Notice number: 2022/00178

COMMISSION OF INQUIRY INTO FORENSIC DNA TESTING

IN QUEENSLAND

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

STATEMENT OF THOMAS NURTHEN

I, **Thomas Edmund Kersey Nurthen**, care of Queensland Health Forensic and Scientific Service, Reporting Scientist, do solemnly and sincerely declare that:

1. On 14 September 2022, I was requested to provide a statement responding to Notice 2022/00178 "Requirement to Give Information in a Written Statement".

Thomas Nurthen

Question 1 - State your full name, current position/title and where you work.

2. My name is Thomas Edmund Kersey Nurthen.

3. I am currently employed by Queensland Health Forensic and Scientific Services as a Reporting Scientist in the Forensic Reporting and Intelligence Team.

Question 2 – List your tertiary qualifications, the year you obtained them, and the institute from which the qualifications were obtained.

4. I obtained a Bachelor of Science from the University of Queensland in 1998.

5. I obtained a Bachelor of Science with Honours from the University of Queensland in 2001.

Question 3 – Identify the duties/responsibilities of your current position.

6. In my current position as Reporting Scientist, the key duties and responsibilities are to:

   (a) Supervise forensic testing and related duties in accordance with relevant forensic protocols and standards within DNA Analysis;

   (b) Supervise the development of scientific practices, procedures and protocols within the DNA Analysis work area;

   (c) Provide the results of forensic DNA Analysis and interpretation to senior staff and key stakeholders with respect to the National Criminal Investigation DNA Database, including the provision of expert testimony in court;

   (d) Monitor and report clinical work practices and outcomes within DNA Analysis and initiating, planning and evaluating scientific and service delivery improvement activities;

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(e) Provide expert technical advice to relevant stakeholders regarding standards and clinical services of a complex nature;

(f) Provide general clinical and technical advice to professional and operational supervisors and relevant service managers regarding service delivery, equipment, technology and the prioritisation and development of clinical services;

(g) Ensure the development of scientific knowledge and expertise by supporting active learning and professional development of DNA Analysis staff;

(h) Provide clinical practice supervision to assistants and clinical support staff, to ensure the maintenance of professional clinical standards; and

(i) Ensure that DNA Analysis complies with all relevant legislative, administrative and professional standards to meet National Association of Testing Authority/International Standardisation Organisation (**NATA/ISO**) accreditation/certification requirements.

Question 4 – Identify what positions you held at Queensland Health Forensic and Scientific Services from 2008 to 2014, and the duties/responsibilities of those positions.

7. The positions I held with Queensland Health Forensic and Scientific Services from 2008 to 2014 are outlined below.

July 2006 to October 2008: Senior Scientist, Automation and Laboratory Information Management System (LIMS) implementation project

8. The Automatic and LIMS implementation project was tasked with:

(a) The validation of automated robotic systems to perform DNA extractions (DNA extractions are the breaking open of cells and cleaning of the DNA from unwanted inhibitors);

(b) Automating the set-up of the DNA quantitation process (which is measurement of the amount of DNA in a sample) and the DNA amplification process (which are the different locations with which a person's DNA is 'copied' or 'amplified'); and

(c) Enhancing AUSLAB to improve efficiency. The LIMS is the software that records all case information, moves samples onto worklists and send the results of the samples back to the Queensland Police Service and other stakeholders. AUSLAB is the LIMS that was used in the Forensic DNA Laboratory.

9. In this role, I was required to lead a team of give scientists and coordinate and manage the overall project. I reported to the Managing Scientist (this was firstly Vanessa Ientile and then later Cathie Allen).

10. In addition to leading a team, my duties and responsibilities included:

(a) Reporting directly to the Managing Scientist with respect to how the project was progressing (including against project milestones) and escalating any issues as necessary;

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(b) Participating in Management Team meetings and decisions about the project and laboratory agenda items;

(c) Developing functional specifications for the enhancements of AUSLAB (i.e. creating new functionality within AUSLAB that allowed, for example, the integration of robotic platforms, new workflows to improve efficiency of case management of samples and improved sample auditing);

(d) Liaising with external laboratories in relation to the development and validation of the robotic systems; and

(e) Overseeing the planning of the validation experiments (which were a necessary party of continuous quality improvement and NATA accreditation requirements) and coordinating of the validation report publication (the validation reports were prepared by my direct reports and provided to me for review, approval and publication).

October 2008 to December 2013: Senior Scientist, Quality & Projects

11. In this role, my duties and responsibilities were to:

(a) Supervise and coordinate a medium-sized team in relation to quality management practices within the multi-speciality discipline of DNA Analysis, Forensic and Scientific Services;

(b) Ensure compliance of forensic DNA testing and related duties and practices with all relevant legislative, administrative and professional standards to meet NATA/ISO accreditation/certification requirements;

(c) Provide independent high level forensic services to all key stakeholders incorporating the interpretation of results, the use of information relating to the National Criminal Investigation DNA Database, and the provision of expert testimony on work performed within the laboratory in accordance with legislative requirements;

(d) Provide scientific judgement in the analysis of specimens and samples in the DNA Analysis Laboratory leading to the provision of forensic test results and advice to clients and stakeholders where appropriate;

(e) Provide clinical advice to practitioners, senior management, clients and relevant stakeholders, in particular regarding the interpretation of internationally recognised standards and forensic quality management systems; and

(f) Represent DNA Analysis in the Forensic & Scientific Services laboratory group which included decision making and strategic planning at a state-wide level.

12. I have been in my current role as a Reporting Scientist from December 2013 up to now. A summary of my duties and responsibilities in this position is summarised above at **Question 3**.

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DNAIQ

Question 5 – Explain what DNAIQ is. Explain the way or ways in which the DNA laboratory used DNAIQ, and/or DNAIQ system(s), as at the start of 2008.

13. The DNAIQ is a commercially available magnetic bead-based DNA extraction kit developed and sold by Promega (an organisation who manufactures certain biotechnological products).

14. There are several different technologies and manufacturers of DNA extraction kits. The general aim of all DNA extraction kits is to break open human cells and purify the DNA inside from external contaminates. There are many different chemicals that can either prevent or reduce the ability to generate a DNA profile.

15. In summary, the DNAIQ kit works as follows:

    (a) An area of interest is sampled;

    (b) The sample has an extraction buffer added to break open cellular material;

    (c) The sample substrate is spun and removed leaving a raw liquid material called lysate;

    (d) A special resin containing magnetic beads is added to the lysate;

    (e) A special buffer is added to allow the DNA to bind to the magnetic beads;

    (f) The sample is added to a magnet to bind the beads and they are washed several times to remove non-DNA material and other chemical inhibitors; and

    (g) The sample has a special buffer (elution buffer) added to allow the DNA to be released from the beads. The most efficient extraction from validation was a double elution.

16. **Annexed and marked Exhibit TN-01** is a protocol published by Promega summarising how DNAIQ works.

17. To the best of my recollection, DNAIQ was being used in the Forensic DNA Laboratory in around early 2008 for the purposes of extracting DNA from:

    (a) cell and blood samples from various substrates for casework samples; and

    (b) buccal cells and blood from FTA (a way of storing DNA) samples for reference samples.

18. The MultiPROBE II was a liquid handling instrument that ran the DNA extraction protocols, including the DNAIQ system. A DNA extraction protocol is the method which is programmed into the instrument and instructs the instrument about how to process a sample.

19. I understand there were three different DNAIQ extraction protocols used in the laboratory during early 2008:

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(a) On deck lysis (this protocol is largely performed on the MultiPROBE II);

(b) Manual DNAIQ extraction (this protocol is a fully manual task without the use of the MultiPROBE II); and

(c) Off deck lysis (this protocol is performed on the MultiPROBE II instrument with some additional tasks performed off the instrument).

20. The 'on deck lysis' protocol was first introduced in around 2007 when the MultiPROBE II was first introduced into the laboratory for the purposes of DNA extraction. **Annexed and marked Exhibit TN-02** is an internal Standard Operating Procedure sets out the 'on deck lysis' protocol.

21. The other two protocols - Manual DNAIQ extraction and off deck lysis – were introduced into the laboratory in around February 2008. The Manual DNAIQ extraction method was validated prior to this time but later formalised in a protocol in February 2008. The off deck lysis protocol was introduced to improve DNA extraction for casework substrates. **Annexed and marked Exhibit TN-03** is an internal Standard Operating Procedure which sets out the Manual DNAIQ extraction and off deck lysis protocols. This Procedure was an updated version of TN-02 intended to collate all DNAIQ extraction methods into the same document.

Question 6 – Explain what problems with DNAIQ were experienced in approximately 2008.

Explain, to the best of your knowledge, how these problems were first detected.

22. All information I have set out below has been derived from my review of emails and files in my possession.

23. To the best of my recollection, sample cross contamination was recorded in an OQI (OQI#19330) on 21 April 2008 on a reference sample extraction batch (**First Contamination Event**). **Annexed and marked Exhibit TN-04** is the OQI#19930 report.

24. An OQI is an opportunity for quality improvement and is raised in circumstances where a potential issue is identified. To be clear, the date an OQI is raised is not necessarily when an issue is first detected. It is sometimes the case that OQIs are raised retrospectively to document an issue. For example, preliminary investigation into a potential issue may occur before an OQI is formally raised.

25. In respect of the First Contamination Event, it is my understanding that a mixed DNA profile (referred to as a 'mixture') was obtained in a reference sample with the contaminating source DNA profile occurring on the same automated extraction batch.

26. For context, reference samples are those samples taken from individuals for the purpose of comparison to casework/crime scene DNA profiles. As reference samples are taken from the mouth (buccal) or from blood samples, these should be single source DNA profiles.

27. The OQI#19330 report concluded that the reason for cross contamination could not be determined on the basis that there were multiple steps in the processing of the reference samples such that any one step could have been the source of the contamination.

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28. It was not my responsibility to investigate the First Contamination Issue and I did not prepare the OQI report. However I may have had discussions with members of the Automation Team (which was the team that I lead at this time) and the Analytical team about the potential cause of the issue.

29. A subsequent contamination event was recorded in OQI#19349 on 23 April 2008 when a below threshold DNA profile was detected in an extraction negative control (**Second Contamination Event**). **Annexed and marked Exhibit TN-05** is the OQI#19349 report.

30. For context, all DNA extraction batches include both positive (samples known to have cells/DNA) and negative (samples that contain no cells/DNA) controls. Before a batch of samples is released for reporting, it must pass the quality procedures in place.

31. The OQI#19349 report indicated that the contaminating source DNA profile was determined to be the adjacent positive control. Based on my understanding of the findings in this report, how the negative control became contaminated (and more specifically the part of the process which may be the source of the contamination), could not be narrowed down to a single process.

32. At the time OQI#19330 and OQI#19349 were raised, I understand that at this stage it was not clear as to whether there was a systemic issue concerning DNAIQ on the MultiPROBE II. Similar with the First Contamination Event, it was not my responsibility to investigate this issue and I did not prepare the OQI. However I may have had discussions with members of the Automation Team and the Analytical team about the potential cause of the issue.

33. A subsequent contamination event was recorded in OQI#19477 on 12 May 2008 when another negative control was found to have been contaminated. **Annexed and marked Exhibit TN-06** is the OQI#19477 report. In this event, several casework samples with the same DNA profile on the same DNA extraction could have been the contaminating source batch.

34. According to the OQI#19477 report, it was observed that there was potential systemic contamination on the MultiPROBE II, including in the context of the First and Second Contamination Events.

35. From my recollection, while the investigation was being undertaken into OQI#19477, there were other subsequent contamination events (as raised in OQI#19767 on 14 June 2008 and OQI#19768 on 30 July 2008). **Annexed and marked Exhibits TN-07 and TN-08** are the reports raised for OQI#19767 and OQI#19768, respectively.

Question 7 – Identify each OQI and adverse event that relates to DNAIQ problems at around this time, or has since been linked to DNAIQ problems from around this time.

36. The table set out below lists the OQIs which were raised in relation to the DNAIQ problems in 2008, including a summary of the contamination detected in the samples. The Preparation batch ID refers to the batch the samples were prepared on, with the Prep date being the date the batch was prepared. The Extraction batch ID refers to the MultiPROBE II processing batch. The extraction date is the date the batch was extracted on the MultiPROBE II.

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OQI#	Preparation batch ID	Prep date	Extraction batch ID	Extraction date	Summary of contamination detected
19330	FTAEXT20080205_01	5/02/2008	RFIQEXT20080205_03	6/02/2008	Single contamination event between reference samples
19349	N/A	27/02/2008	CWIQEXT20080225_02	27/02/2008	Single contamination event between casework samples and negative control
19703	CWIQLYS20080319_02	19/03/2008	CWIQEXT20080319_07	20/03/2008	Single contamination event between casework samples
20351	CWIQLYS20080401_02	2/04/2008	CWIQEXT20080402_01	4/04/2008	Multiple contamination events between casework samples
20925	CWIQLYS20080402_01	3/04/2008	CWIQEXT20080403_01	9/04/2008	Multiple contamination events between casework samples
20615	CWIQLYS20080408_01	9/04/2008	CWIQEXT20080409_01	16/04/2008	Possible multiple contamination events between casework samples
20231	CWIQLYS20080416_01	17/04/2008	CWIQEXT20080417_01	21/04/2008	Multiple contamination events
19477	CWIQLYS20080430_01	30/04/2008	CWIQEXT20080430_01	1/05/2008	Multiple contamination events between casework samples and between casework samples and negative control
19768	CWIQLYS20080502_02	6/05/2008	CWIQEXT20080506_01	7/05/2008	Single contamination event between casework samples and negative control
20422	CWIQLYS20080506_01	6/05/2008	CWIQEXT20080506_02	26/05/2008	Single contamination event between casework samples
19767	FTAEXT20080515_01	15/05/2008	RFIQEXT20080515_01	19/05/2008	Single contamination event between reference samples
21309	CWIQLYS20080530_01	31/05/2008	CWIQEXT20080531_01	4/06/2008	Possible multiple contamination events between casework samples
20617	CWIQLYS20080613_02	13/06/2008	CWIQEXT20080614_02	19/06/2008	Multiple contamination events between casework samples
21222	CWIQLY20080619_01	20/06/2008	CWIQEXT20080620_02	23/06/2008	Single contamination event between casework samples
20690	CWIQLYS20080627_01	28/06/2008	CWIQEXT20080628_01	1/07/2008	Multiple contamination events between casework samples
20437	CWIQLYS20080627_02	30/06/2008	CWIQEXT20080630_01	11/07/2008	Multiple contamination events between casework samples
21589	RFIQLYS20080627_01	30/06/2008	RFIQEXT20080630_01	1/07/2008	Single contamination event between project samples and negative control

37. **Annexed and marked Exhibit TN-09** is a collated bundle of the OQI reports listed in the above table.

Question 8 – What actions did the management committee and/or staff at the DNA laboratory take in response to the discovery of the problem? Provide a clear timeline which covers the problems identified, the decisions taken in response and by whom, and how those decisions were implemented.

38. Information about the actions taken by management and staff and a timeline covering the problems identified have been derived from my review documentation in my possession.

39. To the best of my recollection, a timeline of the actions taken by the management committee and/or staff at the DNA laboratory in response to the contamination issues is provided as follows:

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(a) On 18 April 2008, Allan McNevin sent an email to the Management Team attaching the 'Reference Mixture Investigation Report' in draft for comment in relation to First Contamination Event. The Management Team changed from time-to-time but I believe at this time it likely consisted of myself, Cathie Allen, Justin Howes, Amanda Reeves, Emma Caunt, Paula Brisotto, Shannon Merrick, Allan McNevin and Robyn Smith (acting for Mary Gardam). **Annexed and marked Exhibit TN-10** is a copy of the email dated 18 April 2008 and attached investigation report.

(b) I understand there may have been meetings to discuss the issues in respect of the First Contamination Event but I cannot recall what was discussed in particular nor if I attended all meetings where these matters were being discussed.

(c) On 21 April 2008, OQI#19930 (which was created in response to the First Contamination Event) was raised. I do not recall whether I was notified in our internal reporting system (**QIS**) of this OQI at this time.

(d) On 22 April 2008, after the Analytical scientists observed precipitate in the samples and experiments were undertaken by Dr Vojtech Hlinka (Automation Scientist), it was suggested that the SDS (a detergent used in the DNA extraction process) should be replaced with sarcosyl (i.e. a different detergent). **Annexed and marked Exhibits TN-11 and TN-12** are Dr Hlinka's reports which support the change in detergent.

(e) On 23 April 2008, OQI#19349 was raised in relation to the Second Contamination Event. I do not recall whether I was notified of this OQI in the QIS at this time.

(f) Since the discovery of the contamination issues, there was likely to have been ongoing discussion and further investigation by the Analytical Team into the contamination issues (including as reported in OQI#19767 and OQI#19768 as mentioned above at paragraph 35). In around this time, the Automation Team (which I led) was likely undertaking a review of the DNA extraction protocols on the MultiPROBE II instrument to assist the Analytical team with their ongoing investigation into the cause of contamination.

(g) On 14 July 2008, an extraordinary management meeting (which I attended) was held to discuss the contamination issues. **Annexed and marked Exhibit TN-13** is a copy of the agenda of this meeting.

(h) After the meeting, a memorandum was issued by Vanessa Ientile (who was Chief Scientist at this time) to the entire DNA Analysis laboratory about the contamination events, including the OQIs which had been raised and interim measures that were going to be put into place to address the contamination issue (including troubleshooting, changes to processes and further investigation) (**Memorandum**). For example, it was proposed that from 15 July 2008 the number of blank samples for casework plates (checkerboard patten) would be increased in an effort to identify the source of the contamination issue. A checkboard is a plate which has blank samples interspersed between each casework sample (in a checkboard pattern) to help more effectively identify the possible source of the contamination. **Annexed and marked Exhibit TN-14** is a

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copy of the memorandum dated 14 July 2008 (which includes as diagram of the checkboard pattern).

(i) The QIS indicates that on 15 July 2008, Audit 8227 was conducted. The Memorandum confirmed that this Audit was already underway. **Annexed and marked Exhibit TN-15** is a copy of the QIS record. The purpose of the audit (among other things) was to assess whether the source of the contamination could be identified on the MultiPROBE II instrument. This practically involved direct observation of the application of the DNA extraction protocols on the MultiPROBE II instrument to identify any steps any steps in the DNA extraction protocols where a potential for quality breakdown was present, and also to identify areas of improvement that may benefit the protocols.

The audit was carried out Iman Muharam (Automation Team), Amy Cheng (Analytical Team) and Peter Clausen (Scientific Skills Development). I was the contact for this Audit and the interface between the auditing team and laboratory. This practically meant that if anyone had questions about the Audit, I would be the first point of contact.

(j) On 23 July 2008, Mr McNevin sent an email to the Management Team regarding further contamination events which had been identified. **Annexed and marked Exhibit TN-16** is a copy of this email. The Management Team changed from time-to-time but I believe at this time it likely consisted of myself, Cathie Allen, Justin Howes, Vanessa Ientile, Emma Caunt, Kylie Rika, Robyn Smith, Wendy Harmer, Adrian Pippia, Paula Brisotto, Peter Clausen and Amanda Reeves.

(k) On 28 July 2008, a Management Team meeting was held to discuss the draft Audit 8227 findings. I attended this meeting. **Annexed and marked Exhibit TN-17** is a copy of the agenda for this meeting.

(l) After the meeting, Chief Scientist (Vanessa Ientile) emailed the entire laboratory advising that from that day (28 July 2008), there would no be further DNA extractions on the MultiPROBE II instrument and all extractions which were already commenced were to be finished manually. **Annexed and marked Exhibit TN-18** is a copy of the email update dated 28 July 2008.

(m) The QIS indicates that on 28 July 2008 Audit 8752 commenced. **Annexed and marked Exhibit TN-19** is a copy of this QIS record. The purpose of this audit was to investigate to identify whether there were any additional contamination events that had not been detected to date. The audit team consisted of Susan Brady (lead auditor), Angelina Keller and Rebecca Gregory (all members of the Reporting team). Cathie Allen was the contact for this audit.

(n) On 29 July 2008, a DNA Analysis departmental meeting (which was the whole laboratory) was held to discuss the DNAIQ contamination issues. I attended this meeting.

(o) In around end of July 2008, some members of Management Team (including Cathie Allen and Emma Caunt), Greg Shaw (Executive Director, FSS) and members of the Queensland Police Service attended a meeting to discuss the DNAIQ contamination issues. I was aware of this meeting but I did not attend it.

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Thomas Nurthen Witness

(p) On 5 August 2008, the Management Team met to discuss a specific contamination event which involved possible well-to-well contamination on a plate with an unaffected negative control (an unconfirmed mixture of a hit and run case with a sexual assault). The Management Team changed from time-to-time but I believe at this time it likely consisted of myself, Emma Caunt, Amanda Reeves, Allan McNevin, Kylie Rika, Adrian Pippia, Justin Howes, Paula Brisotto and Cathie Allen. **Annexed and marked Exhibit TN-20** is a copy of the meeting minutes dated 5 August 2008.

(q) On 8 August 2008, the audit report in relation to Audit 8277 was finalised. **Annexed and marked Exhibit TN-21** is a copy of the final audit report dated 8 August 2022.

(r) On 17 September 2008, the auditing team facilitated a presentation outlining the recommendations flowing from the Audit 8227. **Annexed and marked Exhibit TN-22** is a copy of a presentation dated 17 September 2008. I attended this presentation. **Annexed and marked Exhibit TN-23** is a copy of the meeting attendance for this presentation.

(s) On 17 September 2008, the auditing team facilitated a presentation outlining the recommendations flowing from the Audit 8752. **Annexed and marked Exhibit TN-24** is a copy of the presentation dated 17 September 2008. **Annexed and marked Exhibit TN-25** is a copy of the report for Audit 8752.

(t) After the results from the audits were released, an Investigation Team (consisting of Alicia Quartermain, Jacqui Wilson, Shannon Merrrick, Julie Connell, Rhys Parry, Angelina Keller, Claire Gallagher, Ingrid Moeller, Emma Caunt and Paula Brisotto) was set up to report on legal cases which were affected by the MultiPROBE II instrument issues. A report in this context involved interpretating DNA profile results and preparing a statement of witness for Court.

(u) Since the contamination issues first arose, the Automation consulted with PerkinElmer (the manufacturers of the MultiPROBE II instruments) to review the revised DNA extraction protocols to see if they could assist with improving these protocols (in addition to conducting its own review at mentioned below). This practically involved analysing all aspects of the instruments while a DNA extraction process was being performed to ensure there no risks of contamination were being created. For example, the Automation team observed that drops were forming on the tips. The tips are the part of the instrument that draws up the sample or other liquids from one vessel then dispenses them to another. Droplet formation on the ends of the disposable tips when pipetting samples can cross contaminate. In response to this issue, the laboratory asked PerkinElmer to assist with rectifying this issue (in particular, reviewing the programming of the instrument to determine whether any of the liquid handling settings could be improved).

(v) On 3 October 2008, PerkinElmer provided a report outlining the steps which required modification in the extraction process. I understand these steps were implemented. **Annexed and marked Exhibit TN-26** is a copy of the response dated 3 October 2008.

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(w) In parallel with the Automation Team's discussions with PerkinElmer, the Automation Team was also reviewing the DNA extraction protocols to limit the risk of contamination and to improve efficiency. For example, the Automation Team in addition to the Analytical Team were re-extracting the lysates from contaminated batches to identify which step in the extraction process the contamination may have occurred.

(x) On 21 October 2008, Mr Greg Shaw (Executive Director, FSS) emailed Dr Theo Sloots (external scientist) about the possibility of him assisting with a scientific review of the response to the contamination problem. In an email from Cathie Allen on 23 October 2008, Mr McNevin and I were asked to arrange an appropriate person to show Dr Sloots and Dr Whiley around and have a general discussion with him about the process. **Annexed and marked Exhibit TN-27** is an email chain involving Justin Howes, Allan McNevin, Cathie Allen, Paula Taylor and Wendy Harmer between and 21 and 23 October 2008.

(y) On 28 October 2008, the Investigation Team (as listed above) released guidelines for reporting results from DNAIQ batches. **Annexed and marked Exhibit TN-28** is a copy of an email from Emma Caunt to Management Team (Adrian Pippia, Allan McNevin, Amanda Reeves, Cathie Allen, Ingrid Moeller, Justin Howes, Kylie Rika, Paula Brisotto and myself) attaching the reporting guidelines.

(z) On 6 November 2008, PerkinElmer wrote to Forensic Biology team. The letter indicates that the FSS DNA Analysis laboratory requested PerkinElmer to observe and ensure that the liquid handling was optimum. The letter lists the steps which required some modification. I understand these steps were implemented. **Annexed and marked Exhibit TN-29** is the letter dated 6 November 2008.

(aa) Following on from the request from Greg Shaw (as referred to above at paragraph 39(x)), on 12 November 2008, external auditors Dr Theo Sloots and Dr David Whiley (another external scientist) attended Forensic Biology to conduct the review of processes as part an external review. This external review was documented on the QIS as Audit 9175.

(bb) On 14 November 2008, Dr Sloots and Dr Whiley released a report. **Annexed and marked Exhibit TN-30** is a copy of the report dated 14 November 2008.

(cc) In around November 2008, I prepared a report summarising all of the contamination events that were being identified in DNAIQ extraction batches into a single document. I do not recall why I prepared this report but I believe maybe it was to bring all the contamination events, investigations and outcomes into a single document. I never finalised the report. I do not recall why this was the case but to the best of my collection, this may be because each of the individual OQIs had been actioned at this stage and some of the information in the report was available in other documents. The last version was saved by me in 2011. **Annexed and marked Exhibit TN-31** is copy of the draft report.

(dd) I transitioned to another role (Senior Scientist, Quality and Projects) in October 2008. As a result, I had less involvement with the MultiPROBE II issues and was largely responsible for managing operational staff (which were mostly non-scientists) in Quality and Projects.

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Thomas Nurthen Witness

Question 9 – Was the cause of the issues or problems relating to DNAIQ identified? If yes, what was it?

40. Based on my knowledge of the issues relating to DNAIQ arising from the investigations conducted into the issues (including the review work conducted by the Automation Team which I supervised), the investigations arising from OQIs which were raised (as listed in Question 7 above), the retrospective audits to determine the cause of the contamination events (such as Audits 8227, 8752, 9175) and my overall involvement in these processes, I believe:

    (a) there was no issue with the DNAIQ kit itself;

    (b) for most of the contamination events, the issue was more likely related to the use of the plate seals (this is the adhesive which covers the sample plate) (this was consistent with the findings in Audit 9175);

    (c) however, there was no one cause of the contamination such that multiple issues could have been attributed to the contamination. In other words, the plate seals (which being the likely cause of contamination for most of the events) were not the cause of contamination for all events. For example, the contamination identified could have been consistent with the movement of the MultiPROBE II instrument during the extraction, during storage or during plate agitation.

Question 10 – What immediate action was taken after the cause of the issues or problems was identified?

41. The action taken in response to the cause of the contamination issues is outlined above at Question 8.

Question 11 – Outline your role in responding to issues with DNAIQ, and any audits completed in relation to any OQI concerning DNAIQ, including audit 9175.

42. My role in 2008, as the Senior Scientist Automation and LIMS implementation project, was to lead the Automation Team:

    (a) in the troubleshooting of the contamination events (such as the reviews undertaken by the Automation Team set out in response to Question 8 above); and

    (b) the re-optimisation and re-validation of the automated DNAIQ protocols as a result of our troubleshooting efforts (such as our consultation with with PerkinElmer set out in response to Question 8 above).

43. In October 2008, I moved into a different role as Senior Scientist, Quality and Projects. In this role, I was line manger role for all of the operational staff in the Quality and Projects team and reported to the Team Leader Quality and Projects, Paula Brisotto. As mentioned above at paragraph 39, I no longer had an active role in responding to the DNAIQ issues (however I would have likely been aware of the issues given my role in the Management Team).

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44. I was also the contact person for Audits 8227 (as mentioned above at paragraph 39) and 9642 (which was a follow-up audit). The objective of Audit 9642 was to check the changes made to the DNAIQ protocols for the MultiPROBE II sufficiently improved the quality and reliability of results. **Annexed and marked Exhibit TN-32** is the QIS record and report in relation to Audit 9642. During my direct involvement in responding to the DNAIQ issues, I understand that these audits and Audit 9175 (as explained below) are the only audits related to the DNAIQ issues.

Provide an explanation of the findings of audit 9175 and actions taken in response to that audit. When were the follow-up actions finalised?

45. As described above at Question 8, Audit 9175 was related to the external review undertaken by Professor Theo Sloots and Dr David Whiley. **Annexed and marked Exhibit TN-33** is the QIS record for this audit. The objective of this review was to review FSS' internal investigations into the cause of the contamination events.

46. As part of this audit, my role (along with Mr McNevin) was to brief Professors Sloots and Whiley about the nature of the contamination issues, the steps which had been taken by FSS to deal with the issues to date and provide them with any information or material they needed (including for example access to staff members and laboratories) to conduct the audit. I recall attending a meeting in-person at the laboratory with them on 12 November 2008 as part of this briefing process.

47. Professors Sloots and Dr Whiley finalised their report on 14 November 2008. A copy of this report is **Exhibit TN-30**.

48. An excerpt of their findings from the report is extracted below:

The procedures currently in place for the Off-deck Lysis and MPII extraction appeared to be adequate and specifically designed to prevent cross-contamination of test samples.

We agree with the Forensic Services Management team that the previous issue of possible cross-contamination of samples is most likely to related to the use of adhesive film in sealing deep well plates used in the off deck lysis procedure. The type of plate used, and the period of storage at reduced temperatures have in our experience caused similar problems in molecular diagnostics. The subsequent decision to change this procedure to the use of capped tubes has clearly solved the problem.

49. As outlined in the report, Professors Sloots and Whiley identified five items for further consideration. These items are outlined below.

Item 1*: Develop a standard validation protocol for each procedure based on the guidelines described by J Butler (www.promega.com; September 2006). Incorporate these into the Standard Operation procedures for the laboratory.*

Item 2: *We advise that the number of negative controls included in each batch of extractions be increased to comprise at least 10% of the total number of specimens tested. These controls should ideally be distributed randomly over the plate. Currently one negative control is included with 47 samples.*

Item 3: *Quality assessment might be increased by testing a control plate once every 3-4 weeks on each of the MultiPROBE II PLUS platforms. We would suggest alternating between the soccer ball, zebra and checkerboard formats.*

[redacted].....

Thomas Nurthen Witness

Item 4: *It was noted that the magnetic particles used for the nucleic acid extraction had a tendency to settle quickly, thereby blocking the filter tip and potentially producing a false-negative result. At the time of review this was not a problem as the attending operator was diligent in observing all stages of this process. We would like to reiterate however, that constant observation by the operator of all of the processes leading up to and including the addition of magnetic particle is necessary to ensure the failure of the robotic system does not occur.*

Item 5: *Finally, it was noted that the laboratory design allowed traffic from the amplification/post amplification area into the lysis/extraction areas. Presently this carries moderate contamination risk, as the amplification process is limited to 28 -32 cycles. However, if this protocol is changed in the future to detect low copy nucleic acid (greater than 32 cycles) the risk of carrying post-PCR product into the extraction area would be high, and work-flow dynamics must be carefully examined to minimise that risk. Likewise, sample cross-contamination during specimen handling and extraction processes will assume greater relevance when contemplating detection of low copy nucleic acid, and would necessitate stringent validation of all steps.*

50. After the findings were communicated and the external audit was logged in the system, I logged OQI#21718 on 15 December 2008 to internally document the items for further consideration as set out above into our systems. I do not recall if someone instructed me to do this. **Annexed and marked Exhibit TN-34** is OQI#21718 which was raised in response to the items.

51. I do not recall being directly involved in relation to the actioning of these items (at least until 14 February 2014 as explained below) and I do not believe that there was any agreement by the Management Team to fully action these items. I understand the items were for consideration only. The Management Team changed from time-to-time but I believe at this time it likely consisted of myself, Cathie Allen, Justin Howes, Paula Brisotto, Kirsten Scott, Amanda Reeves, Kylie Rika, Sharon Johnstone, Allan McNevin and Luke Ryan. The notes in the OQI record indicate that I was instructed by the Management Team to accept and approve the OQI in order for it to be closed on 14 February 2014 (as the creator of the OQI can only accept an OQI before it is approved).

52. I do not know what follow-up actions were taken in response to the items for further consideration (as identified in the external report) as I do not believe I was directly involved in these processes. However, based on my review of the records in my possession, the below steps appear to align with the items for consideration suggested by Professor Sloots and Dr Wiley:

(a) with respect to Item 1, the reference for Profiles in DNA September 2006 were incorporated into the standard operation procedure Validation (DNA Analysis) (QIS23401V2) on 26 September 2008;

(b) with respect to Item 2, I understand that from 2 October 2010, each off deck lysis batch of 48 contained 5 negative controls;

(c) with respect to Item 3, a number of anticontamination plates were run before the staggered re-implementation of the DNAIQ. After reimplementation, I do not know if this was tested and for what period;

(d) with respect to Item 4, I do not know if any actions were taken in response to this item; and

(e) with respect to Item 5, the Analytical section moved into a new laboratory in July 2010 to minimise traffic and air pressures.

[redacted].....

Thomas Nurthen Witness

Question 12 – Identify each staff member involved in detecting and responding to the problems with DNAIQ, and the nature of each person's involvement.

53. I do not recall the precise involvement of each staff member in detecting and responding to the problems with DNAIQ. However, generally speaking:

(a) the Management Team were responsible for the overall decision making in relation to addressing the DNAIQ issues;

(b) the Analytical Team were responsible for the day to day processing and re-processing of samples and provided feedback on practicalities of protocol changes;

(c) the Automation Team were responsible for the troubleshooting of the instrument protocols, subsequent re-optimisation and re-validation of instrument protocols.

54. Based on my review of the records in my possession, the following staff members likely had knowledge of, and assisted in detecting and responding to the DNAIQ problems:

(a) Greg Shaw, Executive Director;

(b) Vanessa Ientile, Managing Scientist;

(c) Cathie Allen, Team Leader/Managing Scientist;

(d) Allan McNevin, Senior Scientist Analytical;

(e) Iman Muharam, Internal Auditor QIS 8227/Automation Team Scientist/Senior Scientist Automation and LIMS implementation;

(f) Chiron Weber, Automation Team Scientist/Acting Automation Senior Scientist;

(g) Vojtech Hlinka, Automation Team Scientist;

(h) Breanna Gallagher, Automation Team Scientist;

(i) Generosa Lundie, Automation Team Scientist;

(j) Cecilia Iannuzzi, Automation Team Scientist;

(k) Alicia Quartermain, Reporting Scientist;

(l) Jacqui Wilson, Reporting Scientist;

(m) Shannon Merrrick, Reporting Scientist;

(n) Julie Connell, Reporting Scientist;

(o) Rhys Parry, Reporting Scientist;

(p) Angelina Keller, Reporting Scientist;

(q) Claire Gallagher (nee Perrin), Reporting Scientist;

(r) Ingrid Moeller, Reporting Scientist;

[redacted]

(s) Emma Caunt, Reporting Scientist;

(t) Paula Brisotto (nee Taylor), Team Leader Quality and Projects;

(u) Justin Howes, Team Leader Forensic Reporting and Intelligence Team;

(v) Amanda Reeves (nee Storer), Senior Scientist Reporting;

(w) Kylie Rika, Senior Scientist Reporting;

(x) Adrian Pippia, Senior Scientist Evidence Recovery;

(y) Robyn Smith, Quality and Project Senior Scientist;

(z) Amy Cheng, Analytical Scientist/Internal Auditor QIS 8227;

(aa) Peter Clausen, Snr Scientific Skills Development Co-ordinator/Internal Auditor QIS 8227;

(bb) Susan Brady, Reporting Scientist/Internal Auditor QIS 8752; and

(cc) Rebecca Gregory, Reporting Scientist/Internal Auditor QIS 8752.

Question 13 – Identify whether any issue or problem with respect to DNAIQ was audited by an external agency? If yes, when did that occur and in respect of what particular issue or issues.

55. Yes. The issues/problems concerning DNAIQ were externally audited by Professor Theo Sloots and Dr David Whiley in November 2008 as part of Audit 9175 referred to above at Question 11.

Who decided that should occur, provide: instructions, list of material and the report, including any draft report.

56. On 21 October 2008, I understand that Executive Director, Greg Shaw, requested the external audit (Refer to **Exhibit TN-27**). I am not aware of any more formal instructions, for example, a terms of reference,

57. I do not recall the list of material which the external auditors were briefed with. However, as I explained above at paragraph 46, I gave them a verbal briefing about the issues we had experienced to date and provided them with any information they needed to help their review (including, for example, access to staff members and laboratories). As mentioned in the email at **Exhibit TN-27**, Professor Theo asked Mr Shaw for copies of the Standard Operating Procedures.

58. The report is included at **Exhibit TN-30**. I am not aware of any draft report.

Question 14 – How were the results of the audit communicated to the DNA laboratory?

59. Based on my review of my records, the results of Audit 9175 were communicated to the DNA laboratory as follows:

[redacted]

(a) in a Biology Management Team meeting on 14 November 2008. The meeting minutes provide *'The auditors agreed with our actions taken and basic principles. A report will be issued from the external Auditors. Some recommendations are – Locked batches – CJA and Iman to explore this, Reagents to be tested at 35, Strip Cap seals for PCR Plates – Iman will source these, # of QC plates per month (i.e. checkerboard)'*. **Annexed and marked Exhibit TN-35** is a copy of the meeting minutes.

(b) An email from Justin Howes dated 21 November 2008 attaching the external report which says *'FYI-the external auditor's report. Cathie, for your distribution to the team, Greg commented it was complementary'*. This email was forwarded to me by Justin but I was not an original recipient of the email. **Annexed and marked Exhibit TN-36** is the email dated 21 November 2008.

60. It may be the case that there were other meetings and correspondence communicating the results of the audit however I have not found these records in my possession.

Question 15 – What permanent changes, or amendments to SOPs, were made as a result of identifying the problems related to DNAIQ?

61. I was not directly involved in making changes to the SOPs as a result of the DNAIQ issues. However, I may have had peripheral involvement. For example, I may have provided advice to other teams about these changes from a quality perspective.

62. Based on my review of records, I understand that the below changes were made to SOPs based on the amendment history recorded in the SOP and my recollection of the changes more generally:

(a) QIS#24897 DNAIQ Method of Extracting DNA from Casework and Reference Samples (from version 5). Based on the amendment history in the document, it says *'major changes to reflect new procedure. Update to reflect changes in procedure as an outcome of internal and external audits. Created version 6.4 ODLNP2 platforms. Minor changes in procedures using 4titude 4seal heat sealer to seal plates'*. **Annexed and marked Exhibit TN-37** is a copy of this SOP.

(b) QIS#17119 Procedure for the Release of Results; extraction batch checking & inclusion of quality paragraphs (from version 8). Based on the amendment history in the document, *'Added EB checking workflow, added to Quality Flag workflow, moved Quality paragraphs to own Appendix, deleted Pathology and Scientific services logo'*. **Annexed and marked Exhibit TN-38** is a copy of this SOP.

(c) QIS#30800 Adverse Event Standard Operating Procedure was amended to include how to deal with, document and investigate contamination. **Annexed and marked Exhibit TN-39** is a copy of this SOP.

(d) Extraction negative controls were also analysed below the reporting threshold (i.e. at the limit of detection threshold). I do not recall what SOP this was addressed in but it would have likely been an Analytical SOP.

[redacted]

Thomas Nurthen Witness

Question 16 – Explain what communications were made to external agencies, including the Queensland Police Service, the Office of the Director of Public Prosecutions, and the Queensland Courts, about the problems with DNAIQ and when the communications were made. Attach copies of any emails or letters sent to the external agencies.

63. Other than the communications I have referred to below, based on my review of the records, I am not aware of any other communications made to external agencies based on my review of the documentation within my possession.

64. In around July 2008, some members of Management Team (including Cathie Allen and Emma Caunt), Greg Shaw (Executive Director, FSS) met with the Queensland Police Service of the automated DNAIQ issues (as mentioned above at paragraph 38). I did not attend this meeting. **Annexed and marked Exhibit TN-40** is a copy of the agenda of this meeting.

65. On 18 December 2008, I received an email from Cathie Allen advising that Ross Martin, Special Counsel (Major Prosecutions), DPP would like to tour the lab and get clarification on the events. Cathie asked me to have the paperwork handy so she could go through it with Ross. **Annexed and marked Exhibit TN-41** is an email chain between Cathie Allen, Allan McNevin, Iman Muharam, Justin Howes, Paula Taylor and myself between 16 and 18 December 2008.

Question 17 – Did the problems with DNAIQ lead to the retraction or amendment of results in these cases? If yes, how many cases were affected? By what means were the amendment and retraction of results communicated?

66. I was not involved in any retraction or amended of results or statements. At this time, as mentioned above, I was a Senior Scientist in the Quality and Projects Team and issues of this nature were not within my team's remit. This was the remit of the Investigation Team (as referred to above in Question 8).

67. Based on my knowledge and understanding:

    (a) I do not know if there were any results that had to be retracted as a result of the events listed. However, based on the extent of the contamination, I consider that it would have been likely that results had to be retracted. I do not have any documents in my possession in relation to the retraction or amendment of results;

    (b) I do not have knowledge of what the exact number of cases or samples which were affected;

    (c) I do not how the communication of retracted results were communicated to the QPS and DPP; and

    (d) I understand that Statements of Witnesses issued by Reporting scientists had additional paragraphs added to the appendix to disclose affected cases based on QIS#17119 Procedure for the Release of Results as mentioned at Question 15 above.

[redacted]

Thomas Nurthen Witness

Question 18 – Has the DNA laboratory since returned to using DNAIQ processes, systems and/or products? Have there been any further problems with DNAIQ systems or products? Explain all future problems in detail, including what has been done in response to them. Attach any OQIs, Adverse Entry Log's or record of the problem being identified and investigated.

68. Once I moved into my current role in December 2013, I was no longer apart of any decision-making in relation to issues regarding the DNAIQ processes.

69. For this reason, there may be additional problems and/or actions which were taken in relation to DNAIQ systems or products which I am not aware of.

70. Based on my review of the records in my possession, there appears to be adverse entry logs related to contamination. For example, see entry on rows 18,49 (Events 16,46) in the LOG tab, Row 98 BATCH INVESTIGATIONS 2016_2017. I was not involved in investigating these events. **Annexed and marked Exhibit TN-42** is a spreadsheet of the current adverse event log as of 14 October 2022.

71. From the best of my recollection and based on my understanding of events occurring within the Forensic Analysis Laboratory:

    (a) The DNAIQ kit used on the MultiPROBE II instruments were retired from the laboratory in around 2016.

    (b) The DNAIQ kit was validated on the Promega Maxwell in around 2010 and on the Maxwell FSC instruments at a later date.

    (c) Different automated platforms (Qiagen QIAsymphony) replaced the MultiPROBE II instruments on 21 November 2016. I was not involved in this transition to these automated platforms.

All the facts and circumstances declared in my statement, are within my own knowledge and belief, except for the facts and circumstances declared from information only, and where applicable, my means of knowledge and sources of information are contained in this statement.

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

TAKEN AND DECLARED before me at Brisbane this 17th day of October 2022

[redacted]

Thomas Nurthen Witness

.....

Thomas Nurthen Witness

EXHIBITS INDEX

Exhibits Index – Thomas Nurthen Statement

Question	Exhibit	Document Title
5	TN-01	Promega DNA IQ™ System–Small Sample Casework Protocol
5	TN-02	FSS Standard Operating Procedure - Automated DNA IQ™ Method of Extracting DNA from Blood and Cell Substrates
5	TN-03	FSS Standard Operating Procedure - DNA IQ™ Method of Extracting DNA from Casework and Reference Samples
6	TN-04	OQI#19930 Report
6	TN-05	OQI#19349 Report
6	TN-06	OQI#19477 Report
6	TN-07	OQI#19767 Report
6	TN-08	OQI#19768 Report
7	TN-09	~~OQI#19330 Report~~ Collated Bundle of OQI Reports AC.
8	TN-10	Email from Allan McNevin to Management Team re Contamination of a reference sample - feedback please dated 18 April 2008 attaching draft Reference Mixture Investigation Report
8	TN-11	Report titled 'Testing of samples with different detergents in the extraction lysis buffer' by Dr Vojtech Hlinka dated 11 April 2008
8	TN-12	Report titled 'Investigation of gel like substance in extraction buffer' by Dr Vojtech Hlinka dated 11 April 2008
8	TN-13	Minutes of DNA Analysis Management Team meeting dated 14 July 2008
8	TN-14	Memorandum from Vanessa Ientile to DNA Analysis Laboratory dated 14 July 2008
8	TN-15	QIS Record of Audit 8227 DNA Extraction Process dated 15 July 2008
8	TN-16	Email from Allan McNevin to Management Team re Use of Extraction platforms dated 23 July 2008
8	TN-17	Minutes of DNA Analysis Management Team meeting dated 28 July 2008
8	TN-18	Email from Vanessa Ientile to all forensic biology staff re DNA IQ Extraction Update dated 28 July 2008
8	TN-19	QIS Record of Audit 8752 Audit of all extraction batches dated 28 July 2008
8	TN-20	Minutes of Management Team meeting dated 5 August 2008
8	TN-21	Final Audit Report - Audit 8227: Process Audit of the Automated DNA IQ™ System (including Off-Deck Lysis) dated 8 August 2008

[redacted]

Thomas Nurthen Witness

8	TN-22	Auditing Team Presentation re Audit 8227: Process Audit of the Automated DNA IQ™ System (including Off-Deck Lysis) dated 17 September 2008
8	TN-23	Attendance Record for Auditing Team Presentation re Audit 8227: Process Audit of the Automated DNA IQ™ System (including Off-Deck Lysis) dated 17 September 2008
8	TN-24	Auditing Team Presentation re Audit 8752: Extraction Batch Audit dated 17 September 2008
8	TN-25	Final Audit Report - Audit 8752: Extraction Batch Audit dated September 2008
8	TN-26	PerkinElmer response to Forensic Biology Team request for consideration of programming problems with extraction robotics dated 3 October 2008
8	TN-27	Email chain between Justin Howes, Allan McNevin, Cathie Allen, Paula Taylor and Wendy Harmer dated 21-23 October 2008
8	TN-28	Email from Emma Caunt to Management Team re Process for reporting IQ results dated 28 October 2008 attaching Guidelines for Reporting Results from DNAIQ Batches
8	TN-29	PerkinElmer letter to DNA Analysis (Forensic Biology) Team dated 6 November 2008
8	TN-30	Report – External Review of Operations by Theo Sloots and David Whiley dated 14 November 2008
8	TN-31	Report on the Investigation of contamination in the Automated DNA IQ Extraction protocol by Thomas Nurthen dated November 2008
11	TN-32	QIS record and report regarding Audit 9642
11	TN-33	QIS report for Audit 9175
11	TN-34	OQI#21718 Report
14	TN-35	Meeting minutes – Biology Management Team – dated 14 November 2008
14	TN-36	Email from Justin Howes dated 21 November 2008
15	TN-37	QIS#24897 DNAIQ Method of Extracting DNA from Casework and Reference Samples
15	TN-38	QIS#17119 Procedure for the Release of Results; extraction batch checking & inclusion of quality paragraphs
15	TN-39	QIS#30800 Adverse Event Standard Operating Procedure
16	TN-40	Agenda dated July 2008 regarding meeting with QPS
16	TN-41	Email from Cathie Allen dated 18 December 2008
18	TN-42	Adverse log spreadsheet dated 14 October 2022

[redacted]

Thomas Nurthen Witness

DNA IQ™ System–Small Sample Casework Protocol

Technical Bulletin No. 296

INSTRUCTIONS FOR USE OF PRODUCTS DC6700 and DC6701. *PLEASE DISCARD PREVIOUS VERSIONS.*
All technical literature is available on the Internet at www.promega.com
Please visit the web site to verify that you are using the most current version of this Technical Bulletin.

I. Description

DNA analysis is playing an increasingly large role in the identification of both humans and animals. With the advent of large multiplexes such as the PowerPlex® 16 System[a,b, c], the amplification and analysis steps have been streamlined. However, the purification of DNA from a variety of samples is still a rate-limiting step in obtaining useful genotypes.

Several procedures are currently used to purify DNA from samples absorbed to surfaces. Biological material must first be removed from these surfaces. This is typically done by soaking the material, which may result in inefficient recovery of small samples. The DNA must then be purified from inhibitors of PCR amplification and other components that may interfere with accurate quantitation methods.

Purification methods commonly used, such as phenol/chloroform extraction, use hazardous organic chemicals, require multiple centrifugations, may result in significant loss of material and can introduce amplification inhibitors. Chelex® extraction is rapid but can frequently leave amplification inhibitors. Purification on silica matrices uses the affinity of DNA for silica and does not require organic components. Silica filters are convenient when used with a filtration system but tend to give lower yields and require extensive washing to remove the guanidine-based lysis buffer. Currently available silica magnetic particles tend to give higher yield but also need extensive washing.

Promega's DNA IQ™ System[d] for small casework samples includes two steps. For biological material on solid supports, the first step provides an easy, rapid, efficient and almost universal stain extraction method. This step is unnecessary for liquid samples. The second step uses a specific paramagnetic resin that purifies the DNA without requiring extensive washing to remove the lysis reagent. This system is designed to rapidly purify small quantities of DNA, approximately 100ng or less, and becomes more efficient with samples containing less than 10ng of DNA.

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Promega

II. Product Components

Product	Size	Cat.#
DNA IQ™ System	400 samples	DC6700

For Laboratory Use. This system includes:

- 3ml Resin
- 150ml Lysis Buffer
- 70ml 2X Wash Buffer
- 50ml Elution Buffer
- 2 Protocols (Casework and Database)

Product	Size	Cat.#
DNA IQ™ System	100 samples	DC6701

For Laboratory Use. This system includes:

- 0.9ml Resin
- 40ml Lysis Buffer
- 30ml 2X Wash Buffer
- 15ml Elution Buffer
- 2 Protocols (Casework and Database)

Storage Conditions: Store all DNA IQ™ reagents at room temperature.

III. Sample Types Examined

DNA from the following sample types have been successfully purified at Promega or by external forensic laboratories. Due to the nature of casework samples, (i.e, the samples may have been exposed to environmental factors for long periods of time and the amount of biological material may be limiting) DNA yields may vary and DNA may not be obtained from all samples. Please see the most updated list at www.promega.com/applications/hmnid/productprofiles/dnaiq. Tissue masses including hair, bone, and sperm require a Proteinase K digestion to obtain reliable amounts of DNA. Contact Promega Technical Services (techserv@promega.com) for the latest information on available protocols.

Table 1. Types of Samples From Which DNA Has Been Successfully Isolated With the DNA IQ™ System.

Sample type	Promega	External	Comments
Fresh blood	Yes	Yes	Works with the following anti-clotting reagents: EDTA, citrate, heparin, ACD
Frozen blood	Yes	Yes	Old blood may produce lower yields
Bloodstains			
S&S903 paper	Yes	Yes	
FTA® paper	Yes	Yes	
Cotton	Yes	Yes	
Blue denim	Yes	Yes	
Black denim	Yes	Yes	
Soil	Yes		
Leather	Yes	Yes	
Surface to swab	Yes		
Buccal swabs			Fresh swabs can be soaked at room temperature prior to DNA isolation
Cotton	Yes	Yes	
Rayon	Yes		
CEP paper	Yes		

Sample type	Promega	External	Comments
Buccal Swabs (continued)			
Swab to FTA® paper		Yes	
Foam swab to paper		Yes	
Cigarette butt		Yes	Use paper wrapping; filter may form gel if heated
Toothbrush		Yes	Soak bristles in Lysis Buffer at 60°C for 30 minutes
Envelope		Yes	Soak in 0.5% SDS before adding 2 volumes of Lysis Buffer
Urine	Yes		Sample from bladder cancer patient

Samples Requiring a Proteinase K Digestion Prior to Addition of Twice the Recommend Volume of Lysis Buffer

Sample type	Promega	External	Comments
Tissue			
Fresh	Yes	Yes	See Technical Bulletin TB307 for the Tissue and Hair Extraction Kit (for Use With DNA IQ™) Cat.# DC6740
Formalin fixed	Yes	Yes	See Technical Bulletin TB307 for the Tissue and Hair Extraction Kit (for Use With DNA IQ™) Cat.# DC6740
Hair	Yes	Yes	See Technical Bulletin TB307 for the Tissue and Hair Extraction Kit (for Use With DNA IQ™) Cat.# DC6740
Bone*		Yes	From pulverized bone samples.
Antler*		Yes	From drill shavings
Differential extractions		Yes	See Section IV.D.

* Requires the Bone Incubation Buffer containing 1mg/ml proteinase K for DNA purification. Please contact Technical Services (techserv@promega.com) for a protocol for DNA isolation from bone samples.

IV. Protocol for the DNA IQ™ System

Materials to Be Supplied by the User

- 95–100% ethanol
- isopropyl alcohol
- 1M DTT
- 65°C heat block, water bath or thermal cycler
- 95°C heat block, water bath or thermal cycler (for stain or swab extraction)
- vortex mixer
- Microtubes, 1.5ml (Cat # V1231)
- DNA IQ™ Spin Baskets (Cat.# V1221)
- aerosol-resistant micropipette tips
- MagneSphere® Technology Magnetic Separation Stand (twelve-position) (Cat.# Z5342)
- Proteinase K Solution

Use of gloves and aerosol-resistant micropipette tips is highly recommended to prevent cross-contamination.

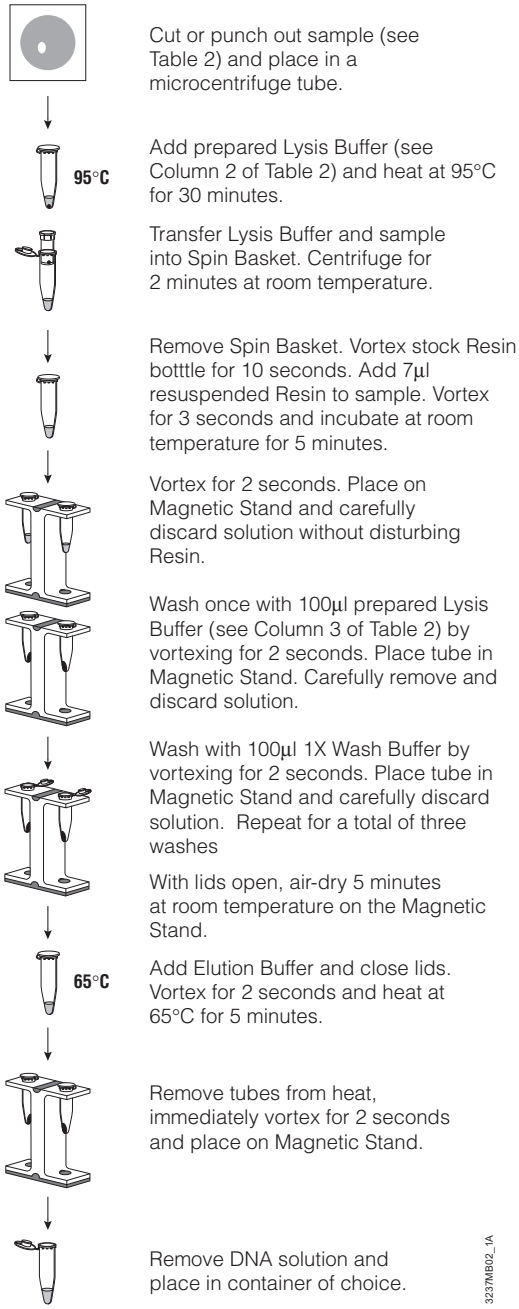

Figure 1. Schematic of DNA isolation from stains on solid material using the DNA IQ™ System. See Section IV.B for a detailed protocol.

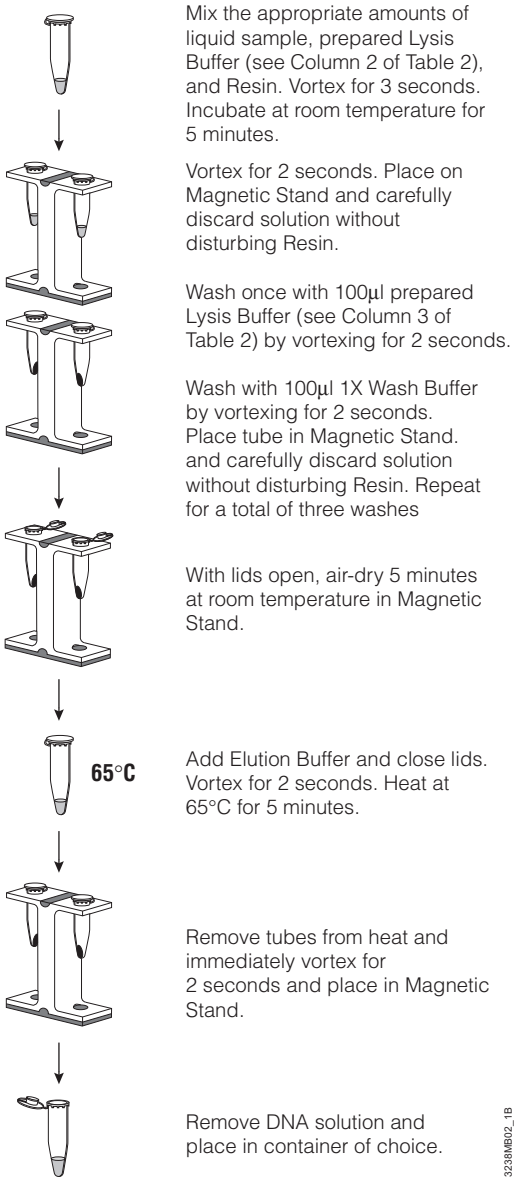

Figure 2. Schematic of DNA purification from liquid samples using the DNA IQ™ System. See Section IV.C for a detailed protocol.

Promega

A. Preparation of 1X Wash Buffer and Lysis Buffer

Note: If Lysis Buffer forms a precipitate, warm solution to 37–60°C.

Preparation of 1X Wash Buffer

1. For DC6700 (400 samples) add 35ml of 95–100% ethanol and 35ml of isopropyl alcohol to the 2X Wash Buffer.

   For DC6701 (100 samples) add 15ml of 95–100% ethanol and 15ml of isopropyl alcohol to the 2X Wash Buffer.

2. Replace cap and mix by inverting several times.

3. Mark label to record the addition of alcohols. Label bottle as 1X Wash Buffer. Solution can be stored at room temperature. Make sure bottle is closed tightly to prevent evaporation.

Preparation of Lysis Buffer

1. Determine the total amount of Lysis Buffer to be used (see Table 2) and add 1µl of 1M DTT for every 100µl of Lysis Solution.

   Table 2. Amount of Lysis Buffer Required Per Sample.

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
15–50mm^2 S&S 903 paper	150µl	100µl	250µl
3–30mm^2 FTA® paper	150µl	100µl	250µl
Cloth up to 25mm^2	150µl	100µl	250µl

   [1]For use in Section IV.B, Step 2 or Section IV.C, Step 1
   [2]For use in Section IV.B, Step 9 or Section IV.C, Step 7.

2. Mix by inverting several times.

3. Mark and date label to record the addition of DTT. This solution can be stored at room temperature for up to a month if sealed.

B. DNA Isolation from Stains on Solid Material (Figure 1)

1. Place sample in a 1.5ml microcentrifuge tube (e.g. Microtubes, 1.5ml, Cat # V1231). The recommended amount of Resin can capture a maximum of approximately 100ng DNA.

Note: Additional Lysis Buffer may be used in order to cover entire sample.

2. Add the appropriate amount of prepared Lysis Buffer. Different samples require different volumes of Lysis Buffer; see Column 2 of Table 2 for the appropriate volume to add at this point. Close the lid and place tube in a heat block at 95°C for 30 minutes.

   Exceptions:

   a. Heat-sensitive fabrics (e.g, polyester and nylon): Extract without heating.
   b. Leather: Lysis Buffer extraction with or without heat may not work on some leathers. Extract in a small volume of aqueous buffer (100–200µl), then add 2 volumes of Lysis Buffer after removing matrix.

   Note: For small stains, an alternative approach is to place the stained material in a DNA IQ™ Spin Basket (Cat.# V1221) seated in a 1.5ml Microtube (e.g, Promega Cat. #V1231) Add 100–150µl of prepared Lysis Buffer to the basket. Carefully close the lid and heat at 95°C for 30 minutes. Most of the Buffer should remain in the basket if the indicated tubes and Spin Baskets are used. Proceed to Step 4.

3. Remove the tube from the heat block and transfer the Lysis Buffer and sample to a DNA IQ™ Spin Basket.
4. Centrifuge at room temperature for 2 minutes at maximum speed. Remove the Spin Basket.
5. Vortex the stock Resin bottle for 10 seconds at high speed or until resin is thoroughly mixed. Add 7µl of Resin to the sample. Keep the Resin resuspended while dispensing to obtain uniform results.
6. Vortex sample/Lysis Buffer/Resin mix for 3 seconds at high speed. Incubate at room temperature for 5 minutes.
7. Vortex for 2 seconds at high speed and place tube in the Magnetic 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 Magnetic Stand and vortex for 2 seconds at high speed.
10. Return tube to the Magnetic Stand and discard all Lysis Buffer.
11. Add 100µl of prepared 1X Wash Buffer. Remove tube from the Magnetic Stand and vortex for 2 seconds at high speed.
12. Return tube to the Magnetic Stand and discard all Wash Buffer.
13. Repeat Steps 11 and 12 two more times for a total of 3 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 Magnetic Stand for 5 minutes.
15. Add 25–100µl of Elution Buffer, depending on how much biological material was used. Lower elution volume ensures a higher final concentration of DNA.
16. Close the lid, vortex the tube for 2 seconds at high speed and place it at 65°C for 5 minutes.
17. Remove the tube from the heat block and vortex for 2 seconds at high speed. Immediately place on the Magnetic Stand.
18. Transfer the solution to a container of choice.

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

Note: It is important to centrifuge Lysis Buffer with the stained matrix to obtain maximum recovery.

Note: If some Resin is drawn up in tip, gently expel resin back into tube to allow reseparation.

Do not dry for more than 20 minutes, as this may inhibit removal of DNA.

Tubes must remain hot until placed in the Magnetic Stand or yield will decrease.

C. DNA Isolation from Liquid Samples (Figure 2)

1. Prepare a stock solution of Resin and Lysis Buffer by using the ratio of 7µl of Resin to 93µl of prepared Lysis Buffer per sample (make extra to allow for losses during pipetting). The following equation will help determine the exact volumes to be made. Vortex the Resin container for 10 seconds at high speed or until Resin is thoroughly mixed.

   (Number of samples + 1) _____ × 7µl = _____ µl Resin

   (Number of samples + 1) _____ × 93µl = _____ µl prepared Lysis Buffer

2. Mix liquid sample gently and place an aliquot up to 40µl into a 1.5ml microcentrifuge tube. The amount of Resin recommended can capture a maximum of approximately 100ng of DNA.

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3. Vortex Resin/Lysis Buffer mixture for 3 seconds at high speed to ensure suspension of Resin and add 100µl of the solution to the tube containing theliquid sample. Resin/Lysis Buffer mixture should be mixed again if Resin begins to settle while dispensing aliquots.
4. Vortex the sample/Lysis Buffer/Resin mix for 3 seconds at high speed. Incubate 5 minutes at room temperature.

Note: If Resin does not form a distinct pellet on the side of the tube, vortex the tube and quickly place back in the stand.

5. Vortex for 2 seconds at high speed. Place tube in the Magnetic Stand. Separation will occur instantly.
6. Carefully remove and dispose of all solution without disturbing the resin on the side of the tube.
7. Add 100µl of prepared Lysis Buffer. Remove tube from the Magnetic Stand and vortex for 2 seconds at high speed.
8. Return tube to the Magnetic Stand, and remove and discard all Lysis Buffer.
9. Add 100µl of prepared 1X Wash Buffer. Remove tube from the Magnetic Stand and vortex for 2 seconds at high speed.
10. Return tube to the Magnetic Stand. Dispose of all Wash Buffer.
11. Repeat Steps 9 and 10 two more times for a total of 3 washes, making sure all of the solution has been removed after the last wash.

Do not dry for more than 20 minutes, as this may inhibit removal of DNA.

12. With lid open, air-dry the resin in the Magnetic Stand for 5 minutes.
13. Add 25–100µl of Elution Buffer, depending on how much biological material was used. Lower elution volume ensures a higher final concentration of DNA.
14. Close the lid, vortex tube for 2 seconds at high speed. Place at 65°C for 5 minutes.
15. Remove the tube from the heat block and vortex for 2 seconds at high speed. Immediately place on the Magnetic Stand.
16. Transfer the solution to a container of choice.

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

D. Processing of Sperm-containing Samples

The following approach describes DNA purification from differentially extracted samples of sperm and non-sperm fractions using the DNA IQ™ System. This approach has been successfully performed in a forensic laboratory setting (1). The sample of interest is first processed using the laboratory's validated differential extraction protocol (2), including the extraction of biological material from the solid support, proteinase K digestion in the absence of DTT and the pelleting and washing of the sperm cells. The following steps are to be followed once the sperm and non-sperm fractions have been separated.

Note: There is no need to digest the sperm pellet with proteinase K and DTT as the prepared DNA IQ™ Lysis Buffer in Step 1 will effectively disrupt the sperm cells after the initial proteinase K digestion.

1. Add at least 2 volumes (minimum 100µl) of prepared Lysis Buffer and 7µl of Resin to the sperm pellet.
2. Vortex 3 seconds on high and incubate at room temperature for 5 minutes.
3. Proceed to Steps 5–16 of Section IV.C to purify DNA from this sperm fraction.

4. Add two volumes of prepared Lysis Buffer and 7µl of Resin to the non-sperm fraction.

   Note: If desired, 100µl of a 500µl extraction can be processed. This amount typically gives sufficient DNA for analysis.

5. Vortex 3 seconds on high and incubate at room temperature for 5 minutes.

6. Proceed to Steps 5–16 of Section IV.C to purify DNA from this non-sperm fraction.

V. Troubleshooting

Symptoms	Possible Causes	Comments
Poor yield	Too much sample	The resin has capacity of about 100ng and begins to lose efficiency above 50ng (1–2µl blood). Use less sample or more resin.
	Poor extraction	After heating stain in Lysis Buffer, centrifuge Buffer with matrix to ensure enough liquid is present to wash out the DNA.
Poor Resin "pellet" formed	Resin settled before tube placed in Magnetic Stand	Samples should be placed in the Magnetic Stand immediately after vortexing/mixing. Repeat vortexing/mixing and place back in stand.
	Excessive input material relative to recommended volumes of reagents	Use less initial sample. Consult protocols for recommended quantities of initial sample.
		A proportional increase in Resin will allow DNA capture from more initial sample. **Note:** Yield increase will be roughly proportional to increase in Resin.
Coloration in final wash or eluted solution (may affect results in down-stream assays)	Insufficient washing	Remove all fluid during washes.
		Ensure a distinct Resin pellet is formed during all washes.
		Use less initial sample. Additional washes with 1X Wash Buffer can be performed.
Resin present in final eluted solution (may affect results in down-stream assays)	Resin is occasionally transferred by rapid pipetting or is caught in the meniscus of the final eluant	Vortex/Mix eluted solution, place in Magnetic Stand and transfer eluant to new tube.
Inconsistent yield (may affect results from downstream assays)	Inconsistent amounts of Resin	Vortex/Mix Resin stock just before making aliquots. Repeat vortexing/mixing Resin/Lysis Buffer while making aliquots.
	Nonhuman contaminating DNA in the initial sample may decrease yield in later human-specific assays	The DNA IQ™ System captures "total DNA", including both single- and double-stranded DNA.

For questions not addressed here, please contact your local Promega Branch Office or distributor. Contact information available at: **www.promega.com**.

E-mail: **techserv@promega.com**

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VI. References

1. Greenspoon, S. and Ban, J. (2002) Robotic extraction of mock sexual assault samples using the Biomek® 2000 and the DNA IQ™ System. Profiles in DNA **5**, 3-5
2. Gill, P. et al., (1985) Forensic application of DNA 'fingerprints'. Nature **318**, 577-9

VII. Composition of Buffers and Solutions

Elution Buffer

10mM Tris (pH 8.0)
0.1mM EDTA

Bone Incubation Buffer

10mM Tris (pH 8.0)
100mM NaCl
50mM EDTA
0.5% SDS

VIII. Related Products

Product	Size	Cat.#
MagneSphere® Technology Magnetic Separation Stand (two-position)	1.5ml	Z5332
MagneSphere® Technology Magnetic Separation Stand (twelve-position)	1.5ml	Z5342
PolyATtract® System 1000 Magnetic Separation Stand	1 each	Z5410
DNA IQ™ Spin Baskets*	1,000/pk	V1221
Microtubes, 1.5ml	1,000/pk	V1231
ART® 20P, Pipet Tip, 20µl	960/pk	DY1071
ART® 200, Pipet Tip, 200µl	960/pk	DY1121
ART® 1000, Pipet Tip, 1,000µl	768/pk	DY1131
AluQuant™ Human DNA Quantitation System (d)*	80 determinations	DC1010
	400 determinations	DC1011
PowerPlex® 16 System(a,b, c)*	400 reactions	DC6530
	100 reactions	DC6531
PowerPlex® 1.1 and 2.1 Systems(a,b)*	400 reactions	DC6500
	100 reactions	DC6501
PowerPlex® 1.2 System(a,b)*	400 reactions	DC6100
	100 reactions	DC6101
Tissue and Hair Extraction Kit (for use with DNA IQ™)	100 reactions	DC6740

*Not For Medical Diagnostic Use.

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[a]U.S. Pat. Nos. 5,843,660 and 6,221,598, Australian Pat. No. 724531, and other patents pending.

[b]STR loci are the subject of U.S. Pat. No. 5,766,847 and German Pat. No. DE 38 34 636 C2, issued to the Max-Planck-Gesellschaft zur Förderung der Wissenschaften, eV, Germany. Exclusive rights have been licensed to Promega Corporation for uses in human clinical research and diagnostics applications and all forms of human genetic identity. The development and use of STR loci are covered by U.S. Pat. No. 5,364,759 assigned to Baylor College of Medicine, Houston, Texas. Rights have been licensed to Promega Corporation for all applications.

U.S. Pat. No. 5,599,666 has been issued to Promega Corporation for allelic ladders for the loci CSF1PO, F13A01, FESFPS, LPL and vWA. U.S. Pat. No. 5,674,686 has been issued to Promega Corporation for allelic ladders for the locus CSF1PO and the combination of allelic ladders for the loci CSF1PO, FESFPS and TH01. U.S. Pat. No. 5,783,406 has been issued to Promega Corporation for allelic ladders for the locus CSF1PO. U.S. Pat. No. 6,156,512 has been issued to Promega Corporation for allelic ladders for the loci D16S539, D7S820, D13S317 and D5S818.

Use of Promega's STR Systems requires performance of the polymerase chain reaction (PCR), which is the subject of European Pat. Nos. 201,184 and 200,362, and U.S. Pat. Nos. 4,683,195, 4,965,188 and 4,683,202 owned by Hoffmann-La Roche. Purchase of Promega's STR Systems does not include or provide a license with respect to these patents or any other PCR-related patent owned by Hoffmann-La Roche or others. Users of Promega's STR Systems may, therefore, be required to obtain a patent license, depending on the country in which the systems are used. For more specific information on obtaining a PCR license, please contact Hoffmann-La Roche.

[c]U.S Pat. No. 6,238,863 and other patents pending.

[d]Patent Pending.

All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

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

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

MultiPROBE® II HT EX Plus with Gripper™ Integration Platform

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

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

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

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

4 REAGENTS AND EQUIPMENT

4.1 Reagents

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

Table 1. Reagent storage locations.

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

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

Table 2. Table of reagent volumes.

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

Extraction Buffer

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

Lysis Buffer with DTT

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

DNA IQ™ Resin

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

1X Wash buffer

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

4.2 Equipment

Table 3. Equipment used and location.

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

5 SAFETY

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

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

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

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

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

6 SAMPLING AND SAMPLE PREPARATION

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

Table 4. Sample storage locations.

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

QC samples

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

Table 5. Extraction Quality Controls

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

Registration of QC

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

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

Create the Extraction Batch

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

Locating Samples

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

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

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

7 PROCEDURE

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

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

Competent Analytical Section staff members perform all the following steps.

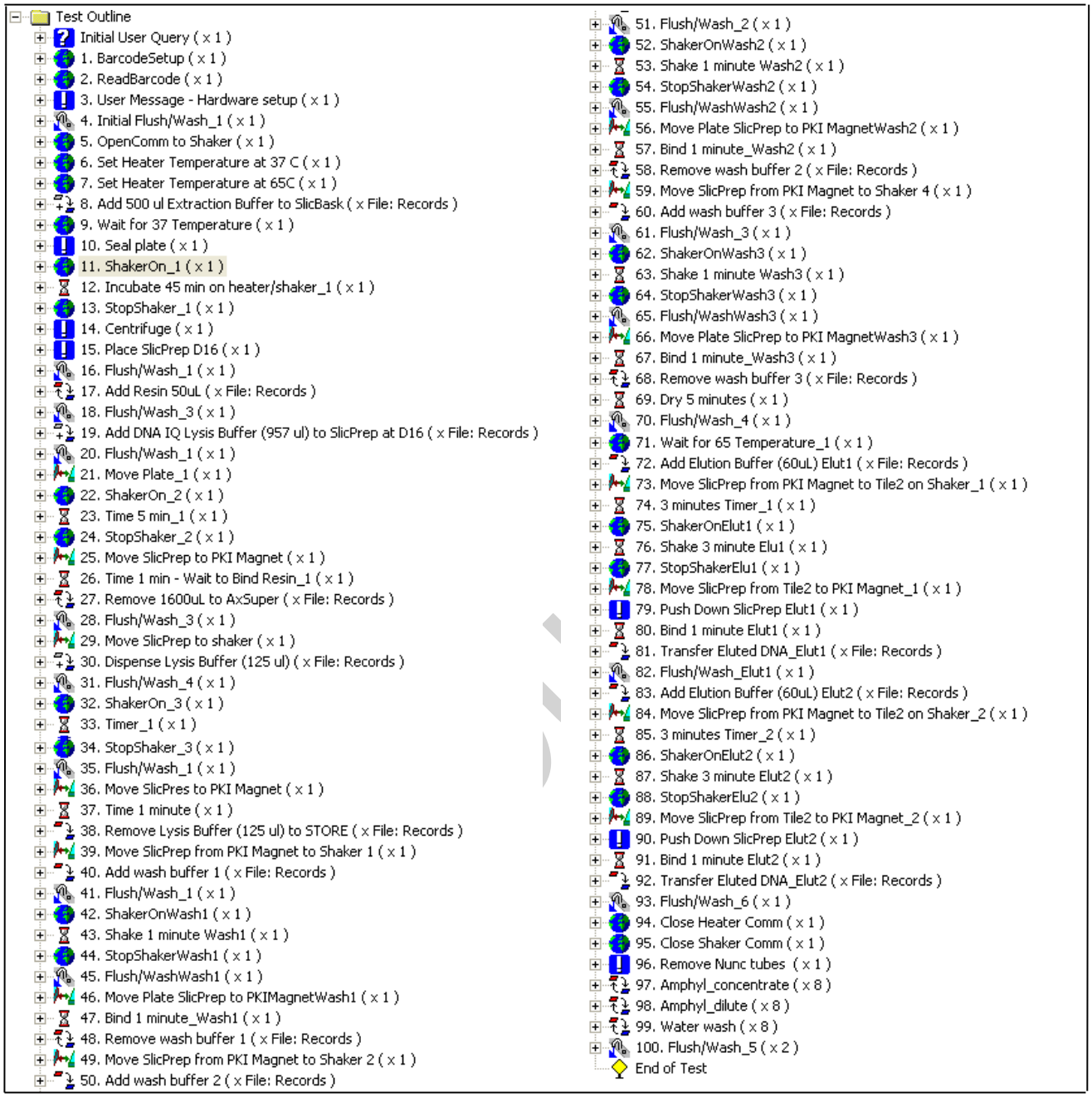


Figure 1. The Test Online of the program **DNA IQ Extraction_Ver1.1**.

Setting up the EP-A or EP-B MPIIs

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

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

Figure 2 The WinPrep® icon.

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

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

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

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

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

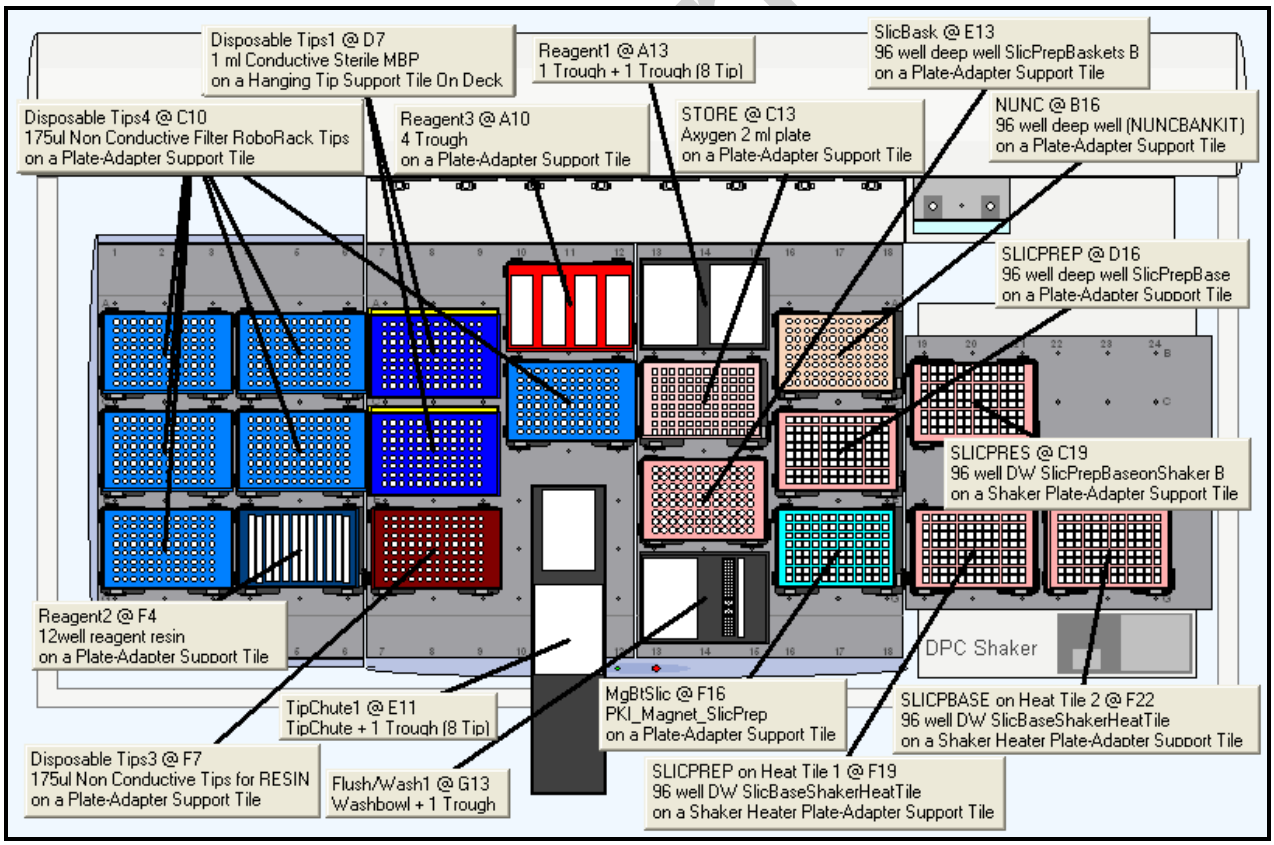


Figure 3. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

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

10. Referring to the table of reagent volumes (table 2), use the volumes of TNE, SDS and Prot K to make up the required amount of Extraction Buffer. Pour the required amounts of Extraction Buffer and Lysis Buffer (with DTT) into the labelled 150mL reagent troughs, using the reagent volume table as a guide to the volumes. Ensure that full PPE is worn, including face shield when handling these reagents.

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

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

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

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

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

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

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

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

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

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

21. Once the extraction buffer has been added to the plate, a user prompt will appear requesting the following:
    "Cover Slicprep with the Aluminium sealing film, then place in position F19. Press OK."
    Once the Slicprep™ 96 device has been covered with an aluminium seal and been placed onto the deck at the correct position, click OK on the user message.

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

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

24. After the first elute where the plate has been heated to 65^0C and moved to the PKI Magnet, a User message (step 79) will appear requesting:
    "**Push down the Slicprep on the PKI Magnet then press OK**."
    Allow to the plate to cool slightly before pushing down on all four corners of the plate to lock it into place on the PKI Magnet. Once it is firmly in place, click OK to continue. After the second elute, the prompt will appear again. Repeat the steps.

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

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

Recording Reagent Details in AUSLAB

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

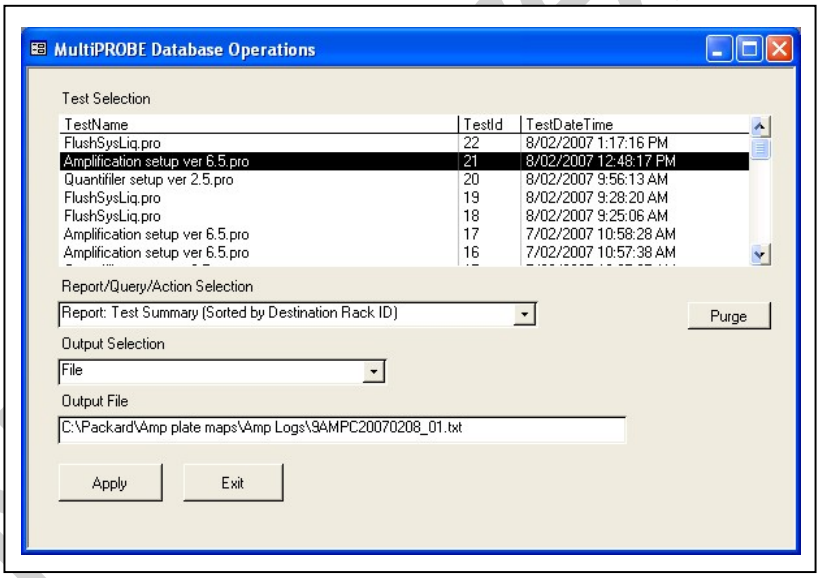
Finalising the MP II run

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

Importing the MP II log file into AUSLAB

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

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



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

Importing Extraction "Results" into AUSLAB

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

8 SAMPLE STORAGE

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

9 VALIDATION

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

10 QUALITY ASSURANCE/ACCEPTANCE CRITERIA

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

11 REFERENCES

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

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

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

MultiPROBE® II HT EX PLUS with Gripper™ Integration Platform

Within DNA Analysis, routine DNA extractions are performed using either one of two MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms (Extraction Platform A, EP-A) and (Extraction Platform B, EP-B) perform casework or reference samples.
Each platform uses a computer – controlled Cartesian X-Y-Z liquid handling system designed for the efficient automation of sample preparation. Liquid transfer is performed by an 8-tip System with VersaTip® and VariSpan™ options. The VersaTip® option allows the use of fixed and/or disposable tips (both clear and conductive). The VariSpan™ option

permits variable probe spacing between each of the individual probes so that a wide range of labware such as micro plates, tubes, vials and reagent troughs can be accessed. Each sample probe is capable of independent motion in the Z direction due to independent Z drives.

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

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

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

4 REAGENTS AND EQUIPMENT

4.1 Reagents

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

Table 1. Reagent storage locations.

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

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

Table 2. Table of reagent volumes.

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

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

Extraction Buffer

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

Lysis Buffer with DTT

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

DNA IQ™ Resin

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

1X Wash Buffer

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

4.2 Equipment

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

Table 3. Equipment used and location.

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

Table 4. Consumables used for extraction

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

5 SAFETY

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

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

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

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

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

6 SAMPLING AND SAMPLE PREPARATION

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

Table 5. Sample storage locations.

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

QC samples

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

Table 6. Extraction Quality Controls

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

Registration of QC

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

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

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

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

1. Log into the **AUSLAB Main Menu**.
2. Select **5. Workflow management**.
3. Select **1. DNA workflow table**.
4. Highlight the appropriate batch type and press **[F5]** ***Batch Allocation***.
5. Press **[F6]** ***Create batch***.
6. Press **[F8]** ***Print menu***.

7. Press **[F7]** ***Print Sample Label***. (print 3 sets)
8. Press **[F8]** ***Print Worksheet***. (print 2 copies)
9. Press **[SF5]** ***Main menu***.
10. Press **[SF11]** ***Print***.
11. Press **[SF6]** ***Accept batch***.
12. Press **[Pause/Break]** to exit to the **Main Menu**.
13. Obtain worksheets **(FBLASER3)** and labels **(FBLABEL13-16)** from the Analytical Section printing bench **(Room 6117)**.

Locating Samples

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

7 OFF-DECK LYSIS PROCEDURE (NO RETAINED SUPERNATANT)

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

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

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

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

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

Note: substrates from each sample need to be retained

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

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

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

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

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

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

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

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

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

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

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

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

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

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

18. Complete batch in AUSLAB.

.

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

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

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

8 OFF-DECK LYSIS PROCEDURE (RETAINED SUPERNATANT)

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

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

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

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

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

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

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

8. Incubate at room temperature for 30 minutes.

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

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

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

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

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

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

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

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

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

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

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

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

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

22. Complete batch in AUSLAB.

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

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

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

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

9 MPII EXTRACTION PROCEDURE

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

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

Summary of DNA IQ EXTRACTION Version 2 ODL

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

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

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

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

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

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

Preparation of Reagents prior to extraction

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

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

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

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

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

Setting up the EP-A or EP-B MPIIs

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

3. Turn on the instrument PC.

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

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

Figure 1 The WinPrep® icon.

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

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

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

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

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

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

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

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

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

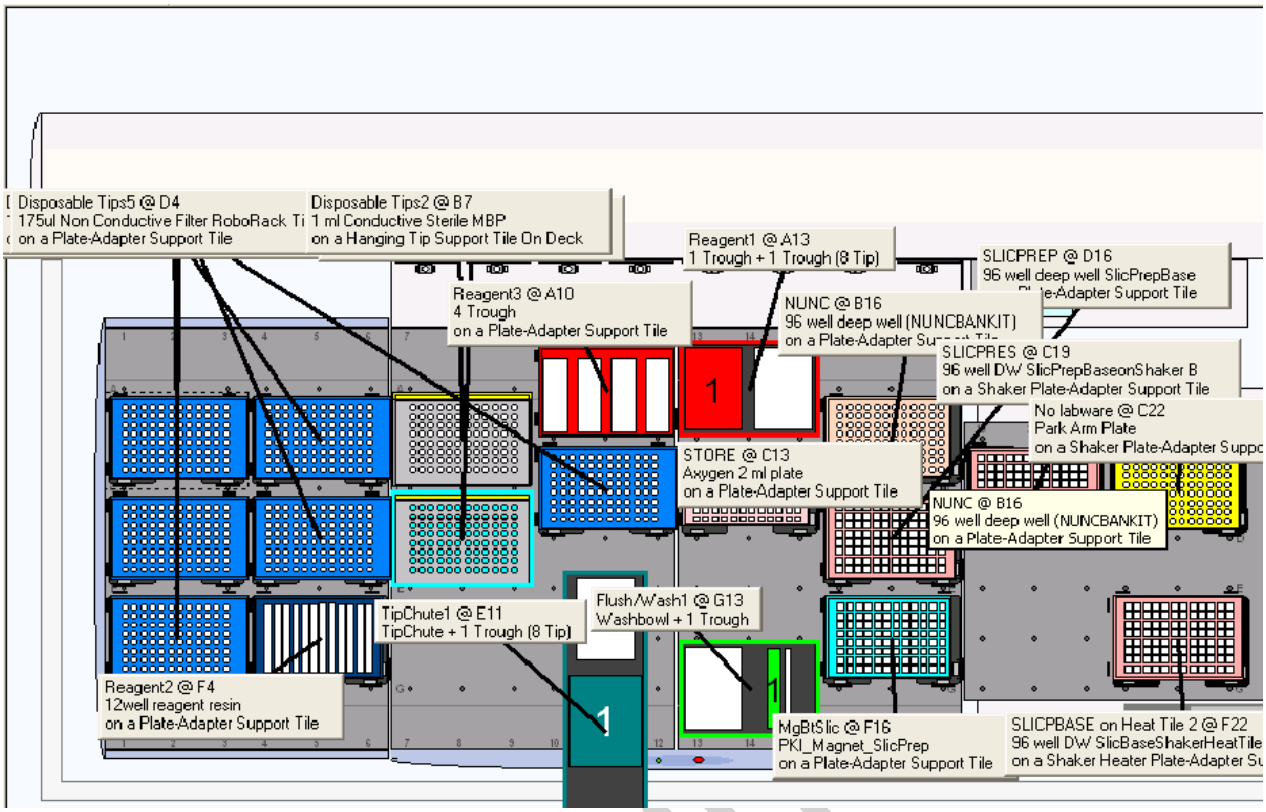

Figure 2. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

13. Ensure that the DPC Shaker and Heater Controller Box are switched on.
    For EP-A: Tile 1 at F22 (85^{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):

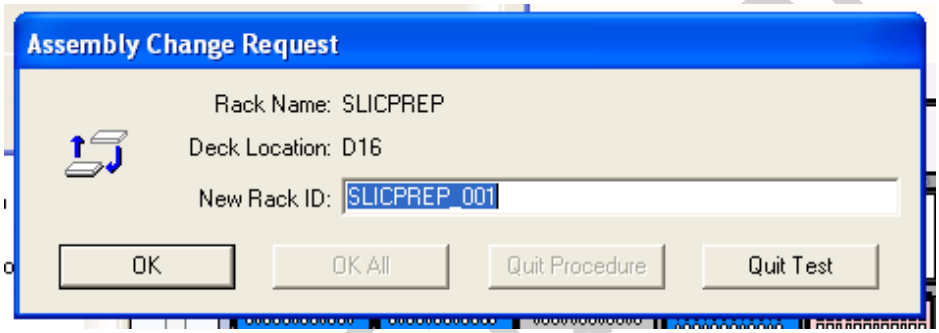

Figure 3. Scan batch ID request

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

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

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

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

Finalising the MP II run

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

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

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

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

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

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

Recording Reagent Details and other information in AUSLAB

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

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

Importing the MP II log file into AUSLAB

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

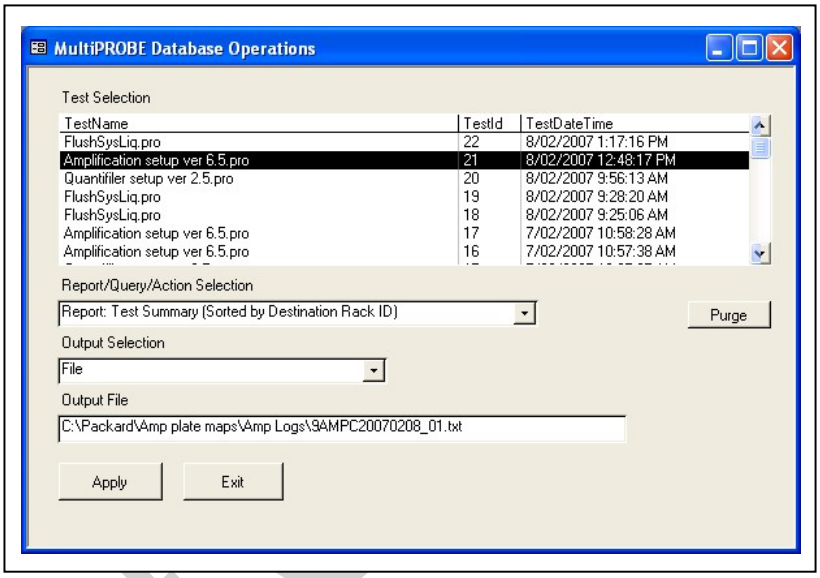

Figure 4. The MultiPROBE log database for collecting MP II run information

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

Importing Extraction "Results" into AUSLAB

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

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

10 SAMPLE STORAGE

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

11 TROUBLESHOOTING

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

Start with the Slicprep at D16.

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

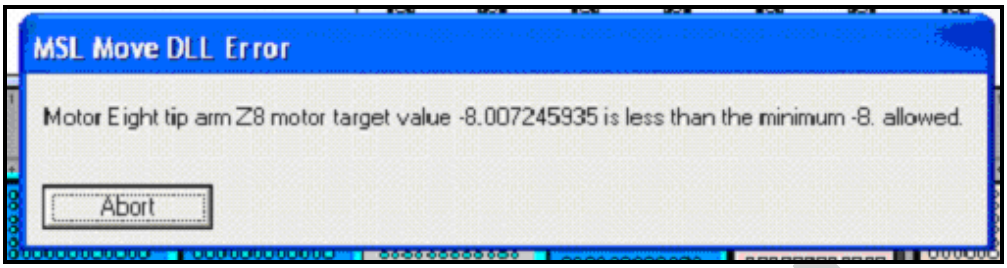

Figure 5. Example of DLL error

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

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

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

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

9. If the message Figure 6 below has appeared:

Figure 6. Example of error

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

12 VALIDATION

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

13 QUALITY ASSURANCE/ACCEPTANCE CRITERIA

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

14 REFERENCES

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.

Figure 7 The WinPrep® icon.

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"**.

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

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

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

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

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

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

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

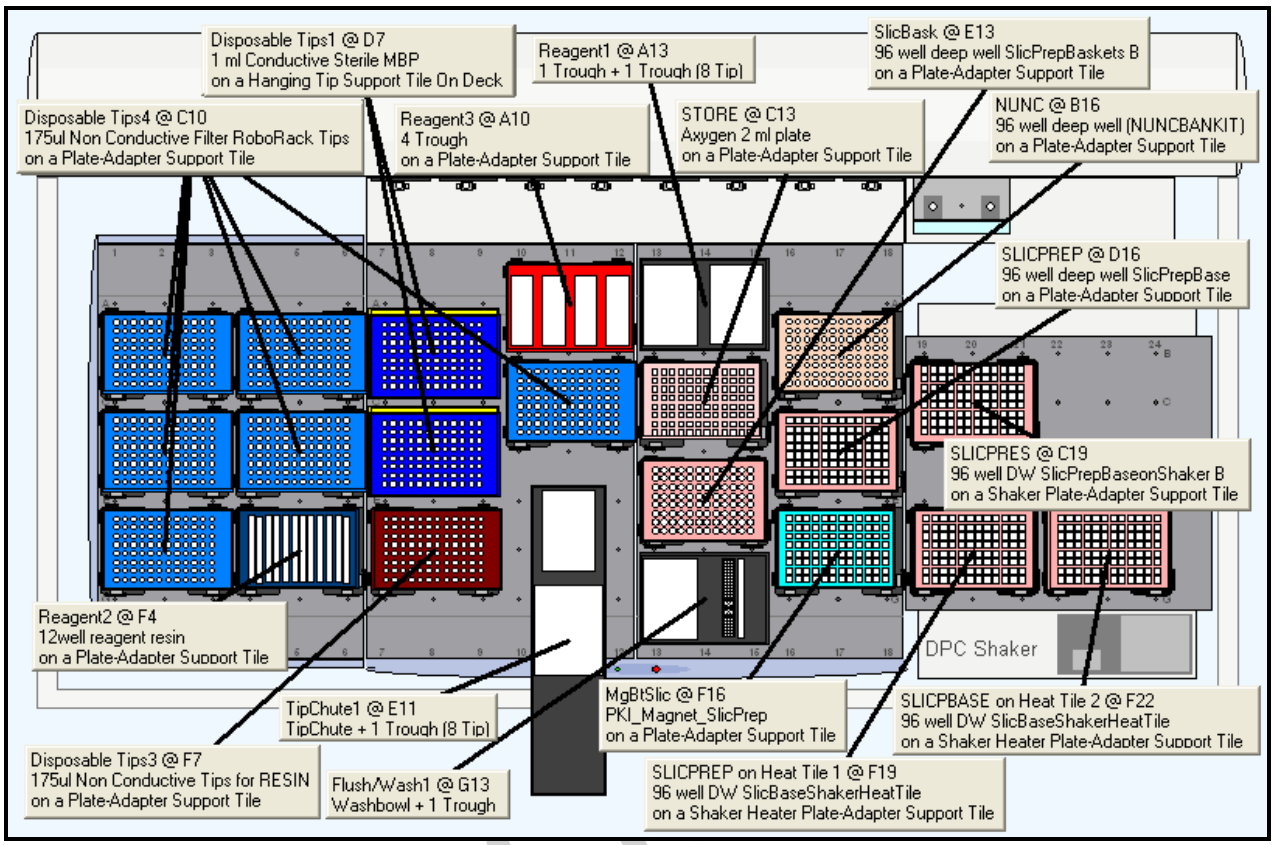

Figure 8. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Finalising the MP II run

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

Recording Reagent Details and other information in AUSLAB

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

Importing the MP II log file into AUSLAB

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

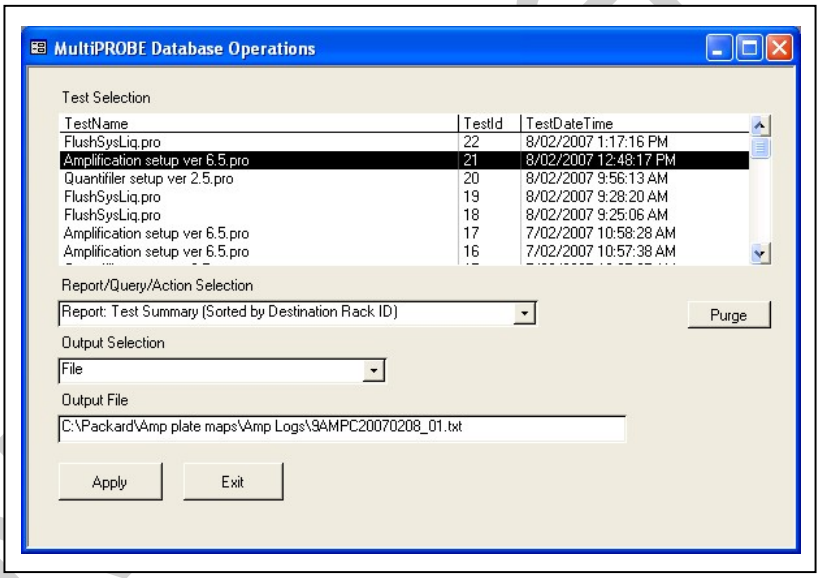

Figure 9. The MultiPROBE log database for collecting MP II run information

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

Importing Extraction "Results" into AUSLAB

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

18.3.3 Sample Storage

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

18.4 Manual method for extraction using DNA IQ™

18.4.1 Sampling and Sample Preparation

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

Table 9. Sample storage locations.

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

QC samples

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

Table 10. Extraction Quality Controls

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

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

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

Create the Extraction Batch

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

Locating Samples

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

When all samples have been located:

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

Sequence Check the tubes

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

18.4.2 Procedure

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

18.4.3 Sample storage

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

TN-04

Report for QIS OQI as of 29/09/2022 12:13:51 PM

Report for QIS OQI -

19330 No Title Provided

OQI Details

Status	Closed Approved
Subject	FTA Evidence sample [redacted] was partial profile after initial processing, when extracted displayed a mixed DNA profile, upon re-extraction a single source profile was observed consistent with the initial profile
Source of OQI	Suggestion
Date Identified	21/04/2008

OQI Creator Contact Details

Creator	Allan MCNEVIN
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigator/Actioner Contact Details

Actioner	Allan MCNEVIN
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigation Details

Investigation Completed	21/04/2008 **Root Cause Type** | Procedure/Method/Process
Investigation Details	During the reading of Genotyper batch GEN9REF20080211_05, it was noted that lab number [redacted] showed a partial mixed DNA profile. The DNA extract of [redacted] was then re-amplified and a mixture was confirmed. At the same time, previous run results for 333993604 were reviewed. It was shown that 333993604 had been processed through the FTA punching process and given a single source profile (Genotyper batch GEN9REF20080128_02). The result from GEN9REF20080211_05 resulted from an extraction of the FTA sample. The FTA sample 333993604 was then re-extracted under barcode 333802806 and yielded a single source profile consistent with one of the mixture contributors and the original FTA processing profile obtained from 333993604.The original profile obtained from FTA processing for sample 333993604 (Genotyper batch GEN9REF20080128_02) was then analysed at lowered peak height RFU thresholds, with no evidence of a mixture present. Re-analysis of original FTA & results obtained after re-extraction (barcode 333802806) using lowered peak height RFU thresholds showed no evidence of a mixture.

	Therefore the mixture was confined to the first extraction of this sample. The second contributor to the mixed DNA profile was then worked out and searched against the staff database. No matches were found. When searched against other profiles obtained from the same extraction batch (FTAEXT20080205_01) a match was found with sample 333941730. As both FTA samples ([redacted]) were extracted, quantified and amplified on the same batches, further investigation was carried out. Due to the vagaries of pipetting order and the particular DNA concentrations of the samples in question, the contamination of one sample with another must have occured prior to the addition of DNA extracts to the initial amplification reaction. In order to determine which step in the process (BSD punching, MPII DNA IQ extraction, re-capping and decapping of extracts at quantification stage, MPII preparation of Quant and decapping of extracts prior to amplification or at any of the workflow handling - storage, removal and re-storage of the extracts) is more likely to be when contamination has occurred, a consideration of how much of DNA extract [redacted] would be needed to transferred into [redacted] to cause the level of contamination that was visualised. With the help of senior scientist Justin Howes, the separation of mixture components and an assessment of the mixture ratio was made. The approximate mixture ratio is 1:1, or at the most conservative value (i.e. the least amount of DNA extract 3[redacted]), 2:1 where the mixture would consist of twice the amount of DNA from 333993604 as from 333941730. The DNA extract 333941730 was quantified at 1.71ng/ul, this is approximately 20x the concentration [redacted]. Given that the method of dual elution that is done with the routine method of DNA IQ extraction performed at FSS DNA Analysis yields a final extract volume of 100?L, to display a mixture ration somewhere between 1:1 and 2:1, approximately 2.5-5?L of DNA extract 333941730 would have to have been transferred to DNA extract [redacted]. This is a very unlikely scenario, especially if considering droplet or aerosol formation. Therefore the determination of exactly where in the process the contamination occured is not possible as each scenario appears to be as unlikely as the next.
Preformed By	Allan MCNEVIN

Action Details

Action Complete	21/04/2008	**Action Fix Type**	Resources
Title	AUSLAB specimen		
Action Description	notes and batch audit entries were made during the investigation phase. The issue was discussed at an Analytical team meeting on two occasions. A written report outlining the investigation (including some EPG screen shots to show mixture and allow for easier visualisation) was sent to the management team for discussion. A review of the DNA IQ extraction protocol is being undertaken as this is the newest part of the processing to ensure that there is no source of potential errors with this processing.		

Task Details

No Tasks found

Follow-up And Approval

Follow-up Status	Accepted
Follow-up Status Comment	21/04/2008 4:52:01 PM Allan MCNEVIN:

	Invewstigation OK - source of OQI was not meant to be suggestion - internal problems would be a better description
Approver	Cathie ALLEN
Approval/Rejection Date	25/04/2008
Approval/Rejection Comment	25/04/2008 12:00:00 AM Catherine ALLEN: No comment was recorded

Associations

No Associations found

Records

No Records found

19330 No Title Provided

TN-05

Report for QIS OQI as of 29/09/2022 12:16:18 PM

Report for QIS OQI -

19349 No Title Provided

OQI Details

Status	Closed Approved
Subject	Negative extraction control 346790262 from extraction batch CWIQEXT20080225_02 has shown a DNA profile below 75 RFU threshold.
Source of OQI	Internal Problems (QHPSS)
Date Identified	23/04/2008

OQI Creator Contact Details

Creator	Allan MCNEVIN
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigator/Actioner Contact Details

Actioner	Allan MCNEVIN
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigation Details

Investigation Completed 23/04/2008 **Root Cause Type** Procedure/Method/Process

Investigation Details

During the Genescan analysis of the negative extraction control (barcode 346790262) of extraction batch CWIQEXT20080225_02, it was noted that there was the potential presence of a DNA profile, however peaks observed were below the 75RFU threshold. The batch was re-prepared and the negative extraction control was re-amplified immediately to confirm the presence of DNA within the sample. Both the original amplification and re-amplified samples were re-analysed with a 30RFU threshold. When analysed at the lowered threshold the partial DNA profiles contained within the negative extraction control were consistent with the positive extraction control used. All alleles within the positive extraction control were present in at least one of the amplifications of the negative extraction control. The two samples, positive and negative extraction controls, were processed in adjacent well positions at each stage of the process up to completion of the first amplification of the negative extraction control. Therefore contamination of one into the other may have occurred at any stage from use of the STORstar, through extraction on the MPII, to processing of the quantification batch (including decapping and recapping) up to the addition

	of DNA extracts during the preparation of the amplification batch on the MPII. At each of these stages, extensive validation and large numbers of routine samples have been processed with no problems detected. It is therefore not possible to determine the exact point where the contamination has occurred. In addition, the level of transference has been very low. When considering the DNA concentration of the DNA extract from positive extraction control (2.59ng/uL), a very small amount of this DNA extract (approximately 0.25uL) may have been sufficient to have been transferred to the DNA extract of the negative extraction control to display the low level of DNA profile observed.
Preformed By	Quality Information System

Action Details

Action Complete	23/04/2008	**Action Fix Type**	Changed Process
Title	Once the		
Action Description	presence of the low-level contamination was confirmed, specimen notes and batch audit entries were made in AUSLAB against the extraction batch and all of the samples contained within the extraction batch. The team leaders of the teams in major crime and volume crime that had samples on the extraction batch were also notified of the presence of the low-level contaminant. The issue was discussed at the next available Analytical team meeting and will be re-visited at the next Analytical team meeting A review of the MPII extraction procedure is currently under way, in addition current processing does not involve the STORstar instrument and involves the use of two positive and two negative extraction controls per extraction batch.		

Task Details

No Tasks found

Follow-up And Approval

Follow-up Status	Accepted
Follow-up Status Comment	23/04/2008 3:37:17 PM Allan MCNEVIN: No comment was recorded
Approver	Cathie ALLEN
Approval/Rejection Date	25/04/2008
Approval/Rejection Comment	25/04/2008 12:00:00 AM Catherine ALLEN: No comment was recorded

Associations

No Associations found

Records

No Records found

19349 No Title Provided

TN-06

Report for QIS OQI as of 29/09/2022 12:31:00 PM

Report for QIS OQI -

19477 No Title Provided

OQI Details

Status	Closed Approved
Subject	A Negative extraction control sample 346795477 was extracted on CWIQLYS20080429_01/CWIQEXT20080430_01 had been profiled twice confirming a partial profile in the DNA extract.
Source of OQI	Internal Problems (QHPSS)
Date Identified	12/05/2008

OQI Creator Contact Details

Creator	Amy CHENG
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigator/Actioner Contact Details

Actioner	Allan MCNEVIN
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigation Details

Investigation Completed	22/07/2008 **Root Cause Type** | Procedure/Method/Process
Investigation Details	Negative extraction control [redacted] showed a single peak above 75RFU at Amelogenin after extraction. This was confirmed after re-amplification. The control was then concentrated via microcon and both the original results and the microcon results were analysed at 30RFU values. A full 9-loci profile was able to be elucidated from the concentrated sample. This was then searched against all profiles from the same extraction batch (CWIQEXT20080430_01). A match was found with samples 320124349, [redacted] which are all from the same case. Further matches were found to a mixture 333810182 and a match was found to sample 288908564 after it had undergone a clean up procedure with the NucleoSpin Tissue Kit. Samples 320124349 (5.61ng/uL), (320124335 8.8ng/uL) & [redacted] (10.53ng/uL) had quite high quantification values and one or more were the most likely source(s) of the contamination. During the course of the investigation two further examples of potential well-to-well contamination have been identified and taken in conjunction with two previously documented events, these events build a picture of potential systematic

	problems. This other events have been documented as OQI's 19330, 19349, 19767, & 19768.
Preformed By	Quality Information System

Action Details

Action Complete	22/07/2008	**Action Fix Type**	Changed ProcessA full process
Title		**Action Description**	audit (audit #8227 ? DNA IQ) has been commissioned to thoroughly review all facets of the automated extraction process. This had been planned as a post implementation review but has been brought forward in view of events mentioned above. A investigation report has been written and stored in I:\AAA Analytical Section\Adverse event investigations\ An extra-ordinary meeting of the DNA Analysis management meeting was held 14/07/2008 and the following actions were agreed upon: i) Processing of Reference samples only on Extraction platform A (initial investigations indicated events were likely related to platform A) ii) Processing of Casework samples on Extraction platform B in a checkerboard pattern with extraction reagent blanks iii) Urgent progression of audit mentioned above and investigation into findings iv) A full information review of results from automated extractions with documented quality events and extractions without documented events to gain further information This OQI has been discussed in the Analytical team meeting. Staff have additionally been individually approached with questions re: concerns, possible solutions etc. by the audit team for audit #8227

Task Details

No Tasks found

Follow-up And Approval

Follow-up Status	Accepted
Follow-up Status Comment	8/08/2008 3:28:01 PM Amy CHENG: Accepted as this OQI will be addressed in the Process Audit 8227
Approver	Cathie ALLEN
Approval/Rejection Date	18/08/2008
Approval/Rejection Comment	18/08/2008 12:00:00 AM Catherine ALLEN: Part of a larger investigation and with Audit 8227.

Associations

No Associations found

Records

No Records found

19477 No Title Provided

TN-07

Report for QIS OQI as of 29/09/2022 12:32:41 PM

Report for QIS OQI -

19767 No Title Provided

OQI Details

Status	Closed Approved
Subject	During the genescan of batch CEPRF20080521_01 (to become GEN9REF20080526_01), it was noted that a mixture was found in a FTA sample (barcode [redacted]). Initially FTA sample [redacted] was processed through routine FTA processing procedures: on batch FTA20080207_01 (to become GEN9REF20080225_03), and due to the initial `NSD? profile obtained on this original process, the sample was re-punched and processed on FTARUN20080318_02 (to become GEN9REF20080423_04). The results after being re-punched resulted in a partial DNA profile of 12 alleles plus Amelogenin. This partial DNA profile necessitated an automated extraction of the FTA card. The FTA sample was again re-punched in batch FTAEXT20080515_01 and was extracted on batch FFIQEXT20080515_01 on the MPII extraction platform. After this extraction process, the sample progressed through to routine quantification and amplification processes. The DNA extract resulted in a quantitation value of 0.0558ng/?L, in which 20?L of the DNA extract was then amplified. The PCR product was then prepared and run through the Applied Biosystems 3130xl Prism Genetic Analyser and analysed using Genescan (version 3.7.2). It was at this stage that the mixture on this sample was noted.
Source of OQI	Internal Problems (QHPSS)
Date Identified	14/06/2008

OQI Creator Contact Details

Creator	Maria AGUILERA
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigator/Actioner Contact Details

Actioner	Allan MCNEVIN
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigation Details

Investigation Completed	22/07/2008	**Root Cause Type**	Procedure/Method/ProcessA
Investigation Details	mixture was located in reference sample 184858899 from extraction batch		

RFIQEXT20080515_01 during the genescan analysis of capillary electrophoresis batch CEPRF20080521_01. The sample was re-prepared and re-analysed and the mixture was still present. The sample was then re-amplified and the the mixture was still present. The mixture contained alleles in common with all alleles seen from a partial profile obtained when the same sample had been processed on FTA batch FTARUN20080318_02. Sample 184858899 was re-extracted under barcode [redacted], and this gave a full single source profile consistent with previously obtained partial profile. The mixture obtained from the original extraction of 184858899 was then separated into the true profile and the second mixture contributor. The mixture contributor was then searched against all profiles obtained from extraction batch RFIQEXT20080515_01 and a match was found with sample [redacted] The presence of a reproducible DNA profile from within the extract and with both samples (184858899 and 308802586) being both processed alongside each other on initial quantification and amplification batches indicates that contamination has occurred at the point of the first Amplification set-up or earlier (e.g. quantification or extraction). During the course of investigation information has been gained that when viewed alongside other previous and subsequent quality events (namely OQI?s #19330, 19349, 19477, & 19768) a picture of potential systematic quality failure of the quality processes from the automated extraction processes is present. Further information is required to further elucidate and rectify if necessary any problems.

Preformed By Quality Information System

Action Details

Action Complete 22/07/2008 **Action Fix Type** Other

Title A full process audit (audit

Action Description #8227 ? DNA IQ) has been commissioned to thoroughly review all facets of the automated extraction process. This had been planned as a post implementation review but has been brought forward in view of events mentioned above. A investigation report has been written and stored in I:\AAA Analytical Section\Adverse event investigations\ An extra-ordinary meeting of the DNA Analysis management meeting was held 14/07/2008 and the following actions were agreed upon: i) Processing of Reference samples only on Extraction platform A (initial investigations indicated events were likely related to platform A) ii) Processing of Casework samples on Extraction platform B in a checkerboard pattern with extraction reagent blanks iii) Urgent progression of audit mentioned above and investigation into findings iv) A full information review of results from automated extractions with documented quality events and extractions without documented events to gain further information This OQI has been discussed in the Analytical team meeting. Staff have additionally been individually approached with questions re: concerns, possible solutions etc. by the audit team for audit #8227.

Task Details

No Tasks found

Follow-up And Approval

Follow-up Status Accepted

Follow-up Status Comment	24/07/2008 1:51:03 PM Maria AGUILERA: No comment was recorded
Approver	Cathie ALLEN
Approval/Rejection Date	15/09/2008
Approval/Rejection Comment	15/09/2008 12:00:00 AM Catherine ALLEN: As this sample was processed with the other sample on extraction, quant and amp, it is difficult to pinpoint the possible root cause. The sample was also processed on the BSD machine with the other sample, so this is a possible source too. A large investigation is being undertaken into this and other OQIs and will be documented.

Associations

No Associations found

Records

No Records found

19767 No Title Provided

TN-08

Report for QIS OQI as of 29/09/2022 12:34:14 PM

Report for QIS OQI -

19768 No Title Provided

OQI Details

Status	Closed Approved
Subject	During the uploading of results for genotyper batch GEN9CW20080513_02, it was noted by the scientist that sample 34679 6064 (which was the negative control for extraction batch CWIQLYS20080502_02 and CWIQEXT20080506_01), was found to have a partial profile result which instead should have resulted in `NSD? which is expected for any negative control. The folder was reviewed through Genescan (version 3.7.2) and Genotyper (version 3.7.1) software, and confirmed that there were peaks visible which were overseen during the initial genescanning of that sample.
Source of OQI	Internal Problems (QHPSS)
Date Identified	14/06/2008

OQI Creator Contact Details

Creator	Maria AGUILERA
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigator/Actioner Contact Details

Actioner	Allan MCNEVIN
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigation Details

Investigation Completed	30/07/2008 **Root Cause Type** Procedure/Method/Process
Investigation Details	Initially, negative extraction control sample [redacted] was extracted as outlined above on batches CWIQLYS20080502_02 and CWIQEXT20080506_01. The DNA extract was then quantified with a quant value 0.00544 ng/uL. This value is above the limit of detection (0.00426ng/uL) but below the limit of reporting (0.0128ng/uL). The DNA extract was then amplified at 20uL. The sample was analysed on CE batch CEPCW20080509_01 and Genotyper batch GEN9CW20080513_02. A single peak at the Amelogenin locus was observed above the peak detection threshold (75RFU) but below the reporting threshold (150RFU) for casework samples. The DNA extract was re-amplified at 20uL with no peaks visible above peak detection threshold, however potential peaks

were visible below threshold. The DNA extract was then concentrated using the standard Microcon procedure. The DNA extract was reduced from approximately 50-60uL in volume to approximately 5uL in volume. This extract was then amplified and no DNA profile was observed. This was thought to be erroneous and the NUNC tube containing the DNA extract was visually reviewed. 3uL of DNA extract was shown to be remaining. This was most likely due to a failure of the MPII to pipette small volumes (see OQI 20113). The DNA extract was re-amplified and a partial DNA profile was observed. The partial DNA profiles obtained from the original amplification, the re-amplification and the repeat amplification of the concentrated extract were then re-analysed using a lowered peak detection threshold of 30RFU. A profile of 15 discernable alleles was then elucidated. This profile was then searched against all profiles obtained from samples on the same extraction batch (CWIQEXT20080506_01). Matches were made to two different samples, [redacted]. Further investigation was then carried out to determine at what processing step the contamination was likely to have occurred. The AUSLAB audit trails for negative extraction control 346796064 and samples [redacted] and [redacted] were reviewed. The quantification and amplification batches samples 342270241 and [redacted] were processed on after extraction were found to be different to the batches negative extraction control [redacted] was processed on. Thus the only stage the samples and the control were processed together was during the extraction process (off -deck lysis and automated extraction). The most likely cause of contamination was during the MPII processing of the extraction batch, however the off-deck lysis component cannot be excluded. The off-deck lysis component is least likely as this is a manual process, during which only one tube is opened at a time and samples are processed sequentially, as negative extraction control [redacted] was the first sample on the lysis batch (position 1) and samples [redacted] and [redacted] were in positions 31 & 32 respectively, a large number of samples were processed in between.

Preformed By Quality Information System

Action Details

Action Complete 30/07/2008 **Action Fix Type** Other **Title** This event has been discussed in an Analytical team meeting and will constitute part of the ongoing investigations and discussions around the automated extraction procedure. AUSLAB audit entries for extraction batch CWIQEXT20080506_01, and AUSLAB specimen notes, and notation in the comments section of the 9PLEX page to refer to specimen notes were made for all samples on this extraction batch. Additionally this OQI was also entered into the UR notes of all samples. A full process audit (Audit #8227 ? DNA IQ) has been commissioned to thoroughly review all facets of the automated extraction process. This had been planned as a post implementation review but was brought forward in view of this OQI and OQI's 19330, 19349, 19477, & 19767. An extra-ordinary meeting of the DNA Analysis management team was held 14/07/2008 and the following actions were agreed upon: Processing of Reference samples only on Extraction platform A (initial investigations indicated events were likely related to platform A) Processing of Casework samples on Extraction platform B in a checkerboard pattern with extraction reagent blanks Urgent progression of audit mentioned above and investigation into findings A full information review of results from automated extractions with documented quality events and extractions without documented quality events to gain further information Initial findings from Audit #8227 have highlighted some pipetting steps within the automated extraction process as being of

particular concern. A second extra-ordinary meeting of the DNA Analysis management team was held on 28/07/2008 and a decision was made to cease processing of samples through the automated extraction process until problems identified could be rectified to the satisfaction of the management team.

Task Details

No Tasks found

Follow-up And Approval

Follow-up Status	Accepted
Follow-up Status Comment	31/07/2008 1:53:19 PM Maria AGUILERA: Note: The full report can be found in I:\AAA Analytical Section\Adverse event investigations\2008 Events
Approver	Cathie ALLEN
Approval/Rejection Date	18/08/2008
Approval/Rejection Comment	18/08/2008 12:00:00 AM Catherine ALLEN: No comment was recorded

Associations

No Associations found

Records

No Records found

19768 No Title Provided

TN-09

Report for QIS OQI as of 29/09/2022 12:13:51 PM

Report for QIS OQI -

19330 No Title Provided

OQI Details

Status	Closed Approved
Subject	FTA Evidence sample 333993604 was partial profile after initial processing, when extracted displayed a mixed DNA profile, upon re-extraction a single source profile was observed consistent with the initial profile
Source of OQI	Suggestion
Date Identified	21/04/2008

OQI Creator Contact Details

Creator	Allan MCNEVIN
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigator/Actioner Contact Details

Actioner	Allan MCNEVIN
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigation Details

Investigation Completed 21/04/2008 **Root Cause Type** Procedure/Method/Process

Investigation Details

During the reading of Genotyper batch GEN9REF20080211_05, it was noted that lab number [redacted] showed a partial mixed DNA profile. The DNA extract of [redacted] was then re-amplified and a mixture was confirmed. At the same time, previous run results for [redacted] were reviewed. It was shown that 333993604 had been processed through the FTA punching process and given a single source profile (Genotyper batch GEN9REF20080128_02). The result from GEN9REF20080211_05 resulted from an extraction of the FTA sample. The FTA sample [redacted] was then re-extracted under barcode [redacted] and yielded a single source profile consistent with one of the mixture contributors and the original FTA processing profile obtained from [redacted].The original profile obtained from FTA processing for sample [redacted] (Genotyper batch GEN9REF20080128_02) was then analysed at lowered peak height RFU thresholds, with no evidence of a mixture present. Re-analysis of original FTA & results obtained after re-extraction (barcode 333802806) using lowered peak height RFU thresholds showed no evidence of a mixture.

	Therefore the mixture was confined to the first extraction of this sample. The second contributor to the mixed DNA profile was then worked out and searched against the staff database. No matches were found. When searched against other profiles obtained from the same extraction batch (FTAEXT20080205_01) a match was found with sample [redacted]. As both FTA samples [redacted]) were extracted, quantified and amplified on the same batches, further investigation was carried out. Due to the vagaries of pipetting order and the particular DNA concentrations of the samples in question, the contamination of one sample with another must have occured prior to the addition of DNA extracts to the initial amplification reaction. In order to determine which step in the process (BSD punching, MPII DNA IQ extraction, re-capping and decapping of extracts at quantification stage, MPII preparation of Quant and decapping of extracts prior to amplification or at any of the workflow handling - storage, removal and re-storage of the extracts) is more likely to be when contamination has occurred, a consideration of how much of DNA extract [redacted] would be needed to transferred into [redacted] to cause the level of contamination that was visualised. With the help of senior scientist Justin Howes, the separation of mixture components and an assessment of the mixture ratio was made. The approximate mixture ratio is 1:1, or at the most conservative value (i.e. the least amount of DNA extract 333941730), 2:1 where the mixture would consist of twice the amount of DNA from [redacted] as from [redacted] 0. The DNA extract 333941730 was quantified at 1.71ng/ul, this is approximately 20x the concentration [redacted] Given that the method of dual elution that is done with the routine method of DNA IQ extraction performed at FSS DNA Analysis yields a final extract volume of 100?L, to display a mixture ration somewhere between 1:1 and 2:1, approximately 2.5-5?L of DNA extract [redacted] would have to have been transferred to DNA extract [redacted] This is a very unlikely scenario, especially if considering droplet or aerosol formation. Therefore the determination of exactly where in the process the contamination occured is not possible as each scenario appears to be as unlikely as the next.
Preformed By	Allan MCNEVIN

Action Details

Action Complete	21/04/2008	**Action Fix Type**	Resources
Title	AUSLAB specimen		
Action Description	notes and batch audit entries were made during the investigation phase. The issue was discussed at an Analytical team meeting on two occasions. A written report outlining the investigation (including some EPG screen shots to show mixture and allow for easier visualisation) was sent to the management team for discussion. A review of the DNA IQ extraction protocol is being undertaken as this is the newest part of the processing to ensure that there is no source of potential errors with this processing.		

Task Details

No Tasks found

Follow-up And Approval

Follow-up Status	Accepted
Follow-up Status Comment	21/04/2008 4:52:01 PM Allan MCNEVIN:

	Invewstigation OK - source of OQI was not meant to be suggestