
		294.1125	*0.1099*	*10.9850*	*0.1017*	*4.5754*	*0.0784*	*11.7563*	*0.0049*	*0.9918*	*0.1405*	*14.0475*
		492.4030	*0.0385*	*3.8501*	*0.0668*	*3.0066*	*0.0200*	*2.9991*	*0.0052*	*1.0517*	*0.0411*	*4.1145*
		6.075	0.0792	7.92	0.0349	1.5705	0.0347	5.205	0.00757	1.52914	0.0766	7.66
		1.56	0.0566	5.66	0.0454	2.043	0.027	4.05	0.00667	1.34734	0.0923	9.23
		5.055	0.0847	8.47	0.0386	1.737	0.0197	2.955	0.00544	1.09888	0.0588	5.88
		4.845	0.109	10.9	0.0276	1.242	0.021	3.15	0.00245	0.4949	0.874	87.4
		4.3838	*0.0824*	*8.2375*	*0.0366*	*1.6481*	*0.0256*	*3.8400*	*0.0055*	*1.1176*	*0.2754*	*27.5425*
		1.9577	*0.0215*	*2.1515*	*0.0074*	*0.3341*	*0.0068*	*1.0274*	*0.0022*	*0.4510*	*0.3993*	*39.9285*

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Comparison of quantitation results for cell samples
Refer to Table 4 for observed data. Using DNA IQ™, neat cell samples displayed higher quantitation results for both cotton and rayon swabs, and also for cotton and denim cloth materials. Only for the FTA® card was the result higher for the Chelex® sample. For 1/4 dilutions, DNA IQ™ results were higher than Chelex® results. For 1/8 dilutions, both protocols showed similar results for most sample types. Rayon swabs produced zero quantitation values for Chelex®, but exhibited consistent results for DNA IQ™. For 1/16 dilutions, most Chelex® samples were undetermined, whereas most DNA IQ™ samples yielded quantitation results.

Only three dilution samples extracted by DNA IQ™ gave zero quantitation values. In contrast, fourteen Chelex® samples gave zero quantitation results. This suggests that the DNA IQ™ sample recovery rate is 111% greater than that of the Chelex® protocol for cell samples.

Comparison of quantitation results for blood samples
Refer to Table 5 for observed data. For this experiment, only rayon samples (in quadruplicate) were tested. Neat blood samples showed higher concentration results when extracted using Chelex®. The 1/4 dilutions showed similar results for both methods. The 1/8 dilutions showed better results for Chelex®, but this was primarily due to an outlier result for one of the replicates (highlighted red in Table 4) that resulted in a concentration value 1300% greater than the remaining samples. This occurrence could be the result of inaccurate pipetting during mock sample creation or variability in the Chelex® method, specifically the inconsistent final elution volumes. For the 1/16 dilutions, the DNA IQ™ results were better. All DNA IQ™ results were more consistent and reproducible than Chelex® results.

Overall, samples that were extracted using DNA IQ™ showed quantitation results that were similar to or better than samples that were extracted using Chelex®. For cell substrates, 44% of Chelex® samples gave zero quantitation results, compared to only 9% for DNA IQ™ samples. All blood substrates generated quantitation results that were similar for both methods. Furthermore, DNA IQ™ generated results that were more sensitive, consistent and reproducible across multiple replicates.

Comparison of DNA profiles
Cell samples that were extracted using the DNA IQ™ method gave DNA profiles with more alleles compared to extractions performed using Chelex® (Table 6). Overall, DNA IQ™ resulted in 282 reportable alleles (excluding Amelogenin), compared to 89 alleles resolved by Chelex®, or in other words samples extracted using DNA IQ™ generated 216% more reportable alleles compared to samples extracted using Chelex®. For neat cell substrates, DNA IQ™ samples generated full profiles in all instances except 2: an X,X+14 for the FTA® substrate and an X,X+16 for a rayon swab replicate. All rayon samples extracted by Chelex® did not produce any profiles at all, in contrast to the full profile results using DNA IQ™. DNA IQ™ also gave more reportable alleles for the lower dilutions compared to Chelex®. Additionally, DNA IQ™ was able to yield full profiles from denim substrates, compared to Chelex® which yielded no profiles at all. This observation indicates the superiority of the DNA IQ™ system for removing and overcoming inhibition due to denim dye. Only one occurrence of allelic imbalance (68% at D13S317) was encountered in all 64 samples.

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Table 6. Comparison of DNA profiles for cell substrate samples extracted using either Chelex® or DNA IQ™.

CELLS **Method: Chelex**

Dilution	FTA	Cotton swabs	Rayon swabs		Cotton	Denim
	Profile	Profile	Sample#	Profile	Profile	Profile
Neat	X,X+18	X,X+18	R14	NSD	X,X+8	NR/NSD
			R15	NR/NSD		
			R16	NSD		
			R17	NSD		
Dil 1/4	X,X+18	X,NR+3	R10	NSD	NR+1	NR/NSD
			R11	NSD		
			R12	NSD		
			R13	NSD		
Dil 1/8	X,X+17	X,X+3	R6	NSD	X,NR+3	NR/NSD
			R7	NSD		
			R8	NSD		
			R9	NSD		
Dil 1/16	NSD	NSD	R2	NSD	NSD	NSD
			R3	NSD		
			R4	NSD		
			R5	NSD		

CELLS **Method: DNA IQ**

Dilution	FTA	Cotton swabs	Rayon swabs		Cotton	Denim
	Profile	Profile	Sample#	Profile	Profile	Profile
Neat	X,X+14	X,X+18	R14	X,X+16	X,X+18	X,X+18
			R15	AI@D13(68%)		
			R16	X,X+18		
			R17	X,X+18		
Dil 1/4	X,X+17	X,X+18	R10	X,NR+3	X,X+18	XX+18
			R11	NR/NSD		
			R12	X,X+6		
			R13	NR/NSD		
Dil 1/8	X,X+8	X,X+18	R6	NR/NSD	X,X+17	X,X+17
			R7	NR/NSD		
			R8	NR/NSD		
			R9	NR/NSD		
Dil 1/16	NSD	X,X+4	R2	NR/NSD	NR/NSD	NR/NSD
			R3	NSD		
			R4	NR/NSD		
			R5	NSD		

For blood samples, only rayon substrates were extracted using the DNA IQ™ system as these were deemed sufficient for observing the effects of heme inhibition (without the need to factor variable substrate types). Almost all samples generated full profiles or a sufficient number of reportable alleles for matching purposes (Table 7). For neat samples extracted by Chelex®, no profiles were resulted from the FTA®, cotton swab or denim samples, indicating possible heme inhibition that could not be removed by the Chelex® protocol. For rayon samples, 19% of those extracted by Chelex® did not generate a profile, whereas DNA IQ™ yielded full profiles for all dilutions except two neat samples. Reworks of the two failed samples were performed but yielded the same NSD results. These failed results appear to be outliers, as all other dilutions yielded the expected results. It was observed that results from blood samples on rayon swabs were more likely (32%) to exhibit allelic imbalance at Amelogenin when extracted using the DNA IQ™ system.

Table 7. Comparison of DNA profiles for blood substrate samples extracted using either Chelex® or DNA IQ™.

BLOOD **Method: Chelex** **Method: DNA IQ**

Dilution	FTA	Cotton swabs	Rayon swabs		Cotton	Denim		Rayon swabs	
	Profile	Profile	Sample#	Profile	Profile	Profile		Sample#	Profile
Neat	NSD	NSD	R14	X,Y+18	X,Y+18	NSD		R14	NSD
			R15	X,Y+18				R15	NSD
			R16	X,Y+18				R16	X,Y+18(AI@AMEL)
			R17	NR/NSD				R17	X,Y+18(AI@AMEL)
Dil 1/4	X,Y+18	X,Y+15	R10	Not Uploaded	X,Y+18	X,Y+18		R10	X,Y+18
			R11	X,Y+18				R11	X,Y+18(AI@AMEL)
			R12	X,Y+18				R12	X,Y+18
			R13	X,Y+18				R13	X,Y+18
Dil 1/8	X,Y+18(AI@D	X,Y+18	R6	X,Y+18	X,Y+18	X,Y+18		R6	X,Y+18(AI@AMEL)
			R7	X,Y+18				R7	X,Y+18
			R8	NR/NSD				R8	X,Y+18
			R9	X,Y+18				R9	X,Y+18
Dil 1/16	X,Y+18	X,Y+18	R2	X,Y+18	X,Y+18	X,Y+18		R2	X,Y+18(AI@AMEL)
			R3	X,Y+18				R3	X,Y+18
			R4	X,Y+18				R4	X,Y+18
			R5	X,Y+18				R5	X,Y+18

We found the DNA IQ™ system yielded results that were either comparable or better than results generated by samples extracted using the in-house Chelex® protocol, both in terms of quantitation values and DNA profile quality and completeness.

6.3 Evaluation of QIAamp® DNA Micro

The QIAamp® DNA Micro kit was designed for the purification of genomic and mitochondrial DNA from small sample volumes or sizes, as often encountered in forensics. The system uses a silica-based membrane to accommodate DNA binding and purification using special buffers, followed by elution in buffer or water, resulting in purified DNA that is free of proteins, nucleases and other impurities.

The QIAamp® DNA Micro system consists of four steps: lysing, binding, washing, followed by elution:

- *Lysis* – Small samples are lysed under highly denaturing conditions at elevated temperatures under the presence of Proteinase K.
- *Binding* – Using Buffer AL and ethanol, DNA is adsorbed into the silica-gel membrane of the column by centrifugation or application of a vacuum. The buffer is formulated so that proteins and other components are not retained in the membrane.
- *Washing* – While DNA is bound to the silica membrane, contaminants are efficiently washed away using a combination of two wash buffers.
- *Elution* – DNA is eluted in a small volume of Buffer AE or sterile water, yielding concentrated DNA.

The QIAamp® protocol involves 5 tube transfers and therefore takes approximately 5 hours to perform a manual extraction of 12 samples. The same set of samples that were used for the DNA IQ™ evaluation was also used to evaluate QIAamp® DNA Micro. Each extraction batch included a positive and negative control, and also a substrate blank. DNA was eluted in 45µL volume.

Comparison of quantitation results for cell samples

Refer to Table 4 for observed data. Twelve samples extracted by QIAamp® gave zero quantitation values, compared to fourteen samples by Chelex®. Despite the low elution volume of 45µL in the QIAamp® protocol that serves to concentrate the purified DNA, quantitation results for all samples were comparable for both DNA extraction methods.

Comparison of quantitation results for blood samples

Refer to Table 5 for observed data. Blood on rayon swab samples displayed wide variation between replicates. For neat samples, the total yield is comparable to Chelex®, however lower dilutions (1/8 – 1/16) suffer from inconsistencies. One of the 1/4 dilution replicates displayed an unexpectedly high quantitation value that was more than 3x greater than the Chelex® average yield, but this can be attributed to inaccurate pipetting, or pipetting of a non-uniform sample mixture, during mock sample creation.

A possible reason as to why the quantitation results for both cell and blood samples were inconsistent is because the QIAamp® DNA Micro protocol uses five sets of collection tubes for supernatant transfer, therefore possibly causing sample lost during multiple sample transfers from one tube to another.

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Comparison of DNA profiles

Cell samples that were extracted using the QIAamp® protocol showed profile results that were either comparable or worse than samples that were extracted using the Chelex® protocol (Table 8). Out of 32 samples, only one QIAamp® sample resulted in a full profile (X,X+18). QIAamp® samples failed to produce full profiles for all but one (n = 8) of the neat samples. Overall, QIAamp® resulted in 86 reportable alleles compared to 89 alleles resolved by Chelex®. Some of the QIAamp® allele calls are inconsistent, e.g. the result for 1/4 dilution on cotton cloth was slightly better than the neat sample. This is further exemplified by the denim substrate samples. The QIAamp® method did not appear to effectively overcome inhibition caused by the denim dye as observed from the resulting profiles.

Table 8. Comparison of DNA profiles for cell substrate samples extracted using either Chelex® or QIAamp® DNA Micro.

CELLS **Method: Chelex**

Dilution	*FTA*	*Cotton swabs*	*Rayon swabs*		*Cotton*	*Denim*
	Profile	Profile	Sample#	Profile	Profile	Profile
Neat	X,X+18	X,X+18	R14	NSD	X,X+8	NR/NSD
			R15	NR/NSD		
			R16	NSD		
			R17	NSD		
Dil 1/4	X,X+18	X,NR+3	R10	NSD	NR+1	NR/NSD
			R11	NSD		
			R12	NSD		
			R13	NSD		
Dil 1/8	X,X+17	X,X+3	R6	NSD	X,NR+3	NR/NSD
			R7	NSD		
			R8	NSD		
			R9	NSD		
Dil 1/16	NSD	NSD	R2	NSD	NSD	NSD
			R3	NSD		
			R4	NSD		
			R5	NSD		

CELLS **Method: QIAamp DNA Micro**

Dilution	*FTA*	*Cotton swabs*	*Rayon swabs*		*Cotton*	*Denim*
	Profile	Profile	Sample#	Profile	Profile	Profile
Neat	X,X+12	X,X+18	R14	NSD	NR,NR+2	NR/NSD
			R15	NR/NSD		
			R16	XNR+6		
			R17	NR/NSD		
Dil 1/4	X,X+14	X,X+15	R10	NSD	NR,NR+3	NR/NSD
	(AI@D18)		R11	NSD		
			R12	NR,NR+1		
			R13	NR/NSD		
Dil 1/8	NSD+2	X,NR+6	R6	NSD	NR/NSD	XNR+7
			R7	NSD		
			R8	NSD		
			R9	NSD		
Dil 1/16	NR/NSD	NR/NSD	R2	NSD	NSD	NSD
			R3	NSD		
			R4	NSD		
			R5	NSD		

Table 9. Comparison of DNA profiles for blood substrate samples extracted using either Chelex® or QIAamp® DNA Micro.

BLOOD **Method: Chelex** **Method: QIAamp DNA Micro**

Dilution	*FTA*	*Cotton swabs*	*Rayon swabs*		*Cotton*	*Denim*	*Rayon swabs*	
	Profile	Profile	Sample#	Profile	Profile	Profile	Sample#	Profile
Neat	NSD	NSD	R14	X,Y+18	X,Y+18	NSD	R14	X,Y+18
			R15	X,Y+18			R15	X,Y+18
			R16	X,Y+18			R16	X,Y+18
			R17	NR/NSD			R17	NR,Y+15
Dil 1/4	X,Y+18	X,Y+15	R10	Not Uploaded	X,Y+18	X,Y+18	R10	X,Y+18
			R11	X,Y+18			R11	X,Y+17
			R12	X,Y+18			R12	X,Y+18
			R13	X,Y+18			R13	X,Y+18
Dil 1/8	X,Y+18(AI@D	X,Y+18	R6	X,Y+18	X,Y+18	X,Y+18	R2	X,Y+18
			R7	X,Y+18			R3	X,Y+18
			R8	NR/NSD			R4	X,Y+18
			R9	X,Y+18			R5	X,Y+18
Dil 1/16	X,Y+18	X,Y+18	R2	X,Y+18	X,Y+18	X,Y+18	R2	X,Y+18(AI@D8,D18)
			R3	X,Y+18			R3	X,Y+18
			R4	X,Y+18			R4	X,Y+18
			R5	X,Y+18			R5	X,Y+18

For blood samples on rayon swabs, 87.5% of QIAamp® samples resulted in full profiles, compared to 81.25% of Chelex® samples (Table 9). Out of all QIAamp® rayon swab samples, only one of the 1/16 replicates displayed allelic imbalance (in D8S1179 and D18S51).

6.4 Evaluation of ChargeSwitch®

The ChargeSwitch® technology (CST) is another magnetic bead-based technology that provides a switchable surface charge dependent on the pH of the surrounding buffer environment to facilitate DNA isolation from small forensic samples. In low pH conditions, the ChargeSwitch® beads have a positive charge that allows negatively-charged DNA to bind. In this environment, proteins and other contaminants are not bound and can be washed away. By using a low salt elution buffer at pH 8.5, the charge on the bead surface is neutralised and DNA can be eluted for immediate use in downstream forensic applications.

The ChargeSwitch® Elution Buffer (E5) that is supplied with the kit is used to provide an environment with a pH of 8.5 that promotes dissociation of bound DNA from the magnetic beads and therefore efficient elution of purified DNA. However, TE buffer with a pH between 8.5 – 9.0 can also be used for elution. TE buffer outside of this pH range should not be used. The use of water for elution is also not recommended.

The manufacturer's method required the use of the MagnaRack™ two-piece magnetic separation rack that consists of two components: a magnetic base station and removable tube rack. The tube rack holds up to 24 microcentrifuge tubes and fits onto the magnetic base station in two different positions associating the row of 12 neodymium magnets with a single row of 12 tubes for simple 'on the magnet' and 'off the magnet' processing. The time to process a batch of 12 samples using the ChargeSwitch® system takes about 3.5 hours, including 30 minutes of incubation time. Each extraction batch included a positive and negative control, and also a substrate blank. Purified DNA samples were eluted in 150µL Elution Buffer (E5).

Comparison of quantitation results for cell samples

Refer to Table 4 for observed data. For cells samples, ChargeSwitch® performed moderately better compared to the current in-house Chelex® method. When comparing the quantitiation values, ChargeSwitch® produced higher quantitation values for cotton and rayon swabs over all dilutions as well as the neat samples of cotton shirt and denim jeans. For other cell samples, ChargeSwitch® performance was comparable to the Chelex® results.

Comparison of quantitation results for blood samples

Refer to Table 5 for observed data. ChargeSwitch® quantitation results for blood samples on rayon swabs were lower but more consistent than Chelex® results.

Comparison of DNA profiles

Cell samples that were extracted using the ChargeSwitch® system showed profile results that were comparable to samples that were extracted using the Chelex® protocol (Table 10). Overall, ChargeSwitch® resulted in 138 reportable alleles compared to 89 alleles resolved by Chelex®. ChargeSwitch® performance for cell samples on FTA® cards was poor for any samples less than the neat dilution. Profiles for both cotton swab and cotton cloth samples were slightly better for ChargeSwitch®, and results for neat samples on rayon

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swabs outperformed Chelex®. However, the ChargeSwitch® system was unable to overcome inhibition in denim samples, and did not yield any DNA profiles at all, despite displaying quantitation results for the neat and 1/4 dilution.

Table 10. Comparison of DNA profiles for cell substrate samples extracted using either Chelex® or ChargeSwitch®.

CELLS **Method: Chelex**

Dilution	FTA	Cotton swabs	Rayon swabs		Cotton	Denim
	Profile	Profile	Sample#	Profile	Profile	Profile
Neat	X,X+18	X,X+18	R14	NSD	X,X+8	NR/NSD
			R15	NR/NSD		
			R16	NSD		
			R17	NSD		
Dil 1/4	X,X+18	X,NR+3	R10	NSD	NR+1	NR/NSD
			R11	NSD		
			R12	NSD		
			R13	NSD		
Dil 1/8	X,X+17	X,X+3	R6	NSD	X,NR+3	NR/NSD
			R7	NSD		
			R8	NSD		
			R9	NSD		
Dil 1/16	NSD	NSD	R2	NSD	NSD	NSD
			R3	NSD		
			R4	NSD		
			R5	NSD		

CELLS **Method: ChargeSwitch**

	FTA	Cotton swabs	Rayon swabs		Cotton	Denim
	Profile	Profile	Sample#	Profile	Profile	Profile
Neat	X,X+17	X,X+18	R14	X,X+8	X,X+11	NSD
	AI@FGA		R15	X,X+15		
	AI@D13		R16	X,X+16		
			R17	X,X+8		
Dil 1/4	X,X+9	X,X+18	R10	X,X+NR's	NRNR+2	NSD
		AI@D13	R11	NR/NSD		
			R12	X,NR+2		
			R13	X,NR+NSD		
Dil 1/8	NR/NSD	X,X+14	R6	NSD	NR/NSD	NSD
			R7	NSD		
			R8	NR/NSD		
			R9	NSD		
Dil 1/16	NSD	NR/NSD	R2	NR/NSD	NSD	NSD
			R3	NSD		
			R4	NSD		
			R5	NSD		

For blood samples on rayon swab substrates, all ChargeSwitch® samples consistently yielded full profiles for all dilutions and therefore outperformed Chelex® (Table 11). Two replicates of the lower, 1/16 dilutions displayed allelic imbalance at two different loci: D3S1358 and D7S820, possibly due to stochastic effects that arise from amplifying low concentrations of DNA.

Table 11. Comparison of DNA profiles for blood substrate samples extracted using either Chelex® or ChargeSwitch®.

BLOOD **Method: Chelex** **Method: ChargeSwitch**

Dilution	FTA	Cotton swabs	Rayon swabs		Cotton	Denim	Rayon swabs	
	Profile	Profile	Sample#	Profile	Profile	Profile	Sample#	Profile
Neat	NSD	NSD	R14	X,Y+18	X,Y+18	NSD	R14	X,Y+18
			R15	X,Y+18			R15	X,Y+18
			R16	X,Y+18			R16	X,Y+18
			R17	NR/NSD			R17	X,Y+18
Dil 1/4	X,Y+18	X,Y+15	R10	Not Uploaded	X,Y+18	X,Y+18	R10	X,Y+18
			R11	X,Y+18			R11	X,Y+18
			R12	X,Y+18			R12	X,Y+18
			R13	X,Y+18			R13	X,Y+18
Dil 1/8	X,Y+18(AI@D	X,Y+18	R6	X,Y+18	X,Y+18	X,Y+18	R6	X,Y+18
			R7	X,Y+18			R7	X,Y+18
			R8	NR/NSD			R8	X,Y+18
			R9	X,Y+18			R9	X,Y+18
Dil 1/16	X,Y+18	X,Y+18	R2	X,Y+18	X,Y+18	X,Y+18	R2	X,Y+18
			R3	X,Y+18			R3	X,Y+18
			R4	X,Y+18			R4	X,Y+18(AI@D3)
			R5	X,Y+18			R5	X,Y+18(AI@D7)

6.5 Evaluation of *forensic*GEM™

*forensic*GEM™ is a novel thermostable proteinase developed as a rapid, cheap and effective single-tube DNA extraction solution for forensic laboratories that was recently released. At the time of testing, the *forensic*GEM™ system was not yet widely used in the field of forensics, however the system has had exposure at various conferences and symposiums, such as the 18th International Symposium on the Forensic Sciences (Fremantle, WA; 2-7 April 2006).

Unlike the other kits that were evaluated, *forensic*GEM™ does not incorporate either magnetic bead or silica membrane technologies, but instead works on the principle action of a thermostable proteinase in an optimised buffer solution. *forensic*GEM™ is based on the work of Moss *et al.* (2003) who developed the use of EA1 proteinase for the DNA extraction of forensic samples. EA1 proteinase comes from the thermophilic *Bacillus* sp. EA1. EA1 proteinase is Ca^{2+} dependent but is unaffected by a concentration of citrate below 5mM and EDTA below 2mM (Moss *et al.* 2003). For EDTA-stabilised blood, the buffer needs to be supplemented to a final concentration of 200µM $CaCl_2$. Heating a sample at 75°C in the presence of *forensic*GEM™ buffer and *forensic*GEM™ lyses the sample and the proteinase hydrolyses nucleases. At 95°C the proteinase is heat-inactivated so that an active form will not be carried over into PCR where it would degrade *Taq* DNA polymerase.

The time to process a batch of 12 samples using the *forensic*GEM™ system takes about 1.5 hours. Each extraction batch included a positive and negative control, and also a substrate blank. The final volume was 100µL for FTA® samples and 200µL for all other samples.

Comparison of quantitation results for cell samples

Refer to Table 4 for observed data. For cells samples, *forensic*GEM™ produced higher quantitation results compared to Chelex® across all dilutions. *forensic*GEM™ also generated the highest yield for all samples, including the 1/16 dilutions. *forensic*GEM™ yielded quantitation results for denim samples (neat and 1/4 dilutions).

Comparison of quantitation results for blood samples

Refer to Table 5 for observed data. *forensic*GEM™ performed very poorly for blood samples on rayon swabs, resulting in the lowest observed yield across all kits that were evaluated. The average yield for all four neat replicates processed using *forensic*GEM™ was 0.6% of the average yield for all Chelex® replicates. The best average yield results were observed for 1/16 dilution samples, where the average *forensic*GEM™ yield was around 25% that of Chelex®. This suggests that the *forensic*GEM™ system is prone to heme inhibition if a neat sample is processed, but can slightly overcome the inhibitory effect if the blood sample is diluted prior to extraction.

Comparison of DNA profiles

*forensic*GEM™ resulted in 209 reportable alleles for cell samples compared to 89 alleles resulting from Chelex® extracts (Table 12). *forensic*GEM™ was able to overcome inhibition in denim samples, producing full profiles (X,X+18) for neat and 1/4 dilutions, accurately reflecting the quantitation results. A partial profile (X,NR+7) was obtained for the 1/8 dilution on denim. *forensic*GEM™ results were also superior than Chelex® for cells on cotton swab down to the 1/8 dilution, but FTA® results were considerably poor.

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Table 12. Comparison of DNA profiles for cell substrate samples extracted using either Chelex® or *forensic*GEM®.

CELLS Method: Chelex

Dilution	FTA	Cotton swabs	Rayon swabs		Cotton	Denim
	Profile	Profile	Sample#	Profile	Profile	Profile
Neat	X,X+18	X,X+18	R14	NSD	X,X+8	NR/NSD
			R15	NR/NSD		
			R16	NSD		
			R17	NSD		
Dil 1/4	X,X+18	X,NR+3	R10	NSD	NR+1	NR/NSD
			R11	NSD		
			R12	NSD		
			R13	NSD		
Dil 1/8	X,X+17	X,X+3	R6	NSD	X,NR+3	NR/NSD
			R7	NSD		
			R8	NSD		
			R9	NSD		
Dil 1/16	NSD	NSD	R2	NSD	NSD	NSD
			R3	NSD		
			R4	NSD		
			R5	NSD		

CELLS Method: forensicGEM

	FTA	Cotton swabs	Rayon swabs		Cotton	Denim
	Profile	Profile	Sample#	Profile	Profile	Profile
Neat	X,X+15	X,X+18	R14	X,X+17	X,X+18	X,X+18
			R15	X,X+13		
			R16	X,X+3		
			R17	X,X+ 15		
Dil 1/4	X,NR+3	X,X+18	R10	X,NR+NR/NS	X,X+18	X,X+18
			R11	NR/NSD		
			R12	NR/NSD		
			R13	X,NR+NR/NSD		
Dil 1/8	NSD	X,X+18	R7	NR/NSD	X,NR+10	X,NR+7
			R8	NSD		
			R9	NR/NSD		
			R6	NR/NSD		
Dil 1/16	NSD	NR/NSD	R5	NSD	NSD	NR/NSD
			R4	NSD		
			R3	NSD		
			R2	NR/NSD		

For blood samples on rayon swabs, only the 1/16 dilutions generated profile results (Table 13). This is indicatory of potential inhibition for higher blood sample dilutions as predicted by the quantitation data.

Table 13. Comparison of DNA profiles for blood substrate samples extracted using either Chelex® or *forensic*GEM®.

BLOOD Method: Chelex Method: forensicGEM

Dilution	FTA	Cotton swabs	Rayon swabs		Cotton	Denim	Rayon swabs	
	Profile	Profile	Sample#	Profile	Profile	Profile	Sample#	Profile
Neat	NSD	NSD	R14	X,Y+18	X,Y+18	NSD	R14	NSD
			R15	X,Y+18			R15	NSD
			R16	X,Y+18			R16	NSD
			R17	NR/NSD			R17	NSD
Dil 1/4	X,Y+18	X,Y+15	R10	Not Uploaded	X,Y+18	X,Y+18	R10	NSD
			R11	X,Y+18			R11	NSD
			R12	X,Y+18			R12	NSD
			R13	X,Y+18			R13	NSD
Dil 1/8	X,Y+18(AI@D	X,Y+18	R6	X,Y+18	X,Y+18	X,Y+18	R6	NSD
			R7	X,Y+18			R7	NSD
			R8	NR/NSD			R8	NSD
			R9	X,Y+18			R9	NSD
Dil 1/16	X,Y+18	X,Y+18	R2	X,Y+18	X,Y+18	X,Y+18	R2	X,Y+15(AI@D13)
			R3	X,Y+18			R3	X,NR+3
			R4	X,Y+18			R4	NR,NR+5
			R5	X,Y+18			R5	NR,NR+2

6.6 NucleoSpin® 8 Trace

The NucleoSpin® 8 Trace kit is designed for extraction of genomic DNA from forensic samples. Cell lysis is achieved by incubating samples in a solution containing chaotropic ions in the presence of proteinase K at room temperature. Adding isopropanol to the lysate creates the appropriate conditions for binding of DNA to the silica membrane, a process that is reversible and specific to nucleic acids. Inhibitors are removed by washing steps using an alcohol-containing buffer. Pure genomic DNA is eluted in a slightly alkaline elution buffer.

The evaluation of this kit was performed with slight alterations in the manual method to incorporate the use of the MultiPROBE® II PLUS PVM vacuum manifold, together with the

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NucleoSpin® 8 Trace Starter Set A containing Column Holders A and Dummy Strips to enable use of the vacuum manifold.

The time to process a batch of 12 samples using the NucleoSpin® 8 Trace system takes about 5 hours, including a 3 hour incubation step. Each extraction batch included a positive and negative control, and also a substrate blank. Purified DNA was eluted in a final volume of 100µL.

Comparison of quantitation results for cell samples
Refer to Table 4 for observed data. NucleoSpin® 8 Trace produced greater mean concentration values and mean yields than the Chelex® protocol.

Comparison of quantitation results for blood samples
Refer to Table 5 for observed data. Mean blood quantitation values for samples extracted using NucleoSpin® 8 Trace were comparable to Chelex® results. Yields were variable but comparable to Chelex®.

Comparison of DNA profiles
NucleoSpin® 8 Trace overall yielded higher allele counts compared to Chelex®, resulting in 202 reportable alleles in contrast to the 89 alleles from Chelex®-extracted samples (Table 14). NucleoSpin® 8 Trace was able to yield profiles for cell samples on denim down to 1/8 dilution, but performed poorly with FTA® samples, resulting only in a partial profile (X,X+5) for the neat cell sample. NucleoSpin® 8 Trace performed better for cells on cotton swabs, and performed moderately better for cells on rayon swabs. Profiles from cells on cotton cloth samples were comparable between the two DNA extraction methods.

Table 14. Comparison of DNA profiles for cell substrate samples extracted using either Chelex® or NucleoSpin® 8 Trace.

CELLS — Method: Chelex

Dilution	FTA	Cotton swabs	Rayon swabs		Cotton	Denim
	Profile	Profile	Sample#	Profile	Profile	Profile
Neat	X,X+18	X,X+18	R14	NSD	X,X+8	NR/NSD
			R15	NR/NSD		
			R16	NSD		
			R17	NSD		
Dil 1/4	X,X+18	X,NR+3	R10	NSD	NR+1	NR/NSD
			R11	NSD		
			R12	NSD		
			R13	NSD		
Dil 1/8	X,X+17	X,X+3	R6	NSD	X,NR+3	NR/NSD
			R7	NSD		
			R8	NSD		
			R9	NSD		
Dil 1/16	NSD	NSD	R2	NSD	NSD	NSD
			R3	NSD		
			R4	NSD		
			R5	NSD		

CELLS — Method: NucleoSpin 8 Trace

	FTA	Cotton swabs	Rayon swabs		Cotton	Denim
	Profile	Profile	Sample#	Profile	Profile	Profile
Neat	X,X+5	X,X+18	R14	AI@D3	X,X+18	X,X+18
			R15	X,X+18		
			R16	X,X+16 AI@D3		
			R17	AI@D13		
Dil 1/4	NSD	X,X+18	R10	X,NR+NR/NS	NSD	X,X+18
		AI @ D13	R11	X,X+3		AI @ FGA
			R12	X,NR+1		
			R13	NR,NR +1		
Dil 1/8	NSD	X,X+17	R6	X,NR+NR/NS	X,NR+2	X,X+13
			R7	NR/NSD		
			R8	X,NR+NR/NSD		
			R9	X,NR+NR/NSD		
Dil 1/16	NSD	NSD	R2	NSD	NSD/NR	NSD
			R3	NSD		
			R4	NSD		
			R5	X,NR+NR/NSD		

For blood samples on rayon swabs, NucleoSpin® 8 Trace profiles were comparable to Chelex®, with several partial profiles being observed in the neat and 1/8 dilutions (Table 15).

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Table 15. Comparison of DNA profiles for blood substrate samples extracted using either Chelex® or NucleoSpin® 8 Trace.

BLOOD	Method: Chelex						Method: NucleoSpin 8 Trace	
Dilution	*FTA*	*Cotton swabs*	*Rayon swabs*		*Cotton*	*Denim*	*Rayon swabs*	
	Profile	Profile	Sample#	Profile	Profile	Profile	Sample#	Profile
Neat	NSD	NSD	R14	X,Y+18	X,Y+18	NSD	R14	AI@Amel.&D18
			R15	X,Y+18			R15	NR,NR+2
			R16	X,Y+18			R16	X,Y+13
			R17	NR/NSD			R17	X,Y+18
Dil 1/4	X,Y+18	X,Y+15	R10	Not Uploaded	X,Y+18	X,Y+18	R10	X,Y+18
			R11	X,Y+18			R11	X,Y+18
			R12	X,Y+18			R12	X,Y+18
			R13	X,Y+18			R13	X,Y+18
Dil 1/8	X,Y+18(AI@D	X,Y+18	R6	X,Y+18	X,Y+18	X,Y+18	R6	X,Y+18
			R7	X,Y+18			R7	X,Y+15
			R8	NR/NSD			R8	X,Y+18
			R9	X,Y+18			R9	X,Y+18
Dil 1/16	X,Y+18	X,Y+18	R2	X,Y+18	X,Y+18	X,Y+18	R2	X,Y+18
			R3	X,Y+18			R3	X,Y+18
			R4	X,Y+18			R4	X,Y+18
			R5	X,Y+18			R5	X,Y+18

6.7 Summary

Findings from the evaluation of various forensic DNA extraction kits, compared to the in-house Chelex® protocol, is summarised in Table 16.

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Table 16. Summary of findings from the evaluation of five forensic DNA extraction chemistries.

	Chelex	DNA IQ™	QIAamp® DNA Micro	ChargeSwitch®	*forensic* GEM™	NucleoSpin® 8 Trace
Processing time f [redacted] ples	2hr	3hr	5hr	3.5hr	1.5hr	5hr
Washing steps in [redacted] remove inhibitors	No	Yes	Yes	Yes	No	Yes
Final extract volu [redacted]	~150	100	45	150	100 for FTA, 200 for other samples	100
% zero quantitati [redacted] or cells	43.750	9.375	37.500	31.250	9.375	24.140
% zero quantitati [redacted] or blood	0.000	0.000	0.000	0.000	0.000	0.000
Cell substrate dis [redacted] ghest quant value for neat cell samples	FTA	Cotton swab	Cotton swab	Cotton swab	Cotton swab	Cotton cloth
Total number of r [redacted] lleles for cells (max 576)	89	282	86	138	209	202
Total number of r [redacted] lleles for blood (max 288)	234	252	284	288	25	264
Total number of a [redacted] oci exhibiting allelic imbalance (max 432)	1	1*	3	5	1	6^
Neat cell samples [redacted] showed inhibition (no profile)	Yes	No	Yes	Yes	No	No
Neat blood samp [redacted] n swabs showed inhibition (no profile)	No	No	No	No	Yes	No
Amenable to auto [redacted]	No	Yes	Yes	Yes	Yes	Yes
Validated MultiPR [redacted] US automated protocol	No	Yes	No	No	No	No

* Five occurrences [redacted] balance were observed in Amelogenin.
^ One occurrence o [redacted] lance was observed in Amelogenin.

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Out of all the chemistries tested, only the Chelex® method and *forensic*GEM™ protocols do not incorporate washing steps for the removal of inhibitors and residual proteins. This is because in these protocols, the DNA is free in solution and not immobilised on to a capture device such as magnetic beads, and therefore washing of the sample cannot be performed. Washing steps result in high quality, purified DNA extracts. As such, Chelex® and *forensic*GEM™ extracts are considered to be crude DNA extracts of suboptimal quality that may not yield the best DNA profiles due to the presence of inhibitors that can affect PCR amplification of multiple STR loci. Although the dye in denim material did not appear to result in inhibition for *forensic*GEM™ samples, only 25/288 alleles (8.7%) from blood samples could be resolved by this extraction method.

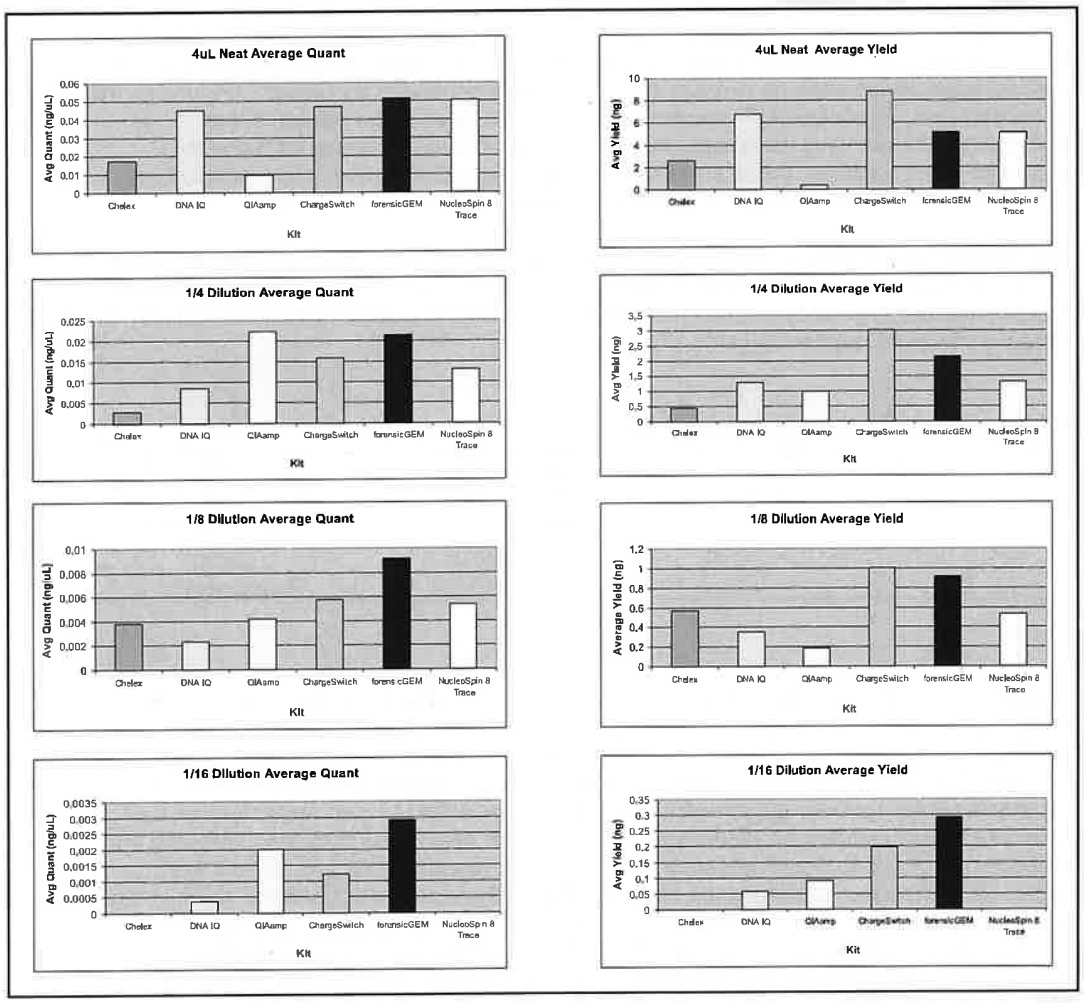

Figure 1. Average quantitation values (ng/µL) and yields (ng) for cell samples extracted using the various extraction chemistries tested, compared to Chelex®.

Although all kits resulted in quantitation results for all blood samples (0% had zero results), the results for cell samples exhibited more variation. Out of the extraction chemistries that incorporate washing steps, the DNA IQ™ system exhibited the best result for zero quantitation values for both cell and blood samples at 9% and 0% respectively. Almost half (44%) of Chelex® cell extracts failed to yield quantitation results. The next worse quantitation results were observed for QIAamp® DNA Micro (37.5% had zero results), followed by ChargeSwitch® (31.25%) and NucleoSpin® 8 Trace (24.14%). For all the different substrate types tested, average quantitation values were comparable for DNA IQ™, ChargeSwitch®, *forensic*GEM™ and NucleoSpin® 8 Trace in neat, 1/4 and 1/8 dilutions (Figure 1). Compared to samples extracted using Chelex®, samples extracted using the evaluated kits displayed higher average quantitation results that were up to 7.7 times higher than Chelex® results. Chelex® and NucleoSpin® 8 Trace were the only two kits that did not result in quantitation values for the 1/16 dilutions. The average yields varied widely due to different elution volumes for the various kits. For neat samples, DNA IQ™, ChargeSwitch®, *forensic*GEM™ and NucleoSpin® 8 Trace resulted in comparable yields for neat samples, which were on average double the yield generated by Chelex® (Figure 1). In all experiments, *forensic*GEM™ resulted in the highest quantitation values, but as discussed in the previous paragraph, this kit produced the least number of reportable alleles for blood samples. It was preferred to have a high quantitation result, coupled with a high yield and high final volume as it allows multiple tests to be performed.

The relationship between quantitation result and the number of resolved reportable alleles is close to proportional. A list of the evaluated chemistries, ranked according to the lowest to highest percentage of zero quantitation results, and also the most to the least number of resolved alleles, is outlined in Table 17.

Table 17. A ranking of the evaluated kits based on quantitation and DNA profile results.

Rank	% zero quantitation values	Total alleles for cells	Total alleles for blood
1	DNA IQ™ & *forensic*GEM™	DNA IQ™	ChargeSwitch®
2	NucleoSpin® 8 Trace	*forensic*GEM™	QIAamp® DNA Micro
3	ChargeSwitch®	NucleoSpin® 8 Trace	NucleoSpin® 8 Trace
4	QIAamp® DNA Micro	ChargeSwitch®	DNA IQ™
5	Chelex®	Chelex®	Chelex®
6		QIAamp® DNA Micro	*forensic*GEM™

The DNA IQ™ system was ranked the highest for most categories and performed the best for both cell and blood samples (see also Figures 2 and 3). For blood samples on rayon swabs, DNA IQ™ received a lower ranking due to 2 outlier results for neat dilutions as discussed above, but overall was considered to produce the best result for all dilutions. In contrast, Chelex® had the lowest rating as it was found to result in the least number of reportable alleles for both cell and blood samples. *forensic*GEM™ also outperformed the other kits for cell samples but performed very poorly for neat blood samples, indicating an inhibitory effect due to dissolved heme, although PCR amplification performance was improved in extracts of diluted blood samples (Figure 3). In contrast, QIAamp® DNA Micro worked well for blood samples, but performed the worst for cell samples. ChargeSwitch®, the alternative magnetic bead system to DNA IQ™, also performed better for blood samples than cell samples. The NucleoSpin® 8 Trace system, another membrane-based technology, performed moderately well and was ranked 3rd for the total number of alleles resolved for both cell and blood samples. Our results did not clearly indicate as to which technology, i.e. magnetic bead or silica membrane, was overall a better DNA extraction technology for forensic samples. However, DNA IQ™ worked the best in our hands as a complete "out-of-the-box" solution for extracting both cell and blood samples on various types of substrates.

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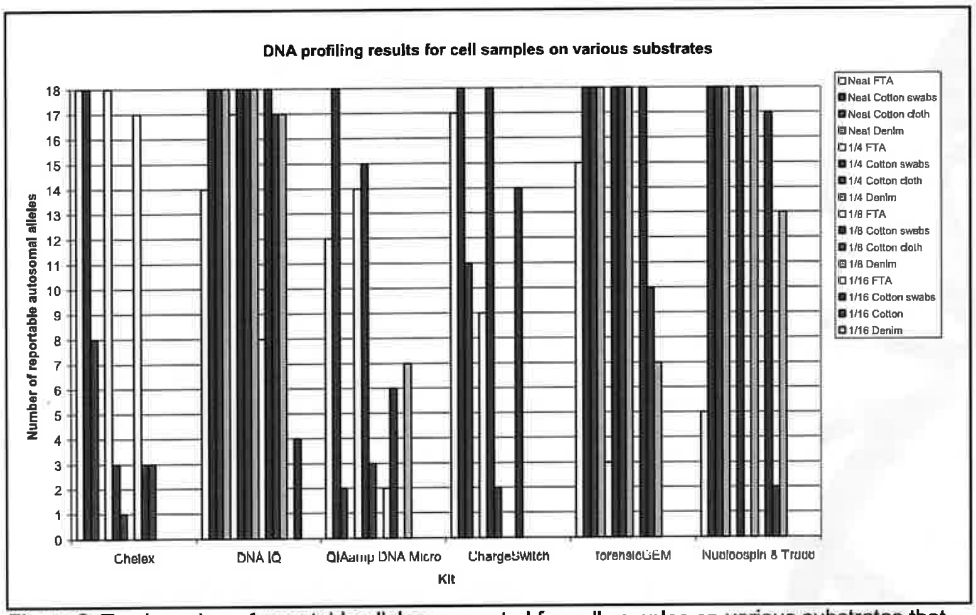

Figure 2. Total number of reportable alleles generated for cell samples on various substrates that were extracted using the various extraction chemistries tested, compared to Chelex®. The kit displaying the most number of full bars (i.e. most full profiles) was found to be DNA IQ™, indicating the superior performance of this kit over the other kits tested. The current in-house Chelex® method did not perform as well as several of the tested kits.

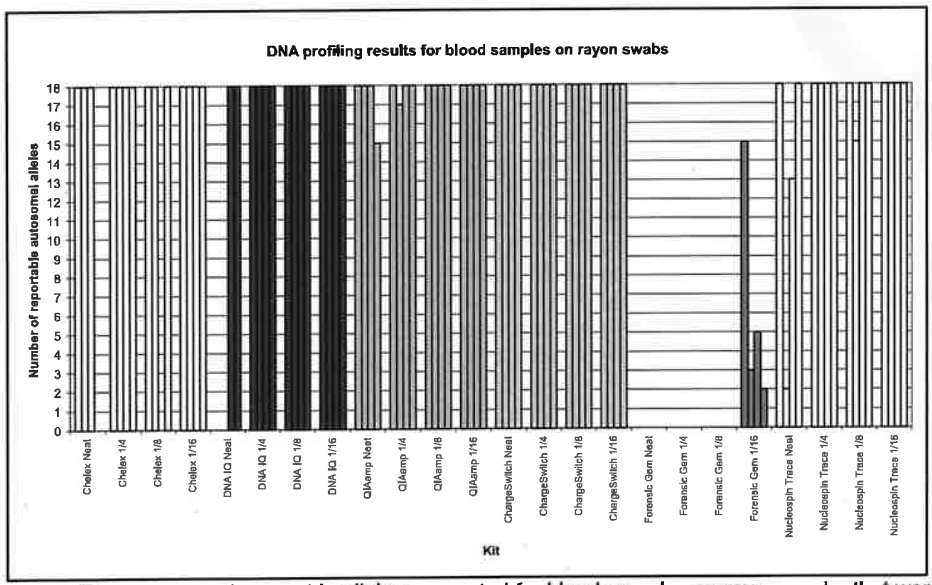

Figure 3. Total number of reportable alleles generated for blood samples on rayon swabs that were extracted using the various extraction chemistries tested, compared to Chelex®. All kits were able to resolve profiles from most dilutions, except *forensic*GEM™ which could only resolve alleles from the 1/16 dilution, indicating an inhibitory effect of heme on the *forensic*GEM™ system.

Out of a total of 432 loci amplified in the assessment of each kit, only one occurrence of allelic imbalance (AI; where peak height ratio is <70%) was detected in each of the Chelex®, DNA IQ™ and *forensic*GEM™ kits (Table 16). QIAamp® DNA Micro and ChargeSwitch® each showed 3 and 5 occurrences of AI respectively, and NucleoSpin® 8 Trace showed the most AI at 6 occurrences observed (Table 16).

Out of 17 occurrences of AI in all samples tested, 9 AI events were observed in cell samples and 8 events were observed in blood samples (Table 18). These results do not suggest any increased likelihood in observing AI in either cell or blood samples. Out of the 9 AmpFℓSTR® Profiler Plus loci interrogated, AI was only encountered in 6 loci: D3S1358, FGA, D13S317, D8S1179, D18S51, and D7S820 (Table 18). Most of the AI (35.29%) occurred in the D13S317 locus, and the least (5.88%) occurred in both D8S1179 and D7S820. The %AI observed was within the range of 52.30% at D13S317 to 69.96% at D3S1358 (data not shown). Most of the AI (58.82%) was ≥60%, and 41.18% of AI was ≥65%. Out of the 7 occurrences of AI that were ≤60%, 4 events (57%) were observed in cell samples extracted using NucleoSpin® 8 Trace. Six additional occurrences of AI were observed in Amelogenin, with all AI events ≥60% (data not shown). The AI data from this evaluation will contribute to further studies on a revised in-house AI threshold.

Table 18. Frequency of all autosomal allelic imbalance observed in the evaluation.

Kit	Number of autosomal AI		Profiler Plus loci exhibiting allelic imbalance					
	Cell	Blood	D3S1358	FGA	D13S317	D8S1179	D18S51	D7S820
Chelex	0	1	1					
DNA IQ	1				1			
QIAamp DNA Micro	1	2				1	2	
ChargeSwitch	2	3	1	1	2			1
forensicGEM		1			1			
NucleoSpin 8 Trace	5	1	2	1	2		1	
Total	9	8	4	2	6	1	3	1
	17		23.53%	11.76%	35.29%	5.88%	17.65%	5.88%

Neat cell or blood samples that were extracted using the various kits displayed varying inhibition results for denim dye and heme (Table 16). In several cases, if a kit did not show inhibition for denim dye, it would show inhibition for heme, or vice versa. Only the DNA IQ™ and NucleoSpin® 8 Trace systems did not indicate inhibition for either inhibitor. There did not appear to be a link between the presence or absence of inhibition and the observation of allelic imbalance, although DNA IQ™ and NucleoSpin® 8 Trace generated the most number of total reportable alleles (534 and 466 alleles respectively). These results suggest that the ability to remove inhibitors (such as encountered in the DNA IQ™ and NucleoSpin® 8 Trace protocols) can result in an increase in the number of resolvable alleles, therefore successfully obtaining more DNA profile results more often.

Cotton substrates (e.g. cotton swabs and cotton cloth) make up a large percentage of samples processed in DNA Analysis FSS. For example, cotton swabs make up around 45% of the total number of sample types analysed for DNA analysis (Figure 4). It was therefore considered important that the DNA extraction kits evaluated could process samples and stains on cotton matrices. It was found that the neat cell samples that displayed the highest quantitation values across all extraction kits originated from cotton swab substrates, except for Chelex® results where the best result came from FTA (Table 16).

All of the forensic DNA extraction kits evaluated are amenable to automation, and automated protocols already exist for several kits. However, only the DNA IQ™ kit has been validated for use on the MultiPROBE® II PLUS HT EX platform and a validated protocol was developed by PerkinElmer (PerkinElmer, 2004).

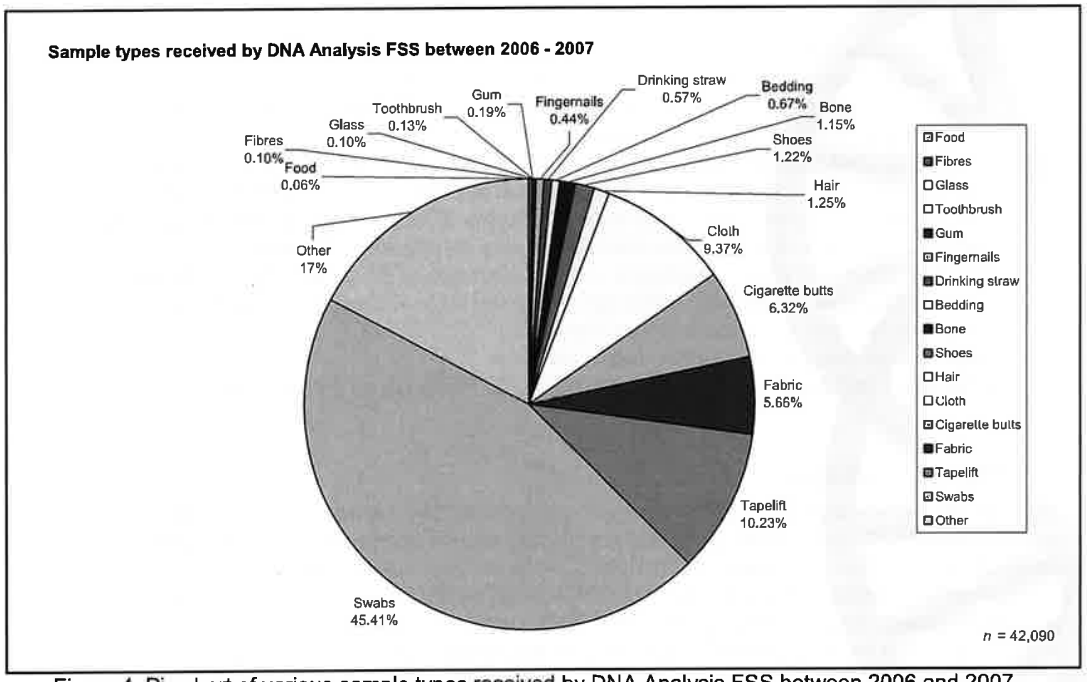

Figure 4. Pie chart of various sample types received by DNA Analysis FSS between 2006 and 2007. Around 45% of samples received for DNA analysis are swab substrates. Data was obtained from AUSLAB on 14 November 2007.

Some of the concerns raised regarding some of the kits tested include:

- QIAamp® DNA Micro involved multiple tube transfers that increased the risk of cross-contamination and also increased processing time to 5 hours for 12 samples.
- An increased risk of contamination was also prevalent in the NucleoSpin® 8 Trace method when coupled with the PVC vacuum manifold, because of the need to fit multiple adapters to ensure seals are maintained for a proper vacuum environment. If the plates and adapters were not assembled correctly, the vacuum environment would fail and possibly cause cross-contamination and, more alarmingly, loss of sample. Furthermore, even when assembled correctly, biohazardous contaminants (e.g. blood) are drawn down the manifold through the vacuum tubing and into the collection containers. Decontamination of the tubing and containers raises serious health and safety concerns.
- The *forensic*GEM™ system was the quickest protocol to perform and yielded crude DNA extracts that produced high allele counts for cell samples. However, the system could not deal with blood samples (and heme inhibition) effectively, therefore causing very low allele counts for blood samples.
- ChargeSwitch® was the alternative magnetic bead system to DNA IQ™. However, ChargeSwitch® did not produce results that were comparable or better than DNA

IQ™. For example, more ChargeSwitch® samples did not yield quantitation results compared to DNA IQ™ and resulted in a lower total allele count. ChargeSwitch® also did not appear to be able to effectively deal with inhibition from the dye in denim material.

Overall, data from the evaluation suggested that DNA IQ™ outperforms all of the forensic DNA extraction kits tested, in addition to the in-house Chelex® protocol. In summary, DNA IQ™:

- Is quick to perform – the amount of time taken to complete the DNA extraction protocol is comparable to the in-house Chelex® method;
- Includes washing steps to remove inhibitors – washing of the immobilised DNA enables purified DNA template to be eluted;
- Produced DNA quantitation values for most (>90%) samples – the percentage of samples that did not yield a quantitation result was one of the lowest for DNA IQ™;
- Generated the highest number of total reportable alleles – samples extracted using DNA IQ™ produced 65% more resolved alleles compared to Chelex®;
- Exhibited minimal allelic imbalance – the occurrence of AI in DNA IQ™ samples was comparable to Chelex®, although increased AI in Amelogenin was observed;
- Was not inhibited by heme in blood samples;
- Was not inhibited by the dye in denim material;
- Has been validated for use on the MultiPROBE® II PLUS HT EX platform.

7. Recommendations

Based on the results from evaluating various commercial DNA extraction kits that were designed specifically for forensic use, and comparing results from each kit to the current in-house Chelex® protocol, we have found DNA IQ™ to be the most suitable kit for extracting cell and blood samples that are analysed in DNA Analysis FSS. We therefore recommend that further studies be performed on the DNA IQ™ system in order to:

1. Validate a manual DNA IQ™ protocol for extracting various DNA Analysis FSS substrate types;
2. Verify an automated DNA IQ™ extraction program on the MultiPROBE® II PLUS HT EX platforms for automated DNA extraction of various DNA Analysis FSS substrate types.

8. References

Butler J (2005). Forensic DNA Typing. Elsevier: Burlington, MA, USA.

Gallagher B, Hlinka V, Muharam I, Iannuzzi C, Lundie G, Nurthen T, Ientile V (2007). Mock sample creation for cell and blood samples [Laboratory Report]. DNA Analysis FSS: Brisbane, QLD, Australia.

Invitrogen Life Technologies (2005). ChargeSwitch® Forensic DNA Purification Kits for purification of genomic DNA from forensic samples [PN 25-0825, Version A, 3 January 2005]. Invitrogen Corporation: Carlsbad, CA, USA.

Macherey-Nagel (2003). NucleoSpin® 8 Trace: Genomic DNA from Forensic Samples [Rev. 0.2, November 2003]. Macherey-Nagel: Düren, Germany.

Moss D, Harbinson S-A, Saul DJ (2003). An easily automated, closed-tube forensic DNA extraction procedure using thermostable proteinase. *Int J Legal Med* 117:340-349.

PerkinElmer (2004). MultiPROBE II Liquid Handling Forensic Workstation Application Guide: Automated DNA IQ™ System for Mixed Casework Sample DNA Isolation [PN 8842157]. PerkinElmer Life and Analytical Sciences: Downers Grove, IL, USA.

Promega Corporation (2006). DNA IQ™ System – Small Sample Casework Protocol [PN TB296, Rev. 4/06]. Promega Corporation: Madison, WI, USA.

Qiagen (2003). QIAamp® DNA Micro Handbook [Rev. 08/2003]. Qiagen GmbH: Hilden, Germany.

QIS 19171 (2007). Method for Chelex® Extraction [Standard Operating Procedure]. DNA Analysis FSS: Brisbane, QLD, Australia.

Scott S, Harbison S-A, Saul DJ (2003). Developmental validation of *forensic*GEM™: a Thermophilic Proteinase for Forensic DNA Extraction [oral presentation at the 18th International Symposium on the Forensic Sciences, Fremantle, WA, 2-7 April 2006]. Institute of Environmental Research Ltd: Hamilton, New Zealand.

Walsh PS, Metzger DA, Higuchi R (1991). Chelex®-100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* 10:506-13.

ZyGEM (2006). DNA extraction from buccal swabs using *forensic*GEM™. ZyGEM Corporation Ltd: Hamilton, New Zealand.

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

August 2008

Automation and LIMS Implementation Project Team,

DNA Analysis

Forensic And Scientific Services

Clinical and Scientific Services

Queensland Health

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

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

1. Abstract

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

2. Introduction

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

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

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

3. Aim

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

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4. Equipment and Materials

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

5. Methods

5.1 Cell and blood collection

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

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

Table 1 lists the donor sample ID's.

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

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

5.2 Cell counting

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

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

5.3 Sensitivity, Reproducibility (Linearity) and Yield

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

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

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

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

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

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

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

5.4 Inhibition challenge

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

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

Table 2. Concentrations of various inhibitors used in the inhibition study.

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

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

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

5.5 Substrates

Swabs

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

Tapelifts

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

Fabric

The material types tested included:

- Denim jeans;

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

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

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

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

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

5.6 Mixture studies

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

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

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

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

5.7 Substrate size

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

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

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

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

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

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

The TNE buffer consisted of:

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

The adapted manual DNA IQ™ protocol is described below:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

5.9 DNA quantitation

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

5.10 PCR amplification

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

5.11 Capillary electrophoresis and fragment analysis

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

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6. Results and Discussion

6.1 Donor sample cell counts

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

6.2 Sensitivity, consistency and yield

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

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

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

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

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

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

Sample type	Dilution factor	Theoretical Input DNA (ng)	Rayon swab yield (ng)	Alleles	Cotton swab yield (ng)	Alleles	Rayon average yield (ng)	Rayon Std Dev	Recovery Rayon (%)	Cotton average yield (ng)	Cotton Std Dev	Recovery Cotton (%)
Cells	Neat	706.56000	110.0000	18	117.0000	18	134.5400	41.30	19.04	95.2800	32.69	13.48
			130.0000	18	124.0000	18						
			160.0000	18	46.8000	18						
			83.7000	7	76.6000	18						
			189.0000	17	112.0000	18						
	1/10	70.65600	10.1000	18	12.8000	18	10.4520	1.44	14.79	10.4820	2.52	14.84
			12.7000	18	6.3100	18						
			9.5500	18	11.5000	18						
			9.0100	18	10.1000	18						
			10.9000	18	11.7000	18						
	1/100	7.06560	0.6350	0	0.0000	0	0.9254	0.64	13.10	0.1270	0.18	1.80
			0.4930	0	0.0000	0						
			1.4000	5	0.2770	0						
			1.7900	14	0.3580	0						
			0.3090	0	0.0000	0						
	1/1000	0.7656	0.0000	0	0.3630	0	0.0166	0.04	2.17	0.0726	0.16	9.48
			0.0000	0	0.0000	0						
			0.0000	0	0.0000	0						
			0.0831	0	0.0000	0						
			0.0000	0	0.0000	0						
Blood	Neat	487.68000	216.0000	18	718.0000	18	317.0000	102.36	65.00	447.0000	196.46	91.66
			447.0000	18	297.0000	18						
			215.0000	18	595.0000	18						
			383.0000	7	326.0000	18						
			324.0000	18	299.0000	18						
	1/10	48.76800	113.0000	18	126.0000	18	124.7800	28.10	255.86	97.6600	21.66	200.25
			107.0000	18	91.9000	18						
			145.0000	18	75.4000	18						
			95.9000	18	81.0000	18						
			163.0000	18	114.0000	18						
	1/100	4.87680	14.3000	18	15.9000	18	12.4800	1.62	255.91	16.7600	4.69	343.67
			12.5000	13	12.1000	18						
			13.2000	18	20.8000	18						
			9.9000	18	22.4000	18						
			12.5000	18	12.6000	18						
	1/1000	0.48768	0.7300	18	2.3700	18	0.8894	0.20	182.37	3.0200	0.85	619.26
			0.6990	18	3.1300	18						
			1.1800	18	3.6300	18						
			0.8670	18	1.9700	18						
			0.9710	18	4.0000	18						

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

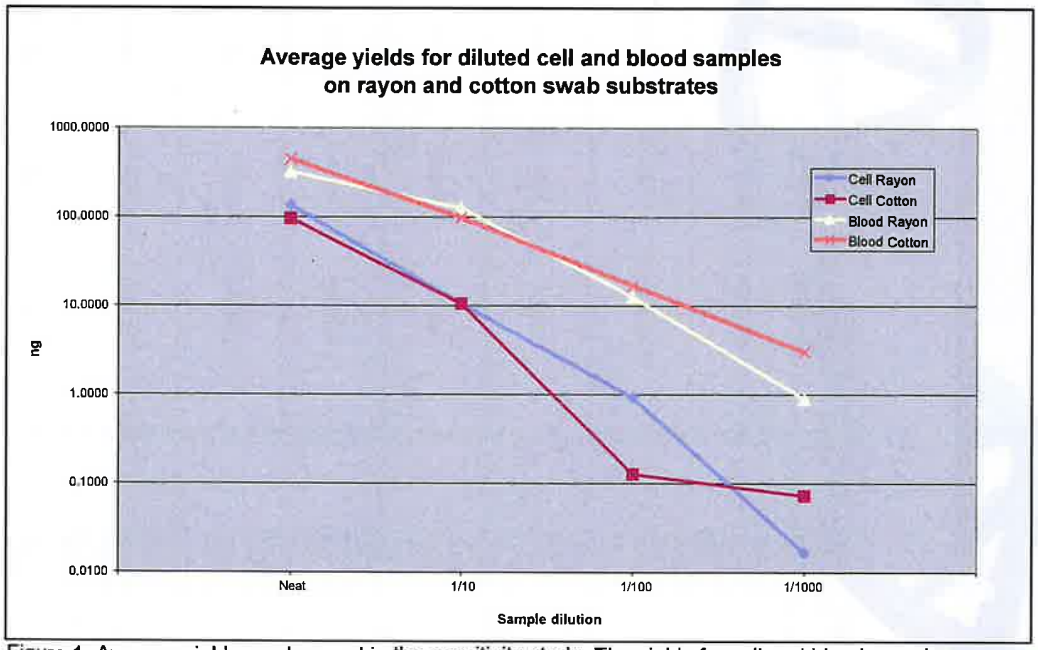

Figure 1. Average yields as observed in the sensitivity study. The yields for cell and blood samples, on two different swab types, were comparable as indicated by overlapping lines on the graph.

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

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

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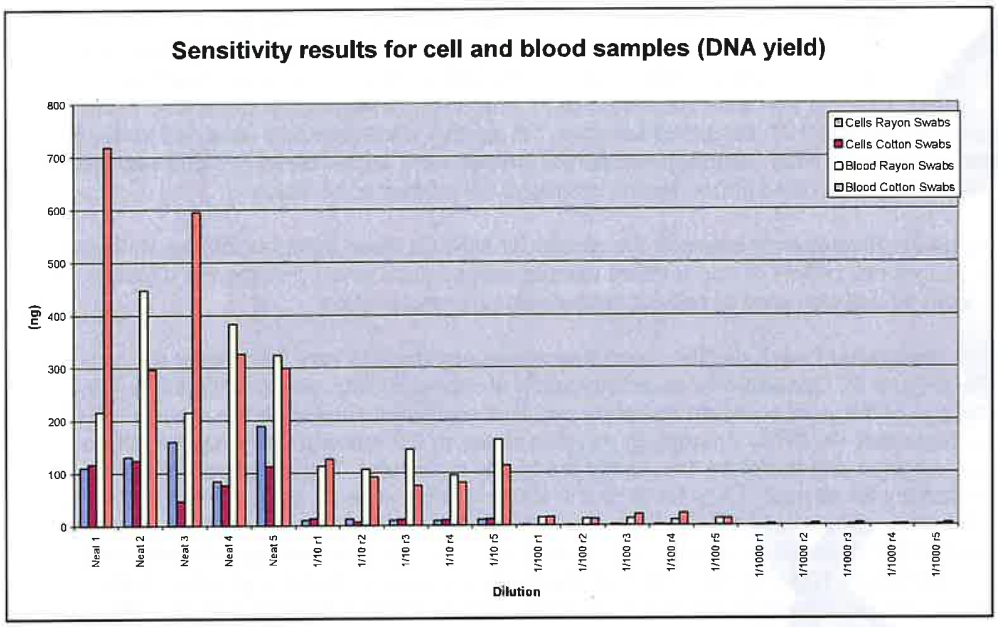

Figure 2. DNA yields (ng) observed for the sensitivity study. As expected, neat samples provided the highest yields. Yields were obtained down to 1/1000 for blood samples and 1/100 for cell samples.

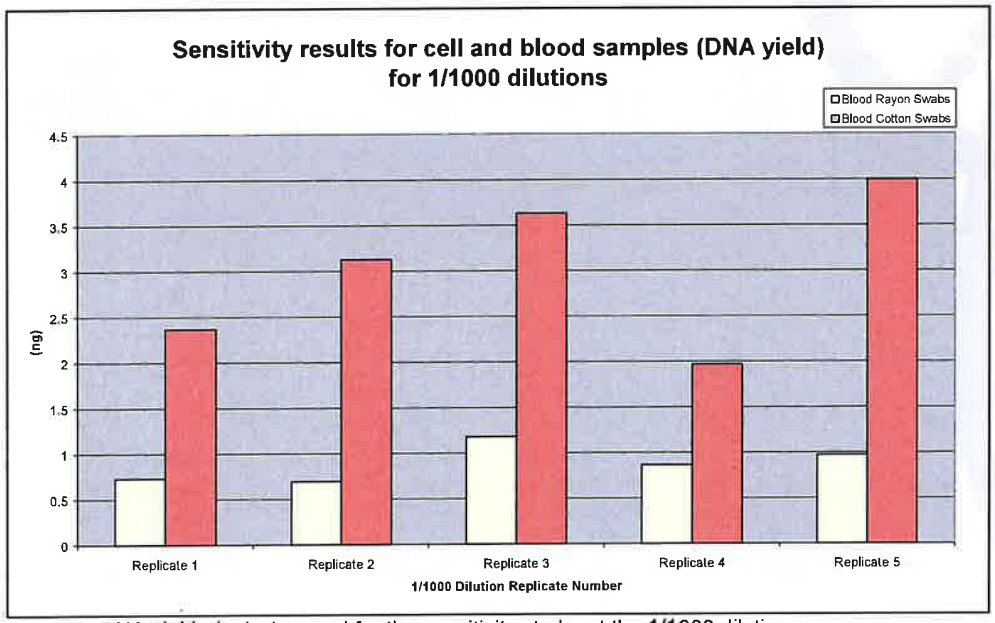

Figure 3. DNA yields (ng) observed for the sensitivity study, at the 1/1000 dilution.

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

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

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

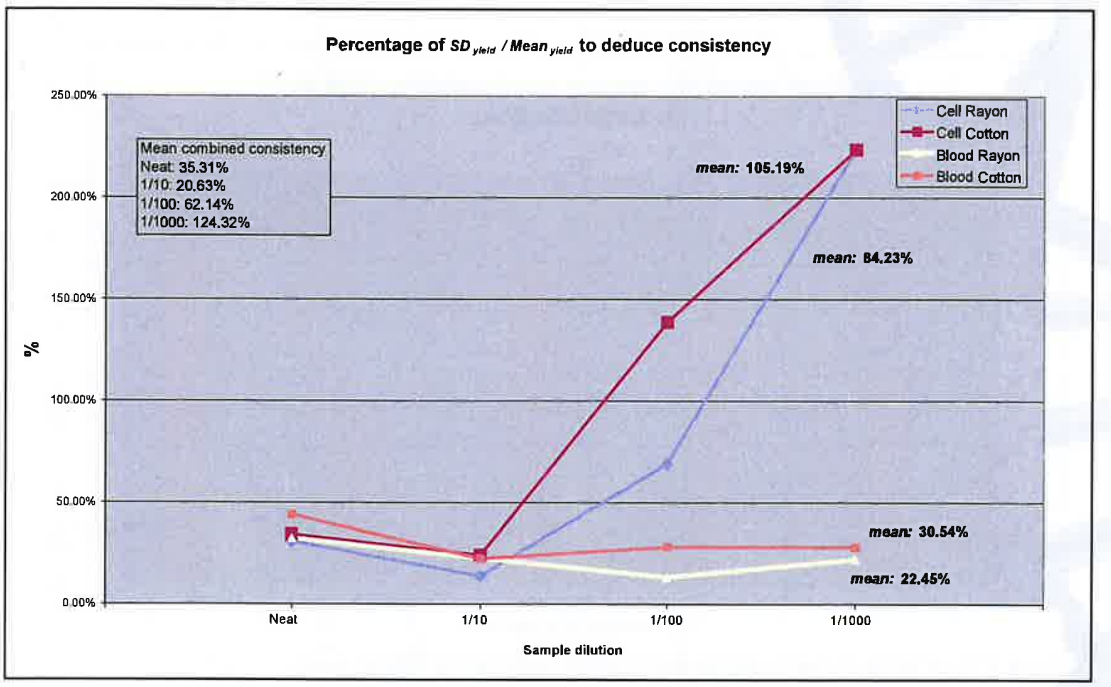

Figure 4. Reproducibility between replicates for cell and blood samples diluted down to 1/1000.

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6.3 Inhibition

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

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

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

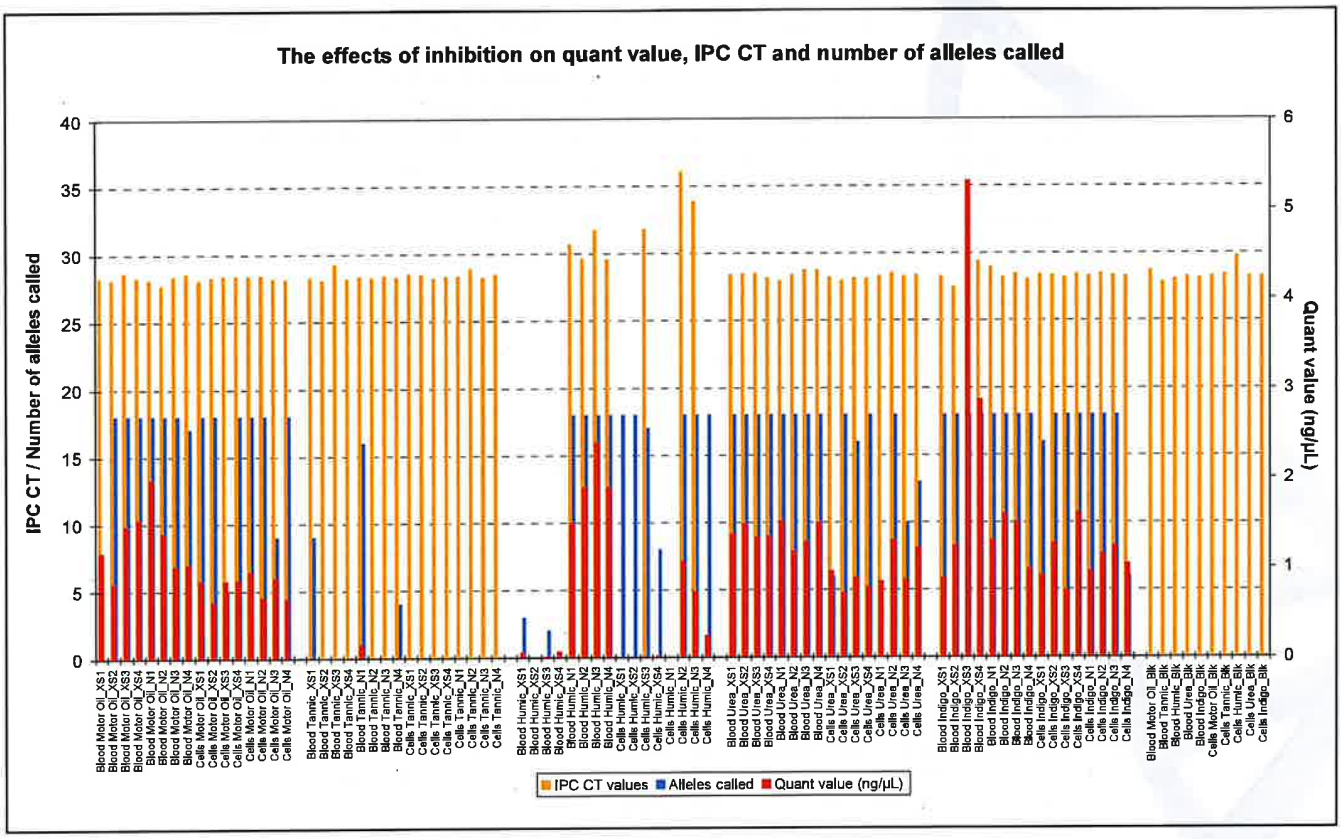

Figure 5. Effects of various inhibitors on quant value, IPC CT and number of resolved alleles.

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

The observations that were made include:

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

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

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6.4 Substrates

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

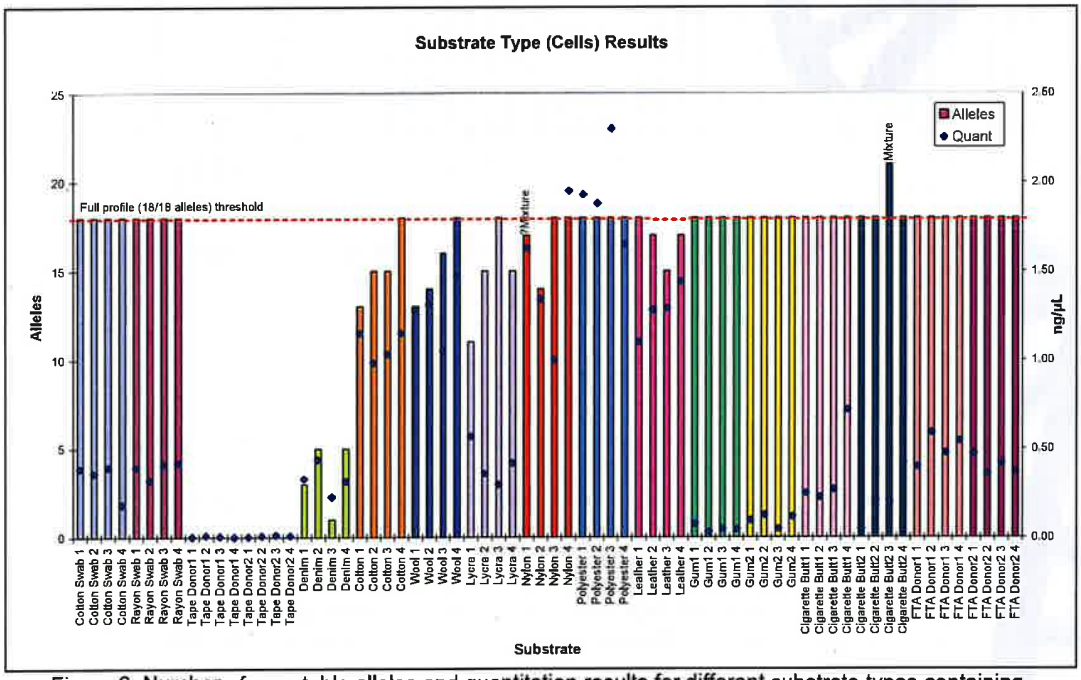

Figure 6. Number of reportable alleles and quantitation results for different substrate types containing cellular material.

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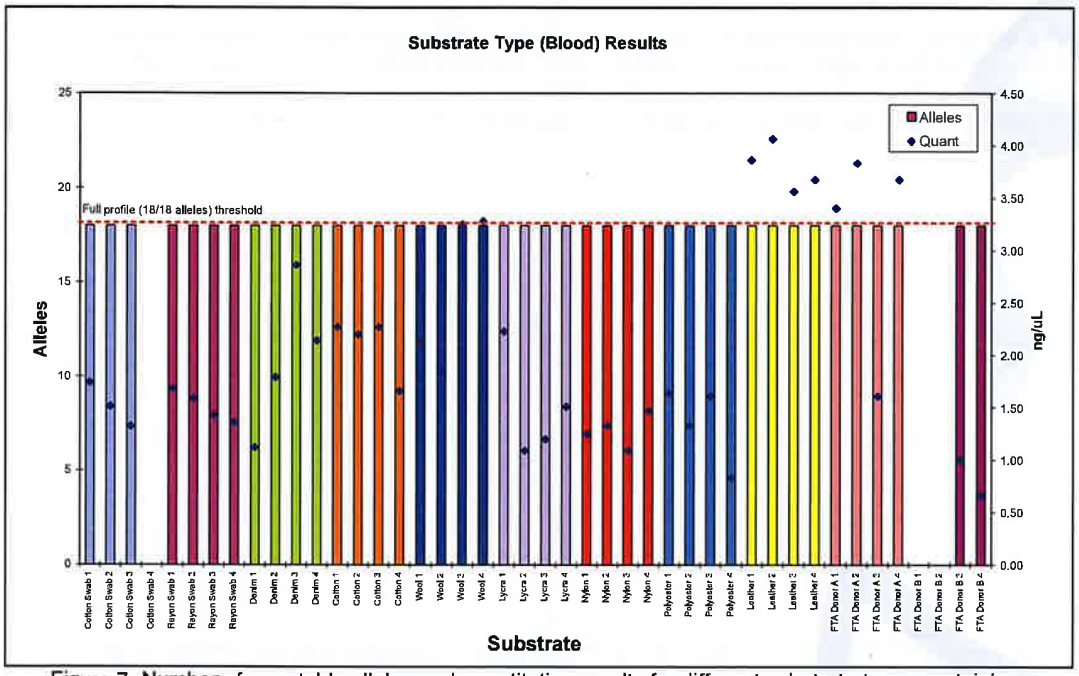

Figure 7. Number of reportable alleles and quantitation results for different substrate types containing blood material.

For cell samples:

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

For blood samples:

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

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

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

6.5 Mixture studies

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

6.6 Substrate size

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

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

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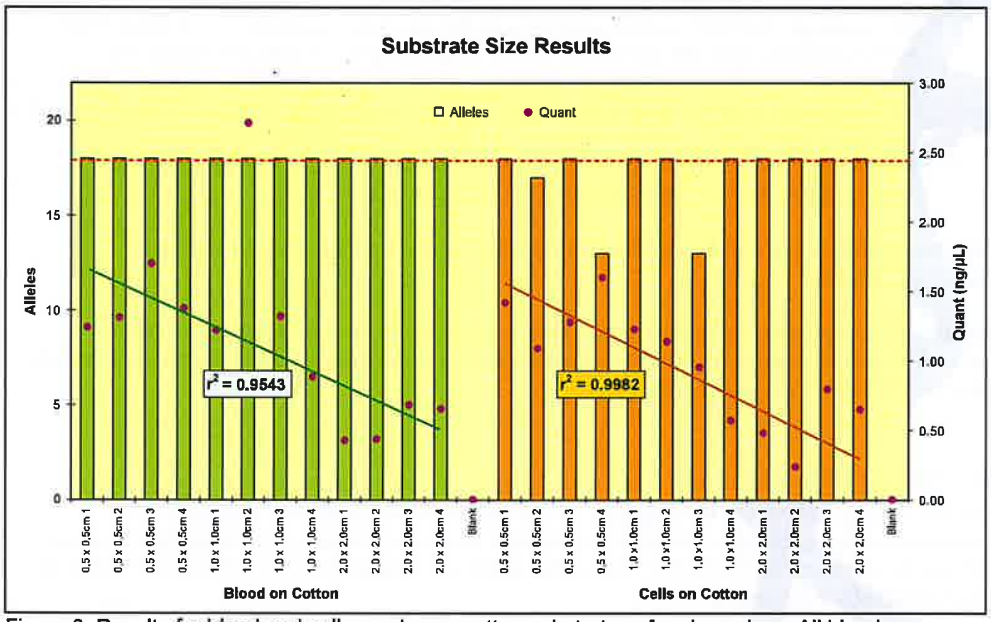

Figure 8. Results for blood and cell samples on cotton substrates of various sizes. All blood samples generated full profiles, but cell samples were more variable. The quantitation results for 0.5 x 0.5cm samples were higher than those for 2.0 x 2.0cm samples (blood r^2 = 0.9543*; cell r^2 = 0.9982;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

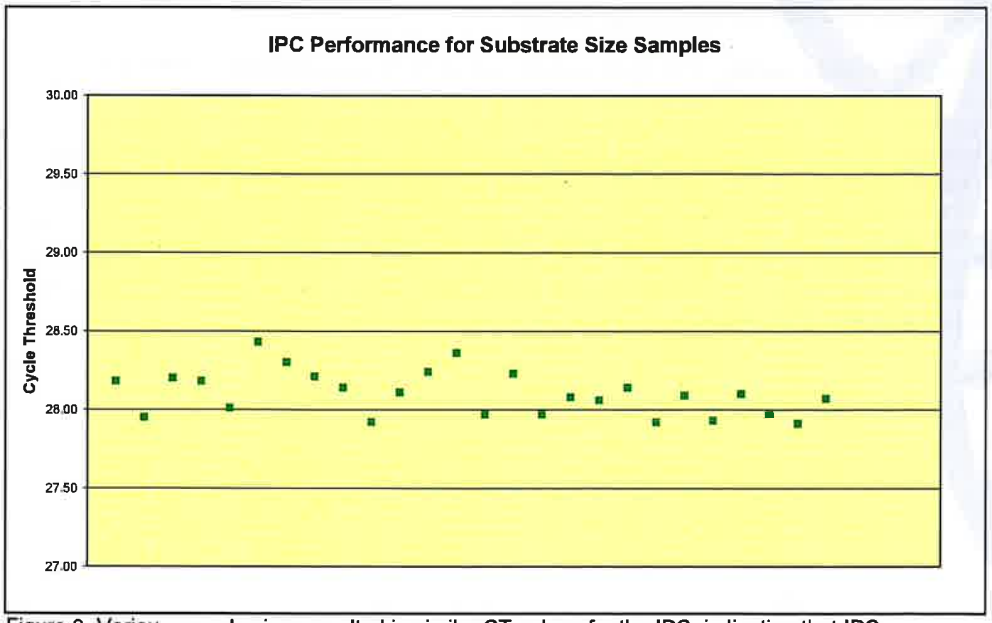

Figure 9. Various sample sizes resulted in similar CT values for the IPC, indicating that IPC performance is not affected by sample size, and that one sample size does not display a level of inhibition that is different to another sample size.

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7. Summary and Recommendations

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

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

8. Acknowledgements

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

9. References

Butler JM (2005). Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers, 2nd Edition. Elsevier: Burlington, MA, USA.

Gallagher B, Hlinka V, Iannuzzi C, Lundie G, Muharam I, Nurthen T, Ientile V (2007a). Project 9: Report on the Evaluation of Commercial DNA Extraction Chemistries [Laboratory Report]. DNA Analysis, FSS: Brisbane, QLD, Australia.

Gallagher B, Hlinka V, Muharam I, Iannuzzi C, Lundie G, Nurthen T, Ientile V (2007b). Mock sample creation for cell and blood samples [Laboratory Report]. DNA Analysis FSS: Brisbane, QLD, Australia.

Hlinka V, Gallagher B, Iannuzzi C, Lundie G, Muharam I, Nurthen T, Ientile V (2007). Evaluation of the effect of inhibitors as assessed by a silica-based DNA extraction method and real-time PCR [in preparation].

Huston K (2002). DNA IQ™ System "Frequently Asked Questions". *Profiles in DNA* 5(1):11-12.

Mahony J, Chong S, Jang D, Luinstra K, Faught M, Dalby D, Sellors J and Chernesky M (1998). Urine specimens from pregnant and nonpregnant women inhibitory to amplification of *Chlamydia trachomatis* nucleic acid by PCR, ligase chain reaction, and transcription-mediated amplification: identification of urinary substances associated with inhibition and removal of inhibitory activity. *Journal of Clinical Microbiology* 36:3122-3126.

Muharam I, McNevin A, Iannuzzi C, Nurthen T, Ientile V (2007). Project 15: Report on the verification of automated capillary electrophoresis setup using the MultiPROBE® II PLUS HT EX platform [Laboratory Report]. DNA Analysis, FSS: Brisbane, QLD, Australia.

Mulot C, Stücker I, Clavel J, Beaune P, Loriot M-A (2005). Collection of human genomic DNA from buccal cells for genetic studies: comparison between Cytobrush, mouthwash and treated card. *Journal of Biomedicine and Biotechnology* 2005(3):291-6.

PerkinElmer (2004). MultiPROBE II Liquid Handling Forensic Workstation Application Guide: Automated DNA IQ™ System for Mixed Casework Sample DNA Isolation [PN 8842157]. PerkinElmer Life and Analytical Sciences: Downers Grove, IL, USA.

Promega Corporation (2006). DNA IQ™ System – Small Sample Casework Protocol [PN TB296, Rev. 4/06]. Promega Corporation: Madison, WI.

QIS 15396 R4 (2007). Method for bronchoalveolar lavage (BAL) cell count and differential cell count. [Standard Operating Procedure]. Anatomical Pathology, RBWH: Brisbane, Australia.

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QIS 19976 R1 (2007). Automated amplification of extracted DNA using the AmpFℓSTR® Profiler Plus® or AmpFℓSTR® COfiler® kit [Standard Operating Procedure]. DNA Analysis FSS: Coopers Plains, Brisbane, Australia.

QIS 19977 R1 (2007). Automated quantification of extracted DNA using the Quantifiler™ Human DNA Quantification Kit [Standard Operating Procedure]. DNA Analysis, FSS: Coopers Plains, Brisbane, Australia.

QIS 19978 R0 (2008). Capillary electrophoresis setup [Standard Operating Procedure]. DNA Analysis, FSS: Coopers Plains, Brisbane, Australia.

Satia-Abouta, King IB, Abouta JS, Thornquist MD, Bigler J, Patterson RE, Kristal AR, Shattuck AL, Potter JD, White E (2002). Buccal cell DNA yield, quality and collection costs: comparison of methods for large-scale studies. *Cancer, Epidemiology, Biomarkers & Prevention* 11:1130-3.

Shutler GG, Gagnon P, Verret G, Kalyn H, Korkosh S, Johnston E and Halverson J (1999). Removal of a PCR inhibitor and resolution of DNA STR types in mixed human-canine stains from a five year old case. *Journal of Forensic Sciences* 44(3):623-626.

Tsai YL and Olson BH (1992). Rapid method for separation of bacterial DNA from humic substances in sediments for polymerase chain reaction. *Applied and Environmental Microbiology* 58(7):2292-2295.

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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.

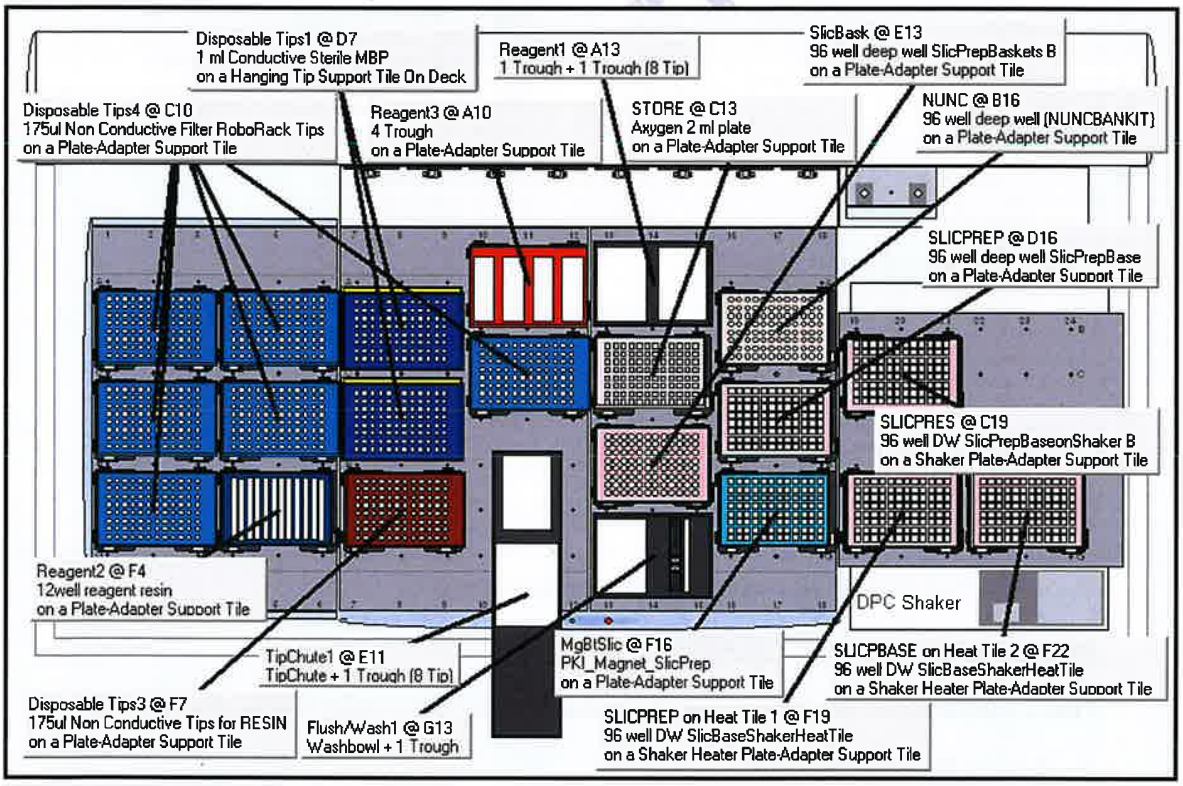


Figure 3. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

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

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

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

Recording Reagent Details in AUSLAB

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

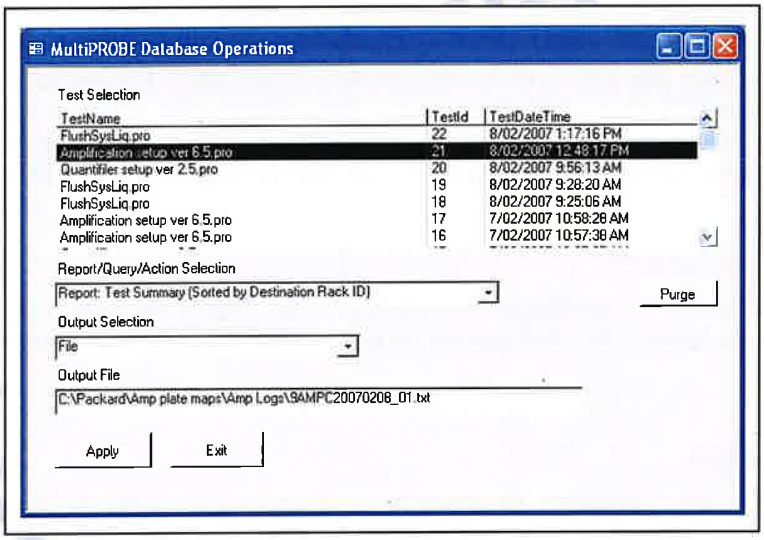
Finalising the MP II run

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

Importing the MP II log file into AUSLAB

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

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



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

Importing Extraction "Results" into AUSLAB

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

8 SAMPLE STORAGE

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

9 VALIDATION

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

10 QUALITY ASSURANCE/ACCEPTANCE CRITERIA

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

11 REFERENCES

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.
3. Cowan, C., The DNA IQ™ System on the Tecan Freedom EVO® 100 Profiles in DNA. Profiles in DNA, 2006: p. 8-10.
4. Eminovic, I., Karamehić, J., Gavrankapetanović, F. & Helijić, B., A Simple method of DNA Extraction in Solving Difficult Criminal Cases. MEDARH, 2005. 59(1): p. 57-58.
5. Greenspoon, S.B., J., Robotic Extraction of mock sexual assult samples using the Biomek® 2000 and the DNA IQ™ System. Profiles in DNA, 2002. 5: p. 3-5.
6. Komonski, D.I., Marignani, A., Richard, M.L., Frappier, J.R.H., & Newman, J.C., Validation of the DNA IQ™ System for use in the DNA extraction of high volume forensic casework. Can.Soc.Forensic Sci.J., 2004. 37(2): p. 103-109.
7. Mandrekar, P., V., Flanagan, L., & Tereba, A., Forensic Extraction and Isolation of DNA Form Hair, Tissue and Bone. Profiles in DNA, 2002: p. 11.
8. Mandrekar, P.V., Kreneke, B. E., & Tereba, A., DNA IQ™: The Intelligent Way to Purify DNA. Profiles in DNA, 2001: p. 16.
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.
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.
11. PerkinElmer, Automated DNA IQ™ System for Mixed Casework Sample DNA Isolation. MultiPROBE II Liquid Handling - Forensic Workstation Application Guide, 2004: p. 1-25.
12. Promega, FAQs –DNA IQ™ System.
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

VI-5
JH-3

Project number	Project name	Implementation date	Comments
238	Evaluation of DBLR v1 2	ongoing	
237	Reduction in Physical Case file creation	Sep-21	
236	Exhibit Result Line Revision	Nov-21	
235	2021 FR version upgrade	ongoing	
234	Process mapping of Interpretation and reporting	closed	
233	Bone sampling and demineralisation protocol	on-hold	
232	Paternity calculations for mixed DNA samples	closed	
231	Verification of STRmix v2.8	Jun-21	
230	Implementation of 3500xL PP21 Casework	Feb-21	
229	Paternity Index Distributions by Locus in PP21	closed	
228	Review of current baseline thresholds 3130xl using PP21	closed	
227	Baseline method trial	ongoing	
226	Collection of sperm from pubic hair	on-hold	
225	Evaluation of DBLR	not implemented	
224	Evaluation of FaSTR DNA	not implemented	
223	DCS v4.0	Apr-22 (3500xL B)	
222	Profiling of Spermatozoa from microscopy slides	ongoing	
221	Impact of magnetic fingerprint powders on bead-based trace DNA extraction (collab with QPS)	closed	
220	Verification of commercial H & E stains	Q1-21	
219	Verification STRmix 2.7 for 3500xL	Mar-21	
218	Verification of BSD600 Ascent A2	Nov-20	
217	Verification of Maxwell FSC Instruments	Jun-20	
216	Validation of Ion Chef & S5	ongoing	
215	STRmix v2.7 - comparison of LRs for 5p mixtures	on-hold	
214	Validation of STRmix v2.7	Feb-20	
213	Verifiler Plus	ongoing	
212	Storage Transition into FR	Aug-19	
211	Streamlining of DNA profile result reporting workflow	on-hold	
210	Verification of GeneMapper v1.6	Jan-20	
209	Verification of SPEX 6775 Freezer Mill	Q4-19	
208	Verification of STRmix v2.6.2	Jun-19	
207	Verification of Pro K	Aug-19	
206	Y Filer Plus	ongoing	
205	Post implementation review of STRmix v2.6.0	nil	
204	Diamond dye collaboration with QPS	not implemented	
203	Number of alleles for SS LR greater than 100billion with STRmix v2.6	closed	
202	Validation STRmix v2.6.0	Jan-19	
201	QIAsymphony QSL3 Verification	Feb-19	
200	Statement format and wording revision	on-hold	
199	Proflex	Jan-22	
198	Assessment of OSD reworking on Intelligence Reference samples	closed	
197	Interpretation of 4 person mixtures using STRmix v.2.0.6	Aug-18	
196	Verifiler Plus Trial	nil	
195	Testing of Quant and Amp reagent stability at room temperature	Apr-18	
194	Verification of QIAsymphony SPAS	Apr-18	
193	Verification of STRmix v2.5.11	not implemented	
192	QIAsymphony Bone Teeth	Apr-18	
191	Effects of HCl on DNA persistence and profiling	nil	
190	Research Project - MPS	nil	
189	Y Filer plus implementation	ongoing	
188	Verification of Maxwell for Retain Supernatant	Jun-18	
187	Verfication of STRmix v2 0.6 for use with the 3500	closed	
186	Analysis of Casework PP21 samples using 3500xL A	not implemented at this time	
185	Validation of QS5	Feb-19	
184	Evaluation of the efficacy of Microcons	Feb-18	
183	Implementation of NCIDD-IFA (bonaparte)	Q3-19	
182	PP21 WEN CW 3500xL Validation	closed	
181	Sperm microscopy sensitivity	Nov-20	
180	Use of STRvalidator for validation or verification	not implemented	
179	DNA sequencing at D18S51 locus	closed	
178	Verification and implementation of STRmix v2.4.03	not implemented	
177	3500 CW PP21-WEN	closed	
176	Investigation of ICMP protocol	closed	
175	Hamilton ID STARlet - CE	May-19 (Starlet C)	
174	Verification of ARTEL (PCS and MVS)	retrospective	MVS introduced to laboratory in 2009, PCS introduced 2013
173	Hamilton ID STARlet - Pre PCR	Jan-17 (A) and Jul-17 (B)	
172	Phadebas testing from suspension in ERT	closed	
171	PP21 Verification of new ILS and Matrix	Q3-16	
170	Reassessment of in-house stutter thresholds and stutter file	not implemented	
169	Verification of swab suspension at RT	closed	
168	Validation of QIAsymphony	Nov-16	
167	Verification and implementation of STRmix V2 3	closed	

Project number	Project name	Implementation date	Comments
166	Verification of TMB Screening Test for Blood	retrospective	
165	Verification of Phadebas paper	retrospective	
164	Case Management improvements	Jan-15	
163	Assessment of results obtained from auto-microcon samples	closed	
162	OSIRIS Freeware for Profile Viewing	closed	
161	FTP processing on OSD plate	Mar-15	
160	Verification of STRmix V2.0.6	Jan-15	
159	M-VAC trial by QPS	not implemented	
158	Statement in Table Format	closed	
157	Quant Standard Data Mining	closed	
156	Verification of 7500A after thermal cycling block change	Aug-14 returned to service	
155	Verification of software for 3130s	closed	
154	Verfication of software for 7500	Did not proceed	
153	Verification of Trigene Advanced	retrospective	
152	Validation of Quantifiler Trio and Y-Filer Plus	Nov-2015 (quantifiler)	Y-filer: additional work is required to complete validation prior to use
151	Verification and implementation of STRmix 2 0.1	Jul-14	
150	Suitability of combining wet and dry swabs from SAIKs	closed	
149	Development of guidelines for number of contributors	Mar-15	
148	Cleaning bone processing equipment	Jul-19	
147	Quantifiler re-validation after manufacturing changes	Aug-14	
146	Globalfiler validation	closed	
145	3500 validation	Mar-15 (A), Jan-16 (B)	
144	Christmas Tree Staining	closed	
143	Foreign DNA on Semen Negative SAIK swabs	Jan-14	
142	Concentration of large items	not implemented	
141	PowerPlex optimisation program	closed	
140	GlobalFiler Express Kit FTA sensitivity study	closed	
139	Extraction negative tube	Not implemented	
138	Batch Case Management	Dec-13	
137	Accepting partial Amel	Mar-14	
136	Frozen AP	Jul-14	
135	Verification of an additional Thermalcycler	Mar-14	
134	Number of alleles for SS LR greater than 100billion	Jul-14	
133	QPFREG - AUSLAB upgrade	Closed	
132	Mantis verification	Nov-15	
131	PP21 post implementation review	nil	
130	GlobalFiler_testing	Not implemented	
129	Reference profile interpretation (EXH lines)_Combined with #126	All information under #126	
128	Trial of QIAGEN Investigator Quantiplex Kit	Not implemented	
127	Verification of GM-IDX software upgrade	Jun-15	
126	DNA profiles without STRmix)	May-13	
125	AUSLAB All Incomplete requests	Jan-14	
124	Generic Instrument interface	Sep-13	
123	Verification of Maxwell DNA Extraction from Bone	Not implemented	
122	Verification ofMaxwell DNA Extraction from Tissue	Not implemented	
121	Verification of new Pro K and DTT	Mar-13	
120	Verification of new Taq in Profiler Plus kits	Q4-12	
119	Validation of Extracting DNA from Concrete	Not implemented	
118	Validation of Extracting DNA from Soil	Not implemented	
117	Creation of animal semen repository	Folder empty	
116	Verification of a New Size Std for GeneScan	Mar-13	
115	Verification of a new membrane for M'con	Jan-13	
114	Change of SAIK booklet and kit	Aug-12	
112	Evaluation of continued competence	closed	
111	Sexual assault reassessment	closed	
110	AUSLAB hardware replacement cutover	Jun-12	
109	Maxwell C & D verification	Jun-12	
108	Pipette disposal	Feb-12	
107	PowerPlex 21 Implemen	Ref Sep-12, CW Dec-12	
106	PowerPlex 21 NCIDD	Ref Sep-12, CW Dec-12	
105	PowerPlex 21 Reporting and STRmix	Ref Sep-12, CW Dec-12	
104	PowerPlex 21 Concordance	Ref Sep-12, CW Dec-12	
103	PowerPlex 21 Mixture	Ref Sep-12, CW Dec-12	
102	PowerPlex 21 Thresholds	Ref Sep-12, CW Dec-12	
101	PowerPlex21 Population	Ref Sep-12, CW Dec-12	
100	PowerPlex 21 Sens	Ref Sep-12, CW Dec-12	
99	PowerPlex 21 program	Ref Sep-12, CW Dec-12	
98	Page numbering of statements	Jan-12	
97	BSD 200uL	Q3-12	
96	Statement Appendix 5	Feb-12	
95	P30 addendum	Not implemented	
94	ESI Pro kit	Not implemented	
93	European Loci AUSLAB changes	Not implemented	
92	Efractions in SAIKS	Feb-12	
91	Maxwell Pre lysis	Jul-13	

Project number	Project name	Implementation date	Comments
90	Population dataset	closed	
89	New Change management	May-12	
88	European population dataset	closed	
87	Autoclave use	Nov-11	
86	Maxwell Hair and Fingernail	Jan-12	
85	Maxwell Diff Lysis	Feb-12	
84	Maxwell Paper & Gum	Sep-11	
83	Maxwell Fabric	Sep-11	
82	Capillary Regeneration	Mar-14	
81	Volume Case management	Jul-11	
80	Volume Undetermined	Jul-11	
79	Maxwell Tapelifts	Sep-11	
78	ABA Cards	Jul-11	
77	GeneAmp 9700 B & D	Mar-11	
76	Manual Staining	Dec-12	
75	QA pend	Apr-11	
74	Destructions	Nov-11	
73	GeneAmp 9700 C & E	Mar-11	
72	GeneAmp 9700 verification	Feb/Mar-11	
71	BSD Series II	Feb-11	
70	Maxwell	Q2/3-11	
69	Sensitivity Amp Vol Euro Loci	closed	
68	New Loci	closed	
67	Tube FBX testcodes	Jun-13	
65	CAPIT-ALL decapper	Nov-10	
64	Modified Off-deck lysis	Not implemented	
63	England Finder	Q1/Q2-11	
62	Re-implement of auto DNAIQ	Aug-09	
61	Theta in Reporting Stats	Dec-12	
60	Change to retention of receipt	Dec-09	
59	Commercial cell line	Not implemented	
58	Half vol P+ reactions	Not implemented	
57	7500	Jun-10	
56	Re-implement of Auto DNAIQ	Aug-09	
55	2uL for CE	Oct-11	
54	400HD ROX	Sep-12	
53	Artel	See project #174	
52	New Software & interp	Proposal not approved	
51	Paperless in-tube cases	Q3/Q4-09	
50	Nuc clean-up double elution	Not implemented	
49	Recal of Quant control ranges	May-10	
48	Fingerprint techniques & DNA	Not implemented	
47	DNAIQ clean-up	Not implemented	
46	Modified chelex from blood & cells	Mar-09	
45	Kinship Stage 3	Jan-09	
44	NCIDD Bulk upload	Feb-09	
	Assessing the success rate of buccal cell controls spotted on FTA		
43	indicating paper	Jul-08	
42	Kinship Stage 2	Jan-09	
41	3130 upgrade	Jul-08	
40	Batch uploading to NCIDD	Jul-09	
39	Semen	Project abandoned	
38	GM ID-x	Feb-09	
37	Tapelift	Project abandoned	
36	Hair	Project abandoned	
35	Quant DUO	Not implemented	
34	Kinship Stage 1	Jan-09	
33	Pk Ht RFU & AI	Feb-09	
32	Off-deck DNAIQ Super retention	Mar-08	
31	One tube testing	Not implemented	
30	RSID Saliva	Not implemented	
29	Swab Submission Improvement	Jul-08	
28	RSID semenogelin	Not implemented	
27	Additional PSA verification	In-house study	
26	Barcodes on receipts	Jul-08	
25	7500 verification	May-08	
24	Supplier change PSA	Not implemented	
23	FTA Destruction checklist	Dec-07	
22	Implementation of the Crime-lite	May-07	
21	Packaging destruction	Jun-08	
20	Upgrade 3130	Feb-08	
19	Tech Admin redesign	Closed	
18	Statement preblurbs	Oct-06	
17	SAIK Improvement	Closed	
16	Cut off limit for statstics and Fst	Closed	
15	Xmas Tree Staining	Not implemented	
14	Statement appendix version 4	Oct-06	
13	Quantifiler Singlicates and Promega Standard	Jun-06	

Project number	Project name	Implementation date	Comments
12	BSD punching for chelex extraction	Apr-08	
11	Blood Clothing test code and list	Dec-06	
10	Digital imaging	Closed	
9	Staff movements	Closed	
8	Move Genotyper Comments Field	Closed	
7	Examination form updates	May-06	
6	Shaking vs Twirling - Cell Extraction	Closed	
5	Movement of DVI Skeletal Analysis to Mortuary Environment	Mar-06	
4	Validation	Feb-06	
3	Inclusion/Review of all Profiles	Aug-05	
2	Court Notification List (AUSLAB)	Oct-05	
1	Change Management	Nov-05	
	Projects without a project number		
Date of report			
Jul-11	Adhesive DNA Collector Trial Report for QPS	Not implemented	
May-10	Oral Swab Submissions - Detecting Foreign Particles	Information for workflow purposes only	
May-10	DNA Profiling of Hair Exhibits	Jun-11	
Nov-09	Differential Extraction using the Iprep ChargeSwitch Extraction Chemistry and Instrument	Not implemented	
Jun-09	PSA kits: Analysis of sensitivity and high-dose hook effect	Jun-09	
May-09	A comparison of DNA recovery and profiling from 4N6 versus rayon swabs: using chelex, Nucleospin and DNAIQ DNA extraction techniques	May-09	
Oct-08	Effectiveness of Nucleospin clean-up where the 9PLEX profile is no sizing data	Information for workflow purposes only	
Nov-07	Phadebas Supernatent Trial: TNE Extraction of Amylase	Mar-08	
Apr-May 2004	Quantifiler initial validation conducted	Jun-04	
	3100 - Validation of Casework samples on the ABI 3100 Genetic Analyser	Validation approval date: Jan-03	
	3100 Validation (Reference Samples)	Validation approval date: Jan-04	
	FTA PFTA Protocol	Validation approval date: Jan-04	
	377/3100 Low level DNA study	Validation approval date: Jan-04	
	STATS - BRB Stats Validation	Validation approval date: Feb-04	
	Investigation into DNA quantitation using Quantifiler sustem (with inhibition data)	Validation approval date: Jan-05	
	Quantifiler Report - Victorian Police	Validation approval date: Apr-05	
	Review of Petricevic Report	Validation approval date: Jun-05	
	STATS - BRB Stats population Data (Caucasion, Aboriginal, Asian)	Validation approval date: 2005	
	NCIDD Validation and Test protocols (4.0.0)	Validation approval date: Mar-06	
	Extended Internal Prospective Valiation of the ABI Prism 7000/Quantifiler system (Forensic Biology) Including external reports	Validation approval date: Jul-06	
	Extended Internal Retrospective Validation of the ABI Prism 7000/Quantifiler System (Forensic Biology)	Validation approval date: Aug-06	
	STATS - BRBStats v1.23	Validation approval date: Apr-07	
	STATS - BRBStats v 1.26	Validation approval date: Apr-07	
	PSA Validation	Validation approval date: Apr-07	
	I3	Not implemented	
	Automation Project		
Project 1	Report on the Verification of automated Quantifiler™ Human DNA Quantification Setup using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform	Feb-07	
Project 2	Report on the Verification of automated AmpFℓSTR® Profiler Plus® and COfiler® amplification reaction setup using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform	Feb-07	
Project 3	Report on the Verification of Automated 3100 Setup using the MultiPROBE® II PLUS HT EX Platform	Refer to #15 and #19	
Project 4	Validation of AUSLAB for Analytical Workflow	Feb-07	
Project 5	Implementation of AUSLAB enhancements (Batch functionality)	Feb-07	
Project 6	Implementation of Pre-PCR platform	Feb-07	
Project 7	Implementation of Post-PCR platform	Not implemented	
Project 8	Report on the Validation of automated FTA® Processing using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform	Not implemented	
Project 9	Report on the evaluation of commercial DNA extraction chemistries	Refer to #11 and #13	
Project 10	Implementation of FTA Processing on Multiprobe II	Not implemented	
Project 11	Report on the validation of a manual method for extracting DNA using the DNAIQ system	Jun-09	
Project 12	25ul Rxn	Not implemented	

Project number	Project name	Implementation date	Comments
Project 13	Report on the Verification of an Automated DNA IQ™ Protocol using the MultiPROBE® II PLUS ht ex with Gripper™ Integration Platform	Oct-07	
Project 14	Implementation of Extraction Chemistries on the MultiPROBE II	Oct-07	
Project 15	Report on the Verification of Automated Capillary Electrophoresis Setup using the MultiPROBE® II PLUS HT EX Platform	Not implemented	
Project 16	Mock sample cleaning: Comparing TriGene™ and bleach and its efficiency in removing DNA.	Nov-06	
Project 17	Report on Automated preparation and testing of Quantifiler standards and controls	Feb-07	
Project 18	Report on the validation of automate.it STORstar system for automated sequence checking of DNA extracts	Jun-08	
Project 19 (A)	Report on the Validation of the Manual 9+1 Method for Capillary Electrophoresis Setup	Feb-08	
Project 19 (B)	Close of Post-PCR MultiPROBE® II PLUS ht ex and the Automated Capillary Electrophoresis Setup Method	Not implemented	
Project 20	Report on the Verification of the RECAP-96M™ Automated Decapper/Recapper System	Jan-08	
Project 21	A Modified DNA IQ™ Method Consisting of Off-Deck Lysis to Allow Supernatant Retention for Presumptive Identification of α-Amylase	Mar-08	
Project 22	A Modified DNA IQ™ Method for Off-Deck Lysis Prior to Performing Automated DNA Extraction	Feb-08	
Project 23	Hair extractions	Not implemented	
Project 24	Sperm Extraction	Not implemented	
Project 25	Investigation and evaluation of tapelift materials and procedures	Not implemented	
Project 26	Report on Whatman® FTA Concentrator PS™ Parasite Purification	Not implemented	
Project 27	DNA IQ Recovery	Jun-09	
Project 28	Semen DNA IQ Validation	Not implemented	
Project 29	DNA IQ Clean-Up Protocol	Not implemented	
Project 30	Rcovery of DNA from IQ Store Plates	Not implemented	
Project 31	Suitability of Lovell cuticle pushers as a substrate for fingernail scrapings for DNA Analysis	Not implemented	
Project 32	Identifiler	Not implemented	

CA-94

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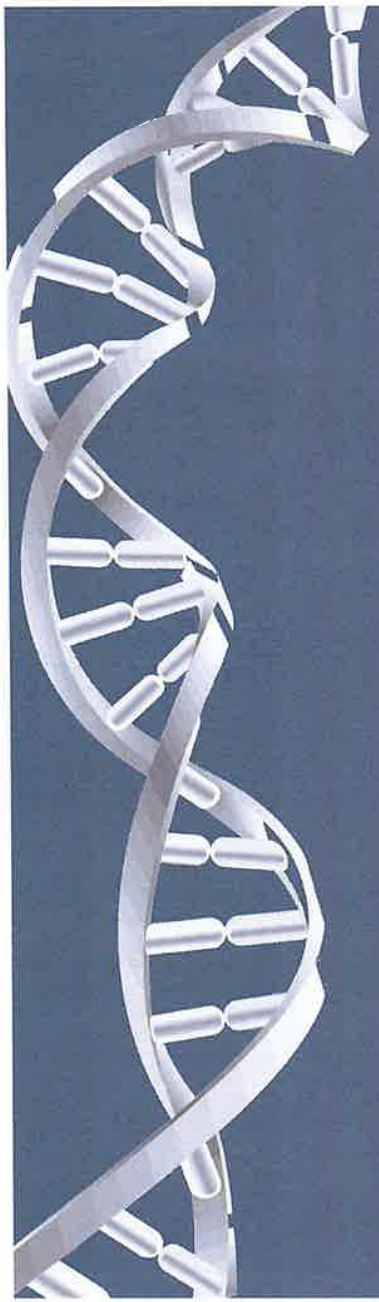
Update on DNA Analysis Issues

31 Oct 2008

Queensland Government

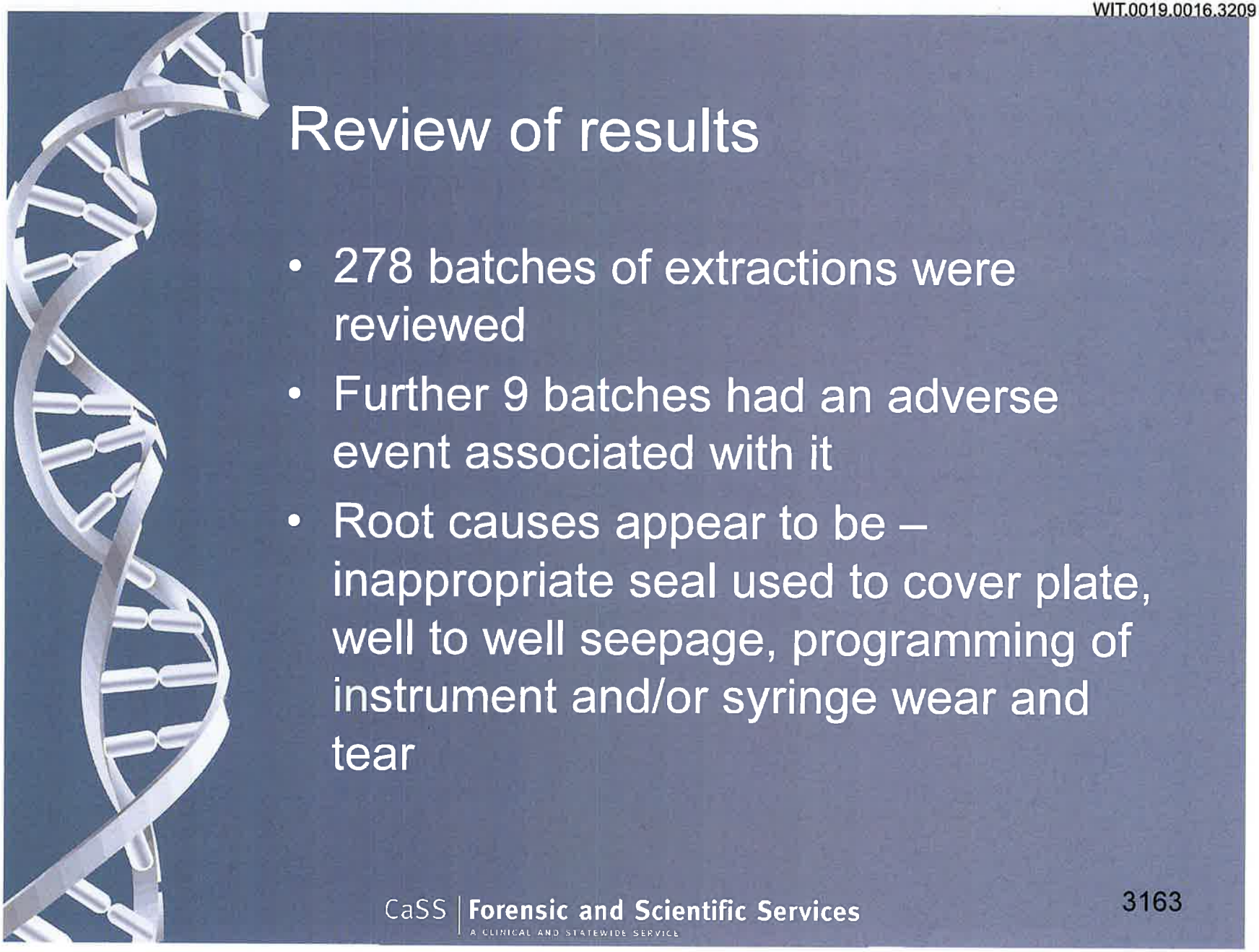
Queensland Health

3161



Background

- 6 Batches in 6mth period (4CW, 2Ref)
- Results deemed 'not reportable'
- Suggested cause: syringe wear & tear and programming of platform
- Cases affected: 113 (Major 27, Vol 86)
- 1 statement & 1 Link affected
- Actions: audit all results from platform, specialist to review programming, revert to manual extractions


Review of results
• 278 batches of extractions were reviewed
• Further 9 batches had an adverse event associated with it
• Root causes appear to be – inappropriate seal used to cover plate, well to well seepage, programming of instrument and/or syringe wear and tear
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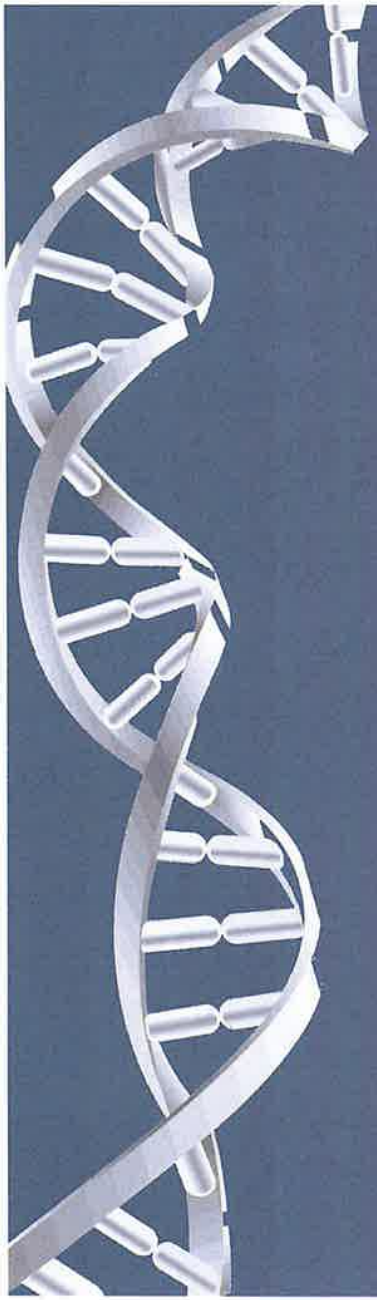

Actions:

- All cases containing DNA IQ results are being assessed using court date as a priority
- ~7,000 cases – 117 reports released – majority checked and passed (32 outstanding)
- Divided Reporting team into 2 – Routine Team and Investigation Team
- 676 profiles loaded to NCIDD – converting to 'Intra', priority of checking Interstate links, major then volume

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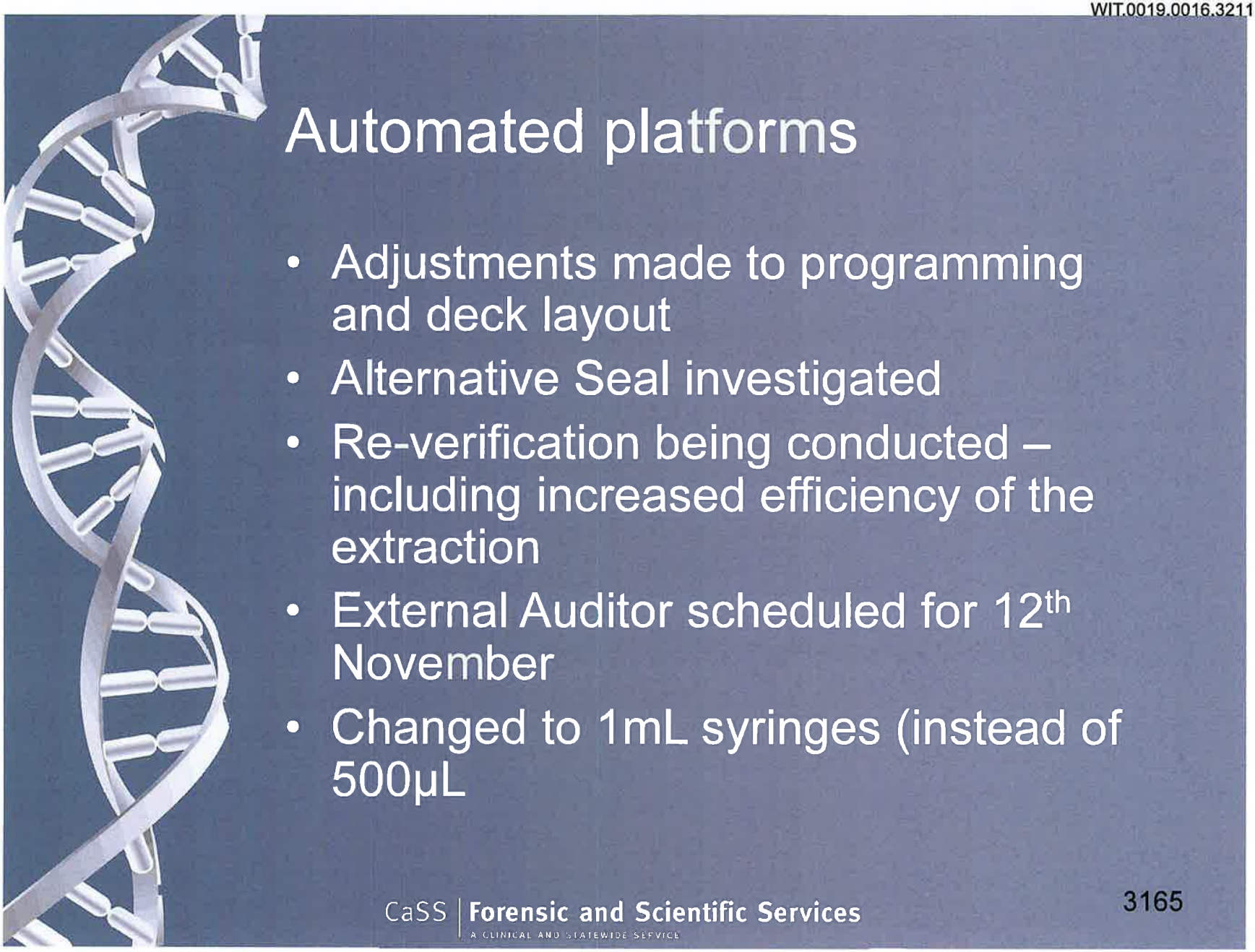
660


Automated platforms
• Adjustments made to programming and deck layout
• Alternative Seal investigated
• Re-verification being conducted – including increased efficiency of the extraction
• External Auditor scheduled for 12th November
• Changed to 1mL syringes (instead of 500µL
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Throughput

- Averaging ~820 extractions/week with manual methods
- Increase in results delivered across interface due to Auslab fix

ARM-104 VI-7

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Implementation Date	Details	Project Leader
14/11/1991	HLA Dq alpha	SMJ (retrospectively added)
23/01/1995	D1S80, HUMTH01, FES and sex typing	SMJ (retrospectively added)
31/05/1995	Polymarker	SMJ (retrospectively added)
01/03/1996	Triplex: CSF1PO, HUMTH01, TPOX	SMJ (retrospectively added)
23/04/1996	Quad: vWA, HUMTH01, F13A, FES	SMJ (retrospectively added)
16/12/1997	Sensitivity test for 9plex (Profiler Plus)	SMJ (retrospectively added)
05/03/1998	First CS entry for 9plex (Profiler Plus)	SMJ (retrospectively added)
	Prior to routine use of NCIDD, the lab used the Unknown Database in FACTS. These unknown profiles were manaually searched against the Known and Unknown databases in FACTS	CJA (retrospectively added)
2001	The Known Offenders Database was used to store and search all ref samples. All unknown crime scene samples were manually searched against KOD as the case was completed. All unknown crime scene samples were still loaded to FACTS to search against other crime scene samples.	CJA (retrospectively added)
09/10/2003	All profiles on the Known Offenders Database were checked by the Queensland Police Service to be included on NCIDD and those profiles were bulk uploaded to NCIDD on the 9th of Oct 2003	CJA (retrospectively added)
10/10/2003	All profiles on the datases in FACTS were bulk uploaded to NCIDD on the 10th of Oct 2003.	CJA (retrospectively added)

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Implementation Date	Details	Project Leader
2nd half of 2004 (b/n Jul and Aug)	Date added to the reviewed field (LRCB1-7) for EXR and LKR testcodes	PMT (retrospectively added)
Jun-04	Implemented use of Quantifiler for routine use for quantitation	CJA (retrospectively added)
08/04/2005	Report issued by ESR regarding review of the use of Quantifiler Human Quantitation System at QHSS	CJA (retrospectively added)
Jun-05	**Western Australia** began using **NCIDD**	CJA (retrospectively added)
15/09/2005	On Thurs the 15th September 2005, QPS RMU advised us that they no longer require Forensic Regiser case details registered on **LKREXT** pages and faxed to them.	Luke Ryan
Oct-05	**Northern Territory** began using **NCIDD**	CJA (retrospectively added)
09/03/2006	For cases registered 05 onwards it has been decided that to create links the case files are not required but only if the admin and tech reviews have been completed. If the admin and tech reviews have not been completed a check of the information in the case file is required therefore the case file is required to generate the link. This also means that the link sticker is not required on these case files.	Luke Ryan/Maryanne Hadfield
13/06/2006	Discussions took place between VKI, MMH and CJA regarding staining of Diff Lysis slides with H&E, and the possible changeover to Christmas tree staining. It was decided that no staining of slides from this extraction type would be done, and if required, they would be stained at a later date (they will still be heat fixed).	Cathie Allen/Maryanne Hadfield/Vanessa Ientile
23/06/2006	Amp volumes changed **from 2ng to 1ng** (as per DNA workbook).	Paula Taylor
27/06/2006	To help Case scientists with amp volumes of casework samples, it has been decided we will now add to the samples with multiple volumes with D1, D2 etc depending on the number of replicates so they will now appear as 12345_15ul_D1, 12345_17ul_D2 so it will be obvious which samples have multiple volumes when plate reading.	Allan McNevin

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Implementation Date	Details	Project Leader
28/06/2006	Change over from Quantifiler standards to Promega Male standard DNA for Quant standards and Promega Female standard DNA for Quant controls (high and low). This will take effect from run id QF#850 onwards (Amp id CW#1320 onwards). From the results of the validation work that has been undertaken this will give us a more reliable quantitation value.	Allan McNevin
17/07/2006	The analytical section will submit one **Quality Control spot** per extraction batch for blood and cellular extractions. There is no need for individual case scientists to submit QC spots after the 17th of July 2006.	Allan McNevin
17/07/2006	The operational staff will only process FTA samples on a 'Link' plate singularly. Previously all samples were punched in duplicate, however since staff are dedicated to this task on a roster, the requirement for duplicates is no longer required. - Project No Longer Required.	Cathie Allen/Luke Ryan
20/07/2006	From the 10th of July 2006 - the initials 'RLS' refer to Rebecca Sonter. A previous staff member Rebecca Smith also had these initials and worked in the section from the 13th of September 2004 to the 5th of August 2005. Any entries in Auslab (RLS) during those dates refer to Rebecca Smith. Any entries after the 10th of July 2006 refer to Rebecca Sonter.	(Brought to CJA's attention from RS)
24/07/2006	The analytical section will register Epithelial Lysates in Auslab. A copy entry will be done of the Sperm Fraction registration, '-E' will be added to the Client Reference field and 'from [barcode of Diff Lysis sample]' will be added to the Sample Info 1 line. The team will also be added to the 9plex page.	Cathie Allen/Allan McNevin/Mary Gardam
25/07/2006	Change over from Quantitating samples in duplicate to singles. This will take effect from run id QF#898 onwards (Amp id CW#1388 & CW#1389 onwards). This is the second part to changes arising from validation/change management work that had previously been undertaken.	Allan McNevin
26/07/2006	Re: Use of **Greiner plates** for FTA processing. Item removed from register as change did not actually occur.	Allan McNevin
25/07/2006	Started using In-house prepared **Diff Lysis controls**, results to be monitored in G:\ForBiol\AAA Analytical Section\Diff Lysis Controls\Diff Lysis controls 2006.xls	Allan McNevin / Justin Howes
07/08/2006	**PDF functionality** for FBSTAT, FBSHRT, FBADD & FBAMEND is live from 8/8/06	Vanessa Ientile / Sam Granato

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Implementation Date	Details	Project Leader
08/08/2006	**MPREM page** created in Auslab for removal of identified missing persons profile off NCIDD (Refer to SOP 17152)	Kirsty Wright
08/08/2006	**EXR2 & EXR3** live in AUSLAB for use when results extend past 7 lines of data. EXR2 & EXR3 information is not transferred across the interface but must be faxed from EXR2 to QPS FIRMU.	Vanessa Ientile
31/08/2006	**Stop** keeping 17050s after upload for samples with and without links after page has been scanned.	Luke Ryan
30/08/2006	**Increase** of amping template from 1ng to 1.2ng. This is effect any CW from CW#1484 onwards and CoREF#32 (CoREF#33 and CoREF#34 were done using 1ng of template)	Allan McNevin / Megan Harvey
19/09/2006	**Addition of "-QC"** suffix to Auslab barcodes of QC dots in DNAmaster	Allan McNevin
06/10/2006	**Moved BRB Stats v1.26 and added a password requirement to open. Made the following changes to v1.23, this version only to be routinely used from now on:** **- Write-protected file** **- Protected all fields except those required for profile entry and kinship type** **- Renamed columns "Father", "Mother", "Child" in Paternity Trio module to "Unknown Parent", "Known Parent", "Known Child" to clarify**	Tim Gardam (for Vanessa Ientile)
23/10/2006	After transferring the completed folder to AAA Results Finalised, the **file must be renamed** to remove the # eg. CW#1632 to CW1632. (To make the files more compatible for the new database)	Mary Gardam
26/10/2006	**All statements in Auslab have been pdf'd by the admin team and VKI.** There are some exceptions where difficulties have been encountered (see list below) Note that some have been accidentally pdf'd twice during this process. Please be aware if printing off any old statements. Justin/Sam are to now progress change of Stat Dec title to Statement of witness, use of new NATA logo and the inclusion of the statement appendix version 4 PDF STATEMENTS - AUSLAB **WOULD NOT VALIDATE** QPS030315107 QPS020411805 QPS030315107	Sam Cave

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	QPS030278763 QPS020277773 QPS020414984 SSF014122 QPS020079224 QPS010414494 QPS030348319 QPS040162797 QPS010148848 QPS050126750 QPS050082825 QPS040281471 QPS050163877 QPS050161928 QPS040192729 QPS050070852 QPS050393608 QPS050312592 F22372 F31528 F17901 QPS050418880 QPS060116432 QPS060069543 QPS060130958 QPS060121966 QPS060118819	
30/10/2006	**Statement Appendix version 4 -** ready for use and should be used for all statements written as of Monday 30th October	Sam Cave
01/11/2006	All Statements are now called **'Statement of Witness'** and the **NATA** logo has been changed.	Cathie Allen
Dec-06	**Commonwealth of Australia** began using **NCIDD**	CJA (retrospectively added)

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22/01/2007	Analytical section will now include a **blank/negative control** to all **Nucleospin clean-up and microcon batches** consisting of 100uL nanopure autoclaved water	Allan McNevin
31/01/2007	For older cases (02/03/04) that have been sample in 2005,2006 and 2007, it has been decided that to create links, the case files are not required BUT ONLY if the admin and tech reviews have been completed. If the admin and tech reviews have not been completed a check of the information in the case file is required therefore the case file is required to generate the link. This process to change from 31/01/07. SOP to be updated.	Paula Taylor
12/02/2007	**AUSLAB Batch functionality** GO LIVE date	Thomas Nurthen
13/02/2007	**Pre PCR MultiPROBE II instrument** GO LIVE date	Thomas Nurthen
03/04/2007	For blood samples received by Property Point staff - the new test code BBCLO is ordered & filled out. The staff no longer have to add 'Blood sample' to the registration screen in the Sample Info 1 line. Biology staff trying to find out if a sample is a blood need to look for the BBCLO testcode.	Thomas Nurthen/Cathie Allen
05/04/2007	All **statements are to include the ref sample source** (eg buccal cell, blood, or hair) in the body of the statement to ensure that all items rec'd are documented as to what rec'd	Sharon Johnstone
05/04/2007	**New lists have been configured to assist in workflow with volume crime cases that have been sampled and results are pending.9PLEXV- (Volume 9plex list) will be the list that you will go to, to assess if all the results are back for a case. If case is ready to go it will progress to the next list. If not it will just be taken off the list to wait for the last sample outstanding VRAPP- (Volume result appraisal list) This list is to be used by results management to look at samples and assess for reworks. If rework required, just taken off the list. If rework not required preferred profiles are selected CCVOL- (Case compilation list) This list you will use to find the files, print results tables, print all EPG's and track physically and electronically to case appraisal drawers.**	Sharon Johnstone

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10/04/2007	**All Quant batches to have results entered in QF QC log**, with a column added to standards tab to indicate batch pass/fail. Log of failed amp bathces created, located I:\AAA Analytical Section\Test Results-AmpsTE\FailedAmpBatchLog2007.xls	Allan McNevin
18/04/2007	**"Items received"** and **"Items tested"** fields on FORREC pages in AUSLAB (forensic receipts) are **no longer mandatory fields**. You are no longer able to edit these fields.	Vanessa Ientile
03/05/2007	**Use of receipt barcode and item number (e.g. 123456-001) in statements is optional.** May not be required if there is only one receipt issued. Exhibit barcode must be used to identify the item in the results section of a statement.	Vanessa Ientile
06/06/2007	**New Allelic Ladder incorporated into ABI Profiler Plus and Cofiler kits. See report I:\AAA Analytical Section\3100 Troubleshooting\Allelic ladder trial May 2007\New Allelic Ladder Report ver 1.doc** **Profiler kits from lot number 0703109 (in-house lot M received June 07) will have the new ladder**	Allan McNevin
04/06/2007	**Use of word "neg"** rather than "-" for any descriptions in any casefiles eg. TMBneg rather than TMB -. Can use "+" or "pos" as the "+" is harder to change than a "-".	Kylie Weller
08/06/2007	Change to NCIDD introduced - **NT profiles are now viewable.**	Vanessa Ientile
15/06/2007	**Pre-Lims** completed Genotyper files were stored in I:\User3100\AAAResultsFinalised\PRE-LIMS. Due to storage restraints on I drive many of these files are now stored in J:\User3100\ResultsFinalised\PRE-LIMS.	Cathie Allen
18/06/2007	Started using the **Generic Interface** (between Auslab and the Forensic Register)	Cathie Allen
19/06/2007	**Change to LKR** reporting for Partial profiles. Statistical calculations will no longer be reported in an LKR.	Paula Taylor
19/06/2007	**DLYS'** added to the specimen notes of SLYS and ELYS samples to allow reworking responsibility to be given to the Yellow Team Case Managers	Justin Howes, Allan McNevin
21/06/2007	**Started using Criteria** (and actions for failures) for Extraction controls as outlined in SOP24012	Allan McNevin
26/06/2007	**Started using Criteria (and actions for failures) for Amplification controls as outlined in SOP17130 (includes minimum average peak height for positive control ≥1200RFU at D3 and ≥600RFU at D7) based on data to be found I:\AAA Analytical Section\AMP RFU monitoring\Amp RFU monitoring 2006-Jan2007.xls**	Allan McNevin

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26/06/2007	**Analytical logs** (including Quantifiler logs) have been moved to I:\AAA Analytical\Analytical Logs, TG has updated the required Q'filer results macro	Allan McNevin / Tim Gardam
28/06/2007	**Interpol/Interstate requests** will now report 9 loci matches as a match instead of "cannot be excluded as a contributor"	Paula Taylor
29/06/2007	**ALL** crime scene profiles will be uploaded to NCIDD.	Vanessa Ientile
13/07/2007	Registering of parent barcodes in Auslab into the **sub-sample registration** page - end of Sample Info field. Interim fax page to be used on 9plex2 page to send to QPS FIRMU for all sub-samples used in final EXR results.	Cathie Allen
TBA	**Networking of 9700** Thermalcyclers to PC	Thomas Nurthen
TBA	**Uploading of Thermalcycler log** into AUSLAB	Thomas Nurthen
TBA	Linking of **QIS 24486** to the 9PLEX,9FTAR,9LINK,COFIL,COFILR screen masks and accessed via the SF1 function	Thomas Nurthen
17/07/2007	**Below threshold peaks** are no longer to be interpreted during plate reading. Eg partial profile with a below threshold mixture is to be reported as a PP. The case scientist is responsible for interpretation.	Mary Gardam
27/07/2007	**Property Tag** on exhibits have been changed - they now include information such as 'seized date' and 'receiving officer'. The font of other fields have been enlarged. The top of the tag now says 'Forensic Biology' instead of Exhibit Tag.	Janine King
01/08/2007	Began **matching with SA, Tas and ACT** on **NCIDD**	Paula Taylor
01/08/2007	**Staff matches for samples** will only be performed by readers and will no longer be checked by the analytical section when importing profiles to AUSLAB.	Megan Harvey
01/08/2007	**Partially inhibited samples** when imported will no longer be automatically reamped. A communication will be sent to the team stating that the sample is partially inhibited and for the cs to order a rework (either reamp or n'spin cleanup).	Megan Harvey
Sep-07	**Victoria** began using **NCIDD**	CJA (retrospectively added)
05/09/2007	Volume Crime will **no longer retain the print outs from NCIDD for person to person links.** Only those matches involving a reference sample we are repeating for confirmation will be retained.	Paula Taylor
06/09/2007	The fix for EXRs was activated in the LIVE system of Auslab. The fix ensures that only reviewed results are sent across the interface (and unreviewed results will not transfer across the interface if 'REV' has not been selected). The act of entering REV ensures that it transfers across the Generic Interface.	Cathie Allen

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20/09/2007	Pre-2003 Volume Crime Cases that have "No Testing Required" in their case status will remain an NTR regardless of whether or not there are UR notes to justify their status. ACTIONS: 1. Still need to enter NWQPS into the EXR page for any exhibits in NTR cases. 2.Find an empty storage box & create a storage location. 3. Store exhibits to the newly created box and transfer the entire box to FBEXR1. There is no longer any need to transfer exhibits to the NTR box. SOP WILL BE UPDATED TO REFLECT THIS CHANGE	Sharon Johnstone Kate Lee Robyn Smith
19/09/2007	**Batch Functionality menu items** changed to remove reference to DNA	Thomas Nurthen
19/09/2007	**Prompts added** to AUSLAB for removing/returning samples in Batch functionality	Thomas Nurthen
19/09/2007	**Modifications to the SF7** Results History screen format in AUSLAB Columns Width increased, Specimen type now displayed	Thomas Nurthen
19/09/2007	**AUSLAB-SF11** Batch Allocation, Batch Creation and Batch details print out to display all storage locations	Thomas Nurthen
24/09/2007	Changes to the general mask **GENREF** mask	Thomas Nurthen
24/09/2007	Changes to the general mask **MICROCON** (Processing Comments)	Thomas Nurthen
24/09/2007	Changes to the general mask **QUANT** (Processing Comments)	Thomas Nurthen
24/09/2007	Changes to the general mask **AMP** (Processing Comments)	Thomas Nurthen
24/09/2007	Changes to the general mask **NUCC** (Processing Comments)	Thomas Nurthen
24/09/2007	Changes to the general mask **NUCB** (Processing Comments)	Thomas Nurthen
24/09/2007	Changes to the general mask **NUCI** (Processing Comments)	Thomas Nurthen
24/09/2007	Changes to the general mask **NUCT** (Processing Comments)	Thomas Nurthen
24/09/2007	Changes to the general mask **HAIR** (Processing Comments)	Thomas Nurthen
24/09/2007	New general mask ReGenescan (**REGS**)	Thomas Nurthen
25/09/2007	**Examination of items** - Scientists are now requried to wipe their gloves with ethonal wipes before conducting tape lifts.	Samantha Cave
27/09/2007	**Print mask** configured for FTAR test	Thomas Nurthen
27/09/2007	**Additional Export analysers** configured for Amplifications batches and Amp split macro cease to be used	Thomas Nurthen
27/09/2007	**Removal of Stor mask** from the bottom of the MPQUANT mask and quant split macro ceased to be used	Thomas Nurthen
27/09/2007	**Autovalidation rules** configured to Save preferred profile automatically if the Comment is OK (GENOT,FTAGEN & LINK masks)	Thomas Nurthen
27/09/2007	**Autovalidation rules** configured to write the Batch ID into the 2nd page of 9PLEX, 9FTAR and 9LINK test panels	Thomas Nurthen

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27/09/2007	**Microcon autovalidation** mask to auto allocate to the Quant batch type if Finvol >26	Thomas Nurthen
03/10/2007	**Verification of new 9700 thermal cycler (9700-F) complete and used for routine use, report located in I:\AAA Analytical Section\Internal Projects\9700 Block F 2007 Routine use agreed upon by management team in meeting Thurs 27-9-07 (no comments received to date)**	Allan McNevin
10/10/2007	**FBEB9-14** (For Biol Examination Boxes) created. These are examination boxes in the stainless steel examination area.	Justin Howes
15/10/2007	**Digital Imaging functionality** for storage and annotation of images	Gail Hargraves
15/10/2007	**Packages** for all examined volume crime swabs, cig butts and small items photographed and destroyed after sampling. Applicable to all items received from 15/10/07	Sharon Johnstone
19/10/2007	From 10/10/07 **Rebecca Sonter(RLS)** has changed her name to Rebecca Gregory(**RLG**). Her AUSLAB login has changed from rlsf1 to rlgf1 on 18/10/07.	N/A
29/10/2007	**First DNAIQ batch extracted on the MPII**	Thomas Nurthen
08/11/2007	From 29/8/07 **Josie Hayward (JEH)** has changed her name to Josie Entwistle (**JE**). Her AUSLAB login has changed from jehf1 to jeef1.	N/A
03/12/2007	**Volume Crime Low priority cases** will no longer have a separate case status or EXR lines, to use started and standard EXR lines	Sharon Johnstone
06/12/2007	As of 5/12/2007 **Kylie Weller**'s new name is Kylie **Rika**. Admin Support, LISS, and IT, have all been informed to change my details to Kylie Rika and initials KDR.	Kylie Rika ne Weller
11/12/2007	**FBEXS661 and FBEXS662** to be used for DLYS slides	Justin Howes/ Rose Higgins/ Amanda Storer
12/12/2007	LISS have changed my login from kdwf2 to kdrf1	Kylie Rika
13/12/2007	When Copying a registration using the SF5 Copy entry function on the Registration screen; The **DNA priority will now copy** to the new barcode.	Thomas Nurthen
14/12/2007	Two people required for **destruction of reference samples process**	Paula Taylor
07/01/2008	Started using **Decapper** (LifeTool Recap 96M), will be used for decapping & recapping tubes in Pre-PCR from today onwards	Allan McNevin
07/01/2008	Started using **thermalcycler networking software**	Allan McNevin
07/01/2008	From 7/01/08 **Angelina Gilbert (AG)** has changed her name to Angelina **Keller** (AK). This name change is not yet effective in AUSLAB or QIS.	NA

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14/01/2008	Crime scene profiles from Sexual Assaults only which match to complainants reference samples **will not** be uploaded to NCIDD. All other crime scene profiles will be uploaded to NCIDD.	SJC
23/01/2008	**FBEXA2 and FBEXA3** are pages to request when further examination of items is required to track bench, time, tests etc as per FBEXAM.	Justin Howes
06/02/2008	Begin routine **use of 3130*xl* A**. Will begin with Reference batches then progress to Casework (9+1 protocol).	Allan McNevin
11/02/2008	Begin routine **use of 7500** for quantification	Allan McNevin
19/02/2008	**Change HiDi / Rox mixture** from 85:1 to 85:2 (i.e. use 850µL HiDi + 20µL Rox or 935µL HiDi + 22µL Rox). Data contained within 3130*xl* upgrade project folder (9+1 protocol).	Allan McNevin
21/02/2008	**ABI7500 mask fixed**, STD7 now says "0.0686ng/uL" so no need to manually fix STD7 on the ABI7500 SDS software.	Iman Muharam
13/03/2008	Switched over from using Barnsted water purifier to **new Millipore Milli-Q** A10 Advantage system	Allan McNevin
18/03/2008	Put **"FTP" in comments field** for all FTA specimens that have failed to profile.	Iman Muharam
18/03/2008	During upload of Genotyper results into AUSLAB, **manual changes** are made for samples that have "FTP" in the comments field. Save the FTP profile as preferred, and change DNA profile result from "Positive" to "Negative", then validate the page.	Iman Muharam
18/03/2008	**Plate readers to source plates to be read each day from Blue "plates to be read" folder located on AS PO4's desk. Plate readers to take plates from the top working down. It is also up to the plate readers to use the batch audit function in AUSLAB to track the whereabouts of the paperwork.**	Allan McNevin
18/03/2008	For specimen types CELLS, BLD, QAB, QAC initial batch type was changed from CWIQEXT to CWIQLYS. For specimen types BRE, QABR, QACR, SPOT, RCELLS the initial batch type was changed from RFIQEXT to RFIQLYS. This is to allow processing of samples through DNAIQ with off-deck lysis.	Iman Muharam
19/03/2008	Start Routine Processing of DNAIQ extraction using Off-Deck Lysis procedure	Allan McNevin
26/03/2008	Changes have been made to the **Destruction Screen** (DEST) in AUSLAB in preparation for enhancements to the Generic System Interface for Forensic Biology.	Vanessa Ientile
26/03/2008	The changes include	Vanessa Ientile
26/03/2008	1. Fields that are **Mandatory** are now displayed with an asterix "*".	Vanessa Ientile

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26/03/2008	2. Display of the fields for **Authorising Officer, Badge ID, QPS Sample Status**. Please note that these fields will be populated by the GSI and so will remain blank for existing samples.	Vanessa Ientile
26/03/2008	3. **Two new fields** for "PROFILE REMOVED FROM AUSLAB:" and "PROFILE REMOVED FROM KINSHIP:". Please note that for existing samples, the registration needs to be re-saved so that these fields are displayed correctly	Vanessa Ientile
11/04/2008	Batch types for capillary electrophoresis have changed from 3100 batches to CE batches to reflect the start of the use of 3130 instruments. e.g. batches that previously would go to 3100CWYYYYMMDD_## are now CEPCWYYYYMMDD_##. CE=capillary electrophoresis, P=Profiler, CW=casework.	Thomas Nurthen / Allan McNevin
22/04/2008	Started to routinely replace SDS with Sarcosyl in the DNAIQ extraction lysis protocol due to gel formation with SDS (does not occur with Sarcosyl).	Thomas Nurthen / Allan McNevin
28/04/2008	Resumed routine use of **ABI Prism 7000** with update software version (to match the 7500) and new T/C block	Allan McNevin
08/05/2008	Received the first volume crime **exhibit by Australia Post**	Cathie Allen
14/05/2008	When using a profile from a previous run the comment is "USE GEN9REFXXXXXXX_XX" only. Other comments eg. OK, PP, AI cannot be included with this comment. The preferred profile should be saved in AUSLAB (<F7> <SF6>), the profile upload form printed (<SF11>), which both readers should sign and include with the plate paperwork.	Susan Gillespie/ Paula Taylor
19/05/2008	**ROBOTICS login increased** to 20 allowable simultaneous logins (Analytical requires 13 logins, including the BSD Duet 600).	Iman Muharam
27/05/2008	New Primary Site available called **"DNA Trace Kit"** with mnemonic "trace". This will be used for trace DNA tapelifts submitted directly from police. Date for commencement yet to be defined. Introduced within the Forensic Register 20th May 2008	Vanessa Ientile
28/05/2008	When requesting a ReGenescan of samples through LIMS, a second person will be required to check the correct 9Amp batch (and sample position number) has been correctly entered into the 9Plex processing comments, and add a specimen note indicating this has been checked (currently we are unable to use the ReGenescan processing comments section on 9plex page)	Shannon Merrick

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12/06/2008	**MIXT & MIXC** will now copy the profile from the 9PLEX and put it in the Profile found fields on the mixture page	Thomas Nurthen
20/06/2008	Commenced using **3130xl Platform B** for routine use.	Allan McNevin
25/06/2008	Sequence check, Pos control lot number and thermalcycler fields added to **AUSLAB amp batch worksheets**, **Transfer/Pool worksheet** now outputs whole sample info field& processing comments (rather than truncating)	Allan McNevin
26/06/2008	**New EXR line** - Mixed profile, unable to load-NCIDD. Ref sample req'd is now available for use in Auslab.	Paula Taylor
01/07/2008	**New specimen types** available in AUSLAB: QPST, EFRAC, SFRAC, DDNA, HDNA, PTISS, CPOOL, CTRAN, CSUP, MICCON, NSCON, FSS.	Thomas Nurthen
01/07/2008	**New Primary Sites available** in AUSLAB: CFAB, BFAB. This will be used for fabrics containing cells and bloods respectively, submitted directly from police.	Thomas Nurthen
11/07/2008	**CWGENP** and CWGENC prefixes added to Main module in Results Table Comparison macro to enable comparions for new samples post-01 July.	Tim Gardam / Iman Muharam
14/07/2008	For microcon samples where the final volume is less than 20µL, TE will be added to make the volume up to 20µL at the end of the microcon batch. E.g. if the final volume after concentration is 7µL, 13µL of TE will be added to the extract tube. This will be reflected in the results file, whereby final volume will be the volume after concentration (e.g. 7µL) and the SV1 (amp volume) will always be 20µL. Comments have been made to the SOP in QIS	Allan McNevin
14/07/2008	In order to facilitate trouble shooting, extraction platform A will be used to process reference batches only and extraction platform B will be used to process casework batches in a checkerboard configuration. This will consist of samples and extraction blanks as communicated to the Biology management, Analytical and Operational teams via email. This will be done from 14/7/08 until sufficient time as for trouble-shooting has been completed and any issues detected resolved.	Allan McNevin
23/07/2008	Further information has shown adverse events have occurred equally across platform A and platform B, therefore casework and reference processing will not be limited to either instrument. The checkerboard pattern mentioned above will be continued	Allan McNevin

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28/07/2008	**Missing audit trail data** from January 2008 in AUSLAB cannot be restored from back up tapes as these back up tapes are not available. If required for audit trails, missing data up to the 31/12/07 and after January 2008 can be restored.	Vanessa Ientile
28/07/2008	Outcome from extraordinary managament meeting 28/7/08 - suspend processing of samples through automated DNAIQ. Samples that are already lysed (i.e. processed through off-deck lysis) will be extracted through manual DNAIQ procedure. All extractions from 28/7 will be through chelex process until resolution of DNAIQ troubleshooting	Allan McNevin
07/08/2008	**CSRC list enabled**. FSLU to list insert if a Case Manager or Case Scientist is required due to case of high priority/media interest, or serious enough to warrant a contact point and close attention.	Justin Howes
07/08/2008	**CASEFC list enabled**. This is a list to contain cases that are complete in terms of examination and results and is requiring case compilation and peer review.	Justin Howes
11/08/2008	Once profiles have been imported to AUSLAB, AS will manually list insert each barcode from the Genotyper batch (excluding Controls) onto the following lists according to the team name entered into the TEAM field on the 9PLEX page: no team - BLCM Yellow team - YELCM Blue team - BLUECM Red team - REDCM Green team - GREECM Volume team - VOLCM Orange team - BLCM This process will be in place until it can be done automatically by AUSLAB	Allan McNevin
15/08/2008	Any samples that have the "STAFF" flag shown by the auto-validation rules when importing profiles into AUSLAB will be noted by the person importing the results file in the specimen notes ... the wording used will be "Staff flag X/Z alleles". Where X/Z represents the number of matching alleles out of possible alleles	Allan McNevin
18/08/2008	New batch type configured "Casework manual Amp Profiler" (**mnemonic CWMAMP**). This batch type will be used to manually amplify sampels that have less than or equalt to 20µL of extract remaining after microcon.	Allan McNevin

C:\Users\blburges\Documents\Other Documents\DNA Inquiry\Allan McNiven\ARM-92\Copy of Change Register - Minor Changes and emerging or novel practices as at 20-09-2022.xls

Implementation Date	Details	Project Leader
21/08/2008	Case managers to go through checklist as set out by working party to ensure there is no evidence of contamination at levels below what would have been detected by the extraction audit. Case manager to enter Specimen Note 'Quality Checklist Passed/Failed' for each sample that has been checked.	Justin Howes
21/08/2008	There will be a new process in ordering reworks for Volume Crime samples only effective immediately.This means no MICROCONS or NUCLEOSPINS are to be ordered for Volume Crime samples only. Reamps can be ordered if the first 9Plex was amped at less than 20uL. Re-genescans can be ordered as normal.	Justin Howes
21/08/2008	This means no MICROCONS or NUCLEOSPINS are to be ordered for Volume Crime samples only. Reamps can be ordered if the first 9Plex was amped at less than 20uL. Re-genescans can be ordered as normal. If 12 alleles are obtained and is loaded to NCIDD and eventually linked, a rework by any means can be ordered to get the best match probability possible for statement purposes.	Justin Howes
25/08/2008	The **following line will be added to statement preblurbs** in line with NATA guidelines. 'This document is issued in accordance with NATA's accreditation requirements'.	Justin Howes
27/08/2008	As of this date, **Qld will match with NSW** - thereby meaning that Qld matches with all states and territories.	Intell Team
29/08/2008	**All microcon to full samples** will have a final volume of 27uL. Case scientist's will only order microcon to full or microcon to half.	Megan Harvey
04/09/2008	**FTAGEN mask** - new autoval rule, looks for comment of 'REGS', adds RRREF	Thomas Nurthen
04/09/2008	**Dilution Volumes** - changes to stop 9FTAR reworks overwriting dilution volumes	Thomas Nurthen
04/09/2008	**EXTRES mask** - new autoval rule, looks for comment ELINK, returns to QUALNK batch type	Thomas Nurthen
08/09/2008	**REQC result added** to 9PLEX cummulative table, REQR added to 9FTAR cummulative table	Thomas Nurthen
11/09/2008	**Syringes** on MPII EP-A changed from 500µL to 1000µL.	Iman Muharam
19/09/2008	As of 10 September, all DNAIQ results to be put on hold. Urgent results for statements to be worked on using macro, final results for samples on batches, autofilter function of spreadsheet - any that cannot be reported can be either reported in addendum (if committal) or quality failed (if trial or no other opportunity for investigative analysis).	Justin Howes
22/09/2008	**Syringes** on MPII EP-B changed from 500µL to 1000µL.	Iman Muharam

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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.

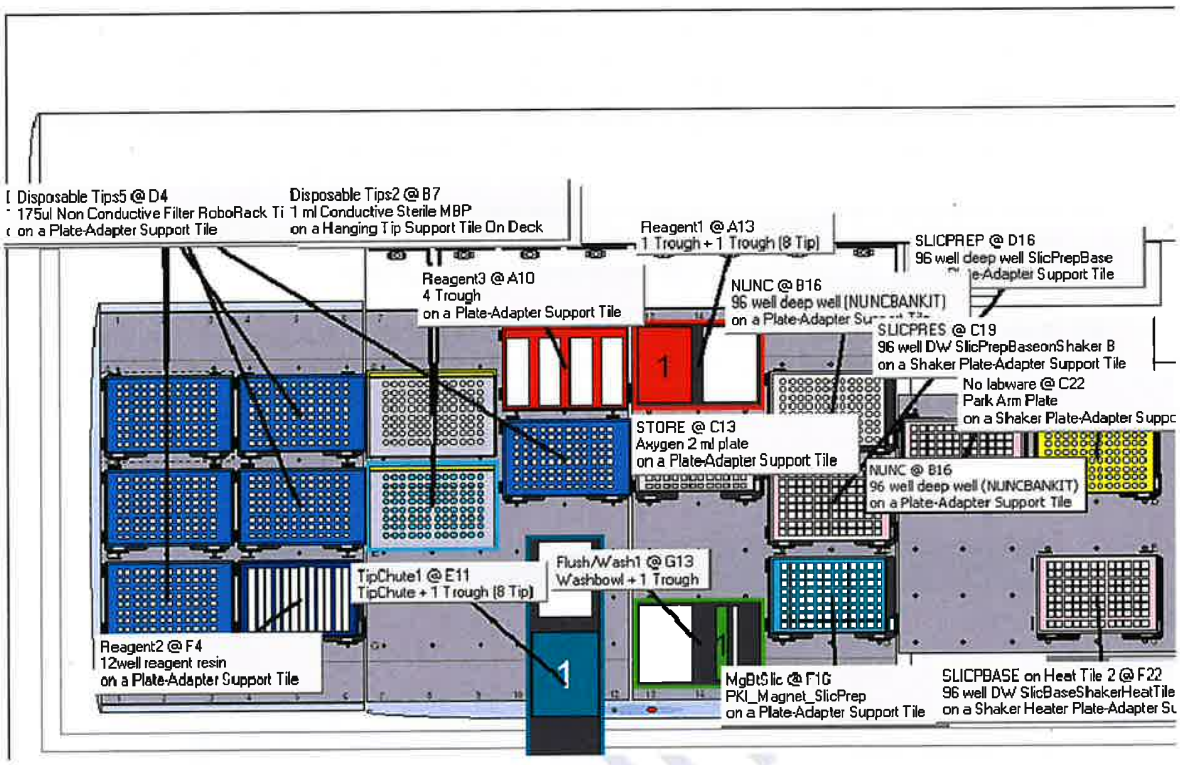

Figure 2. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

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

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

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

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

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

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

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

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

21. Message will appear (Figure 3 below):

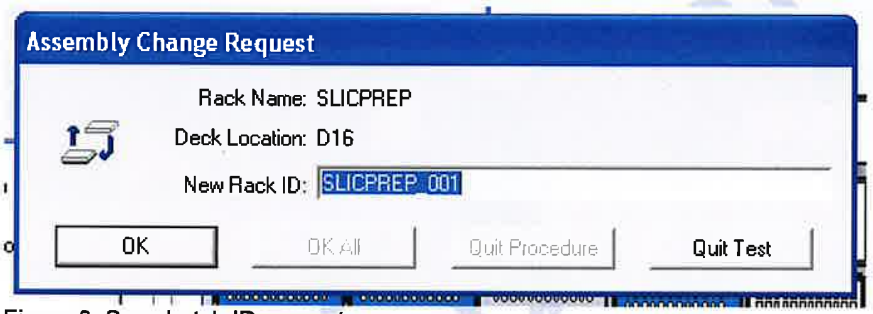

Figure 3. Scan batch ID request

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

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

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

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

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

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

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

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

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

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

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

Finalising the MP II run

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

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

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

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

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

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

Recording Reagent Details and other information in AUSLAB

38. To record reagent lot numbers, log into the **AUSLAB Main Menu.**
39. Select **5.Workflow Management.**
40. Select **2. DNA Batch Details.**
41. Scan in the Extraction Batch ID.
42. Press **[F6]** ***Reagents***.
43. Press **[SF8]** ***Audit***.

44. Press **[F5]** ***Insert Audit Entry***, enter the lot number details, operator name and Extraction platform the batch was run on and press **[Enter]**.

Importing the MP II log file into AUSLAB

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

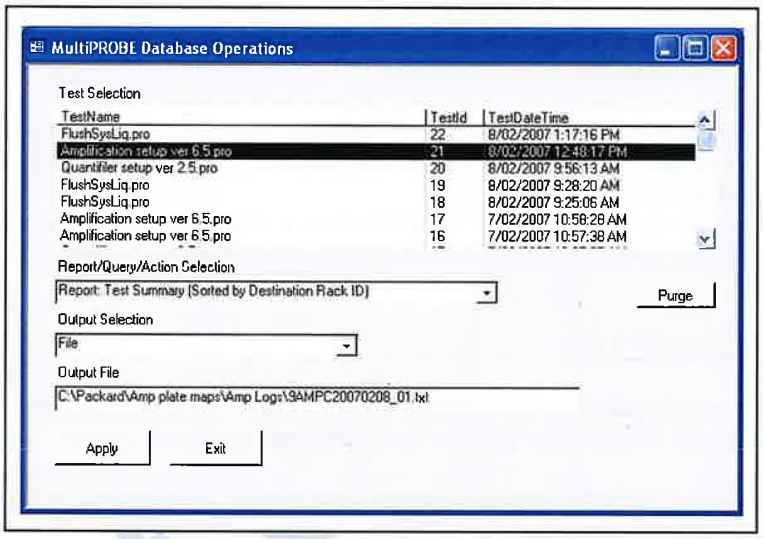

Figure 4. The MultiPROBE log database for collecting MP II run information

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

Importing Extraction "Results" into AUSLAB

59. Log into the **AUSLAB Main Menu**.
60. Select **5. Workflow Management**.
61. Select **2. DNA Batch Details**.
62. Scan the Extraction batch ID barcode located on the worksheet.
63. Press **[SF6]** ***Files***.

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

10 SAMPLE STORAGE

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

11 TROUBLESHOOTING

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

Start with the Slicprep at D16.

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

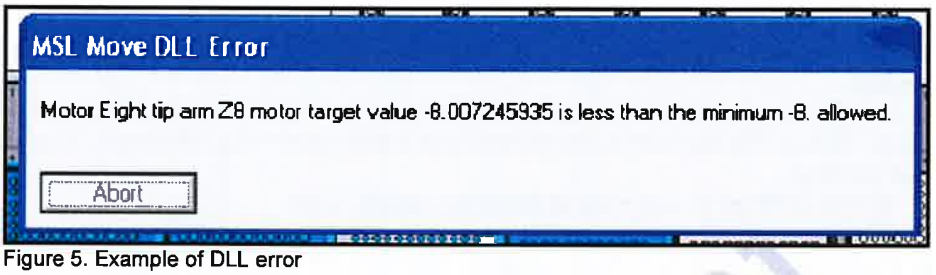

Figure 5. Example of DLL error

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

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

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

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

9. If the message Figure 6 below has appeared:

Figure 6. Example of error

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

12 VALIDATION

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

13 QUALITY ASSURANCE/ACCEPTANCE CRITERIA

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

14 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.
3. Cowan, C., The DNA IQ™ System on the Tecan Freedom EVO® 100 Profiles in DNA. Profiles in DNA, 2006: p. 8-10.
4. Eminovic, I., Karamehić, J., Gavrankapetanović, F. & Helijić, B., A Simple method of DNA Extraction in Solving Difficult Criminal Cases. MEDARH, 2005. 59(1): p. 57-58.
5. Greenspoon, S.B., J., Robotic Extraction of mock sexual assult samples using the Biomek® 2000 and the DNA IQ™ System. Profiles in DNA, 2002. 5: p. 3-5.
6. Komonski, D.I., Marignani, A., Richard, M.L., Frappier, J.R.H., & Newman, J.C., Validation of the DNA IQ™ System for use in the DNA extraction of high volume forensic casework. Can.Soc.Forensic Sci.J., 2004. 37(2): p. 103-109.
7. Mandrekar, P., V., Flanagan, L., & Tereba, A., Forensic Extraction and Isolation of DNA Form Hair, Tissue and Bone. Profiles in DNA, 2002: p. 11.
8. Mandrekar, P.V., Kreneke, B. E., & Tereba, A., DNA IQ™: The Intelligent Way to Purify DNA. Profiles in DNA, 2001: p. 16.
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.
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.
11. PerkinElmer, Automated DNA IQ™ System for Mixed Casework Sample DNA Isolation. MultiPROBE II Liquid Handling - Forensic Workstation Application Guide, 2004: p. 1-25.
12. Promega, FAQs –DNA IQ™ System.
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.

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

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.

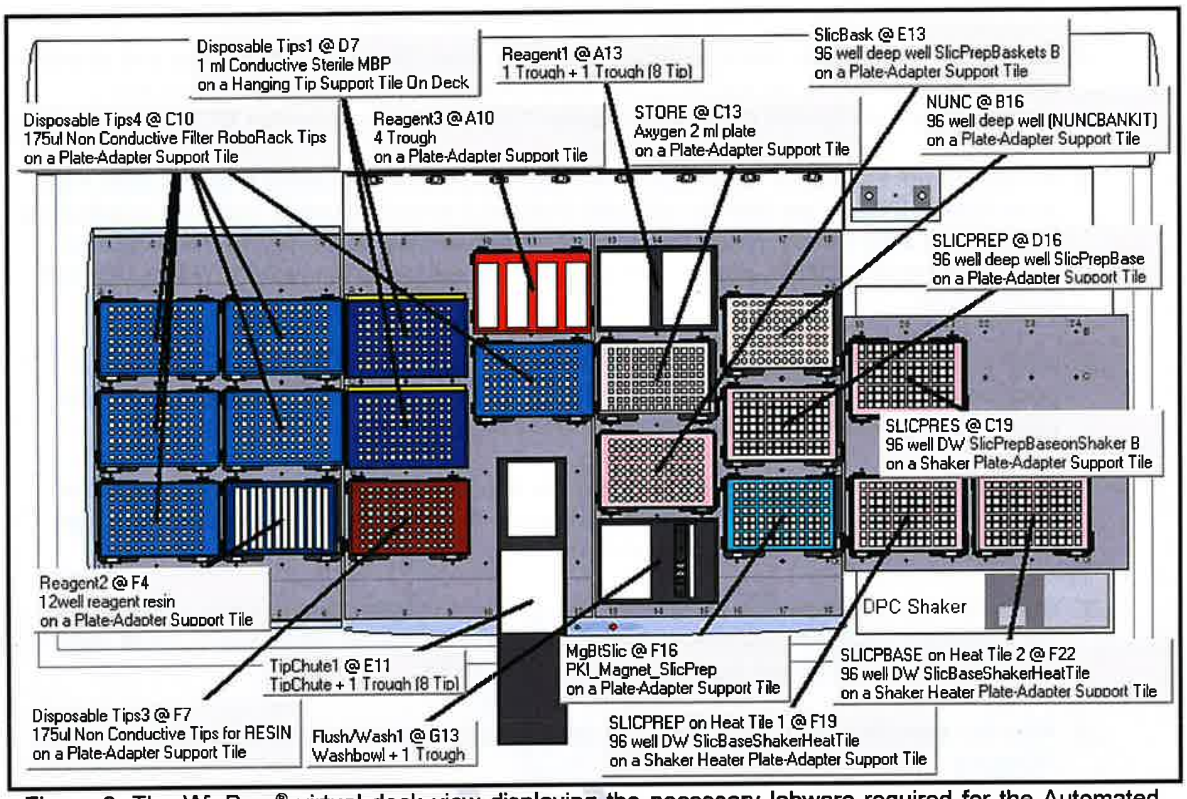

Figure 8. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

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^{0}C (real temp 37^{0}C). When current temperature reaches 50^{0}C click "**Continue**".

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

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

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

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

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

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

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

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

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

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

Finalising the MP II run

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

Recording Reagent Details and other information in AUSLAB

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

Importing the MP II log file into AUSLAB

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

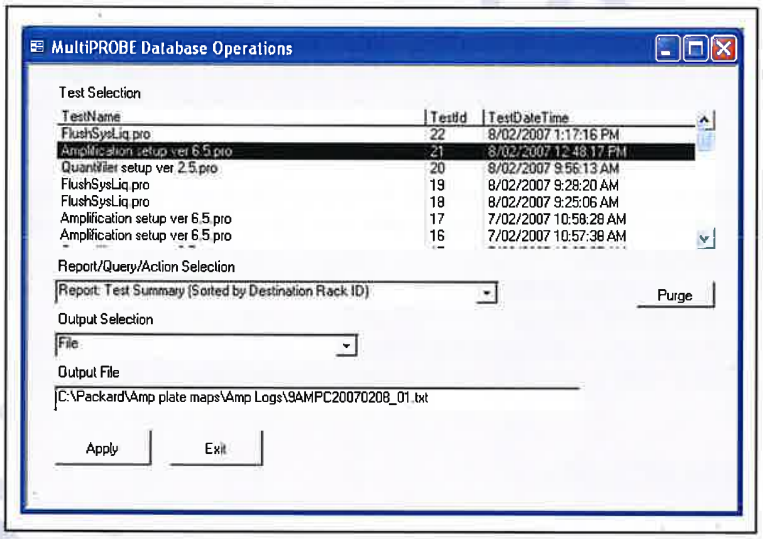

Figure 9. The MultiPROBE log database for collecting MP II run information

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

Importing Extraction "Results" into AUSLAB

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

18.3.3 Sample Storage

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

18.4 Manual method for extraction using DNA IQ™

18.4.1 Sampling and Sample Preparation

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

Table 9. Sample storage locations.

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

QC samples

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

Table 10. Extraction Quality Controls

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

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

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

Create the Extraction Batch

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

Locating Samples

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

When all samples have been located:

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

Sequence Check the tubes

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

18.4.2 Procedure

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

18. Wipe down a Biohazard hood with bleach followed by ethanol. Uncap the tubes, placing the lids inside down onto a clean rediwipe in consecutive order and place the

tubes in the same order into a clean plastic rack. Air-dry the resin in the hood for 15 minutes at Room temperature. Do not dry for more than 20 minutes, as this may inhibit removal of DNA during the elution phase.

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

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

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

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

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

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

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

18.4.3 Sample storage

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

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

1. PURPOSE AND SCOPE

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

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

2. DEFINITIONS

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

3. PRINCIPLE

Sample Pre-lysis

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

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

DNA IQ™ Kit

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

The in-house protocol includes:

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

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

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

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

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

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

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

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

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

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

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

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

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

4. REAGENTS AND EQUIPMENT

4.1. Reagents

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

- 40% Sarcosyl
- Nanopure Water

These reagents are stored in locations as per Table 1.

Table 1. Reagent storage locations.

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

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

Table 2. Table of reagent volumes.

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

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

4.2. Extraction Buffer

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

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

4.3. Lysis Buffer with DTT Solution

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

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

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

4.4. DNA IQ™ Resin

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

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

4.5. 1x Wash Buffer

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

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

4.6. Elution Buffer

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

5. EQUIPMENT

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

Table 3. Equipment used and location.

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

Table 4. Consumables used for extraction.

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

6. SAFETY

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

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

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

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

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

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

7. SAMPLING AND SAMPLE PREPARATION

7.1. Sample Locations

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

Table 5. Sample storage locations.

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

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

7.2. QC Samples

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

Table 6. Extraction Quality Controls

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

7.2.1. Registration of QC Samples

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

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

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

7.4. Locating Samples

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

8. OFF-DECK LYSIS PROCEDURE

8.1. Off-Deck Lysis (No Retained Supernatant)

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

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

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

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

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

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

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

5. Prepare Extraction Buffer as per Section 4.1.1.

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

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

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

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

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

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

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

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

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

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

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

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

8.2. Off-Deck Lysis (Retained Supernatant)

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

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

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

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

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

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

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

5. Add 450µL of TNE buffer and vortex.

6. Incubate at room temperature for 30 minutes.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

9. AUTOMATED EXTRACTION OF LYSED SAMPLES

9.1. Create the DNA IQ Extraction batch

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

9.2. Locating samples

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

9.3. Sequence checking the Nunc Bank-It™ tubes

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

9.4. MPII Extraction Procedure

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

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

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

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

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

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

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

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

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

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

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

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

9.6. Preparation of reagents for the automated extraction process

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

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

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

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

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

2. Restart or turn on the instrument PC.

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

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

Figure 1. The WinPrep® icon.

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

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

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

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

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

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

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

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

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

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

15. Refer to section 4.1 for the preparation of reagents. Record all lot numbers onto the worksheet and in AUSLAB. Note, for batches of <48 samples, use volumes for 48 samples.

16. Check the syringes and tubing and perform a Flush/Wash if required.

17. Add Wash Buffer to the far right hand side trough of the Amphyl wash station in position **A10**. Add Lysis Buffer to the labelled 150mL reagent trough on the left hand side of the 2 trough holder in position **A13**.

18. Place the 12 channel plate into position **A16**. Add Elution Buffer to the plate by dividing the Elution Buffer between channels 11 and 12. Add Resin to channel 1. It is important to add the resin in a uniform fashion to ensure equal distribution of resin along the channel.

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

    a. Add a B1-Lite generated **'LYSATE'** barcode on the **right hand side** of the Nunc™ Bank-It™ tube rack.
    b. Place the rack onto the MixMate to shake for 1 minute at 1000rpm.
    c. Centrifuge the rack at 3000rpm for 2 minutes in the Eppendorf 5804 centrifuge and then place into position **C13**.

    Note: Do not uncap lids until prompted by program.

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

21. **2mL 96-deep well storage plate:** Label the **left hand side** of the plate with both the correct **AUSLAB batch ID** and **batch ID barcode**. Label the **right hand side** of the plate with a B1-Lite generated "**STORE**" barcode. With a marker, print the word "FRONT-store" on the front of the plate. Place the plate in its correct orientation in position **E16**.

22. **Nunc Bank-It™ extract tubes:** Ensure that the rack is labelled with the correct **AUSLAB batch ID** on the **front** of the Nunc™ Bank-It™ tube rack. Label the **right hand side** of the plate with a B1-Lite generated "**EXTRACT**" barcode. Additionally, randomly check some visible barcodes on the side of lysate tubes against the positions on the worksheet to ensure the correct batch of samples is to be processed. Place the rack in position **G16**.
    Note 1: Do not uncap lids during this step.
    Note 2: If B1-Lite generated barcodes are not available hand-write the labels.

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

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

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

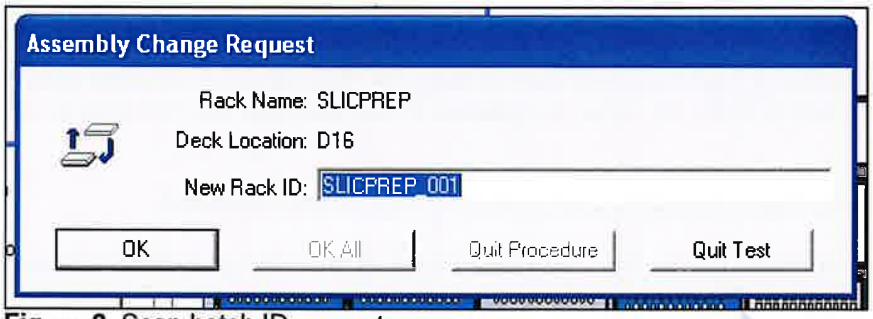

Figure 2. Scan batch ID request

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

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

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

28. For a full batch of 96 samples, ensure that all nodes are checked. For a batch of 48 samples or less, uncheck the node: "Add resin to second half of plate". Click "**Next**" to check all other nodes.

29. Click "**Start**" to continue.

30. The MPII instrument will proceed to scan the required plates on the platform deck in the below order. If barcode reading fails or if B1-Lite barcodes are not available (and hand-written labels have been used), the user is prompted to enter a plate ID. A plate ID can be entered manually into the "Read failed" prompt window for:
    a. Nunc extract tubes, type in **EXTRACT** and press **"Enter".**
    b. 96-deep well storage plate, type in **STORE** and press **"Enter".**
    c. Nunc lysate tubes, type in **LYSATE** and press **"Enter".**

31. After the plates have been identified, two user prompts will appear as a reminder to confirm the deck setup. **Always decap tubes from positions H1 to A1, H2 to A2 etc.**
    a. Ensure all steps on the first prompt have been complete, Click **OK** to continue.
    b. Ensure all steps on the second prompt have been complete, Click **OK** to continue.

32. The program will progress to transfer the lysates followed by automated addition of the Lysis-DTT buffer and the DNA IQ™ Resin solution. The next user prompt will appear. Follow the steps as outlined in the user prompt. Then click **OK** when ready.
    Note: Ensure that plate is sealed properly with the Pierce Seal. Once the Pierce Seal film is pierced, the PCR Microplate is then discarded (new plate used each time).

33. Once lysis steps have been completed, a user prompt will appear. Follow the directions as outlined in the user prompt. Then click **OK** when ready.
    Note: Nunc lysate tubes (including the rack) should be placed into a clipseal bag, sealed and discarded into a biohazard waste bin.

34. Once the wash steps have been completed, a user prompt will appear. Follow the directions as outlined in the user prompt. Then click **OK** when ready.
    Note: The Nunc extract tubes can be decapped on the bench in the MPII cabinet while the samples are drying and the heating tile is stabilising. At this point, the operator has approximately **12 minutes** to decap the tubes. If the operator has been interrupted and requires more time to decap the tubes, the program may be paused at this step.

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

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

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

38. Once all plates are removed from the deck and sealed, place into a clipseal plastic bag. Click "**OK**" to proceed to the Amphyl wash step.
    Note: Before placing the supernatant storage plate into a clipseal bag, check the plate for the transfer of beads. To do this, place the plate onto the ABI magnet (if beads are present they will settle to the bottom of the plate) and then inspect the bottom of the plate. If beads are present refer to the Section 15, Troubleshooting.

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

9.8. Finalising the MP II Run

1. Transfer left over Resin solution from the 12 channel plate and the Lysis Buffer-DTT (wearing safety glasses) into the glass Lysis-DTT bottle previously used. Discard the 12 channel plate in the biohazard waste bin. Take the bottle to room 6122 and transfer left over reagents into the brown Winchester bottle located in the fume hood.
2. Discard the contents of the Amphyl wash station into the sink and rinse the troughs with Nanopure water.
3. Remove all labware from the deck and clean with 5% TriGene™ followed by 70% Ethanol, and setup for the next run if necessary. In addition, clean the work area around the MPII instrument with 10% (v/v) Bleach 7x and 70% Ethanol.
4. Remove the tip chute and funnel, rinse with warm tap water to remove any residue inside the chute before cleaning with 5% TriGene and 70% Ethanol.
5. Move the platemap to **C:\PACKARD\EXT PLATE MAPS**\Completed Extractions.

9.9. Importing MP II Log File into AUSLAB

1. Click on the Microsoft Access icon in the WinPrep® main menu to open the MultiPROBE log database.
2. Click on the relevant run Test ID in the Test Selection box. In the Report/Query/Action Selection dropdown menu, select "Report: Test Summary (Sorted by Destination Rack ID)"
3. In the Output Selection dropdown menu, select "File". Save the output file as *.csv format to **C:\PACKARD\EXT PLATE MAPS\EXT LOGS** with the same name as the AUSLAB batch ID and click "**Apply**".
4. Open the log file and check for any errors that may have arisen during the extraction process. Compare the listed errors to any that were encountered during the run. Report any critical errors to the line manager.
5. Copy the log file to **I:\EXTRACTION\EXT A MPII\LOGS or I:\ EXTRACTION\EXT B MPII\LOGS** for uploading to AUSLAB.

6. Import the log file, entering the path, filename and extension (e.g. I:\EXTRACTION\EXTA MPII\Logs\CWIQEXT20071115_01.csv) and press **[Enter]**. For more details on importing files into AUSLAB refer to Batch functionality in AUSLAB SOP (QIS 24469).

9.10. Importing Extraction "results" into AUSLAB

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

9.11. Sample Storage

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

10. TROUBLESHOOTING

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

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

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

11. VALIDATION

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

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

12. QUALITY ASSURANCE/ACCEPTANCE CRITERIA

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

13. REFERENCES

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.
3. Cowan, C., The DNA IQ™ System on the Tecan Freedom EVO® 100 Profiles in DNA. Profiles in DNA, 2006: p. 8-10.
4. Eminovic, I., Karamehić, J., Gavrankapetanović, F. & Helijić, B., A Simple method of DNA Extraction in Solving Difficult Criminal Cases. MEDARH, 2005. 59(1): p. 57-58.
5. Greenspoon, S.B., J., Robotic Extraction of mock sexual assult samples using the Biomek® 2000 and the DNA IQ™ System. Profiles in DNA, 2002. 5: p. 3-5.
6. Komonski, D.I., Marignani, A., Richard, M.L., Frappier, J.R.H., & Newman, J.C., Validation of the DNA IQ™ System for use in the DNA extraction of high volume forensic casework. Can.Soc.Forensic Sci.J., 2004. 37(2): p. 103-109.
7. Mandrekar, P., V., Flanagan, L., & Tereba, A., Forensic Extraction and Isolation of DNA Form Hair, Tissue and Bone. Profiles in DNA, 2002: p. 11.
8. Mandrekar, P.V., Kreneke, B. E., & Tereba, A., DNA IQ™: The Intelligent Way to Purify DNA. Profiles in DNA, 2001: p. 16.
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.
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.
11. PerkinElmer, Automated DNA IQ™ System for Mixed Casework Sample DNA Isolation. MultiPROBE II Liquid Handling - Forensic Workstation Application Guide, 2004: p. 1-25.
12. Promega, FAQs –DNA IQ™ System.
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. Promega Corporation 2006 Material Safety Data Sheet. Lysis Buffer. Article number: A826.
18. 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.
19. 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.

14. STORAGE OF DOCUMENTS

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

15. ASSOCIATED DOCUMENTS

QIS 17120 Operational Practices in the DNA Dedicated Laboratories
QIS 17171 Method for Chelex Extraction
QIS 17165 Receipt, Storage and Preparation of Chemicals, Reagents and Test Kits
QIS 23939 Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform
QIS 24255 Analytical Sample Storage
QIS 24256 Sequence Checking with the STORstar Instrument
QIS 24469 Batch functionality in AUSLAB
QIS 24919 DNA Analysis Workflow Procedure

16. AMENDMENT HISTORY

Version	Date	Author/s	Amendments
R0	23 Oct 2007	B. Gallagher, T. Nurthen, C. Iannuzzi, V. Hlinka, G. Lundie, I Muharam.	First Issue
R1	12 Dec 2007	M Harvey, C Iannuzzi, A McNevin	Reviewed and updated after initial training
R2	19 March 2008	M Harvey, B Andersen, C Iannuzzi, A McNevin	Addition of Off-deck Lysis procedure, Retention of fully automated method as Appendix, addition of reagent record tables into Appendix
R3	April 2008	QIS2 Migration Project	Headers and Footers changed to new CaSS format. Amended Business references from QHSS to FSS, QHPSS to CaSS and QHPS to Pathology Queensland
4	13 March 2009	QIS2 migration	Version incremented by one on migration to QIS2
5	03 June 2009	M Aguilera, B Micic, C Iannuzzi, A. Cheng, V. Hlinka, I. Muharam, G. Lundie, C. Weber	Major changes to reflect new procedure. Updated to reflect changes in procedure as an outcome of internal and external audits. Created ver.6.4 ODL in MPII Platforms. Minor changes in

			procedures using 4titude 4seal heat sealer to seal plates.
6	29 June 2009	A McNevin, K Lancaster	Removed references to retaining lysate and beads, fixed minor formatting errors. Created ver6.5 ODL in MPII Platforms. Substrates now to be retained in 2mL tube

17. APPENDIX

17.1. Manual method for extraction using DNA IQ™

17.1.1. Sampling and Sample Preparation

Refer to section 9 above.

17.1.2. QC samples

All extraction batches require two controls to be registered. The registration of control samples is covered in the DNA Analysis workflow procedure (QIS 24919)

17.1.3. Creating the Extraction Batch and Locating Samples

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

17.1.4. Procedure (No Retain Supernatant)

1. Turn on the Eppendorf Thermomixer and set the temperature to 37°C. Alternatively an appropriately calibrated hot block may be used.

2. Remove DTT 1M (small aliquot) and Prot K 20mg/mL (small aliquot) from freezer to thaw.

3. Label for each sample:
   - Original sample tube; 2mL SSI tube (if original sample is not in a 2mL tube); Spin basket or 2mL tube; and Nunc™ Bank-It™ storage tube.

   Note: Spin baskets are not required for the Negative Extraction control, tape lifts, nails and other non absorbent substrates. For these samples, excluding the Negative Extraction control, label a 2mL tube instead of a spin basket. Substrates will be retained into original 1.5mL or 2mL after being processed in a spin basket.

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

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

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

Reagent (volume per sample)	Constituent	Volume per sample (µL)	Volume for 12 Samples (mL)	Volume for 24 Samples (mL)
Extraction Buffer (300µL/sample)	**TNE Buffer**	277.5	4.0	8.0
	Prot K (20mg/mL)	15.0	0.216	0.432
	Sarcosyl (40% w/v)	7.5	0.108	0.216
Lysis Buffer – DTT (726µL/sample)	**Lysis Buffer**	660	10.0	20.0
	DTT	6.6	0.1	0.2
Resin-Lysis Solution (50µL/sample)	**Lysis Buffer with DTT** (from above)	43	0.645	1.29
	DNA IQ RESIN	7	0.105	0.210
DNA IQ 1X Wash Buffer (300µL/sample)	See Reagent preparation		4.0	8.0
DNA IQ Elution Buffer (100µL/sample)	Use directly from Kit		1.4	2.8

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

6. Add 300µL of Extraction Buffer. Ensure that large substrates including tape lifts are fully submerged. Vortex, then incubate at 37°C on the Thermomixer at 1000 rpm for 45 minutes.

If no thermomixer available, incubate samples within a hotblock, vortex mix at the start and end of the incubation and at least one during the incubation.

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

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

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

10. Retain the spin basket and transfer the flow through back into sample tube. Transfer the substrate into a labelled 2mL tube.
Note: If original sample tube is not a 2mL tube, transfer flow through from spin basket and the supernatant from the original tube into a 2mL tube.

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

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

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

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

15. Carefully remove and discard the solution, ensuring that the resin is not disturbed. Remove from the magnetic stand.
Note: If some resin is drawn up in the pipette tip, gently expel resin back into tube to allow re-separation.

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

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

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

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

20. In a Biohazard hood, uncap the tubes and place the lids down onto a clean rediwipe. Air-dry the resin in the hood for 15 minutes and then recap tubes and remove from the Biohazard hood.
Note: Do not dry for more than 20 minutes, as this may inhibit the elution of DNA.

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

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

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

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

25. Remove from the magnetic stand and repeat the Elution Buffer steps (step 21-24). The final volume after the double elution is approximately 95µL of DNA extract.

26. DNA extracts are stored in temporary storage in freezer 6117-2 (-20°C) located in the workflow area. Tubes containing the original substrate are to be stored in spin basket boxes in freezer 6117-5 located in the workflow area.

17.1.5. Procedure (Retain Supernatant)

1. Turn on the Eppendorf Thermomixer and set the temperature to 37°C. Alternatively an appropriately calibrated hot block may be used.

2. Remove DTT 1M (small aliquot) and Prot K 20mg/mL (small aliquot) from freezer to thaw.

3. Label for each sample: Original sample tube; 2mL SSI tube (if original sample is not in a 2mL tube); 1.5mL tube (for supernatant) these tubes should not be in contact with the substrate; Spin basket or 2mL tube; an extra 2mL tube for spin baskets; Nunc™ Bank-It™ storage tube.
   Note: Spin baskets are not required for the Negative Extraction control, tape lifts, nails and other non absorbent substrates. For these samples, excluding the Negative Extraction control, label a 2mL tube instead of a spin basket. Substrates will be retained into original 1.5mL or 2mL after being processed in a spin basket.

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

5. Using Table 8, prepare Lysis Buffer & Resin solution. Ensure that the DNA IQ™ Resin solution is thoroughly vortexed prior to use.
   Note: Reagents need to be prepared fresh before each run and Lysis Buffer-DTT solution and Resin solution need to be prepared in the fume hood.

Table 8. Table of reagent volumes for DNA IQ Manual Extraction

Reagent (volume per sample)	Constituent	Volume per sample (µL)	Volume for 12 Samples (mL)	Volume for 24 Samples (mL)
Lysis Buffer – DTT (726µL/sample)	**Lysis Buffer**	660	10.0	20.0
	DTT	6.6	0.1	0.2
Resin-Lysis Solution (50µL/sample)	**Lysis Buffer with DTT** (from above)	43	0.645	1.29
	DNA IQ RESIN	7	0.105	0.210
DNA IQ 1X Wash Buffer (300µL/sample)	See Reagent preparation		4.0	8.0
DNA IQ Elution Buffer (100µL/sample)	Use directly from Kit		1.4	2.8

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

6. Add 450µL of TNE buffer and vortex.

7. Incubate at room temperature for 30 minutes.

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

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

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

11. Vortex, then incubate at 37°C on the Thermomixer at 1000 rpm for 45 minutes. If no thermomixer available, incubate samples within a hotblock, vortex mix at the start and end of the incubation and at least one during the incubation.

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

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

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

15. Retain the spin basket and transfer the flow through back into sample tube.
Note: If original sample tube is not a 2mL tube, transfer flow through from spin basket and the supernatant from the original tube into a 2mL tube. Transfer the substrate into a labelled 2mL tube.

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

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

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

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

20. Carefully remove and discard the solution, ensuring that the resin is not disturbed. Remove from the magnetic stand.
Note: If some resin is drawn up in the pipette tip, gently expel resin back into tube to allow re-separation.

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

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

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

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

25. In a Biohazard hood, uncap the tubes and place the lids down onto a clean rediwipe. Air-dry the resin in the hood for 15 minutes and then recap tubes and remove from the Biohazard hood.
    Note: Do not dry for more than 20 minutes, as this may inhibit the elution of DNA.

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

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

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

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

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

31. DNA extracts & retained supernatants ("sup" tubes) are stored in temporary storage in freezer 6117-2 (-20°C) located in the workflow area. Tubes containing the original substrate are to be stored in spin basket boxes in freezer 6117-5 located in the workflow area.

17.1.6. Sample storage

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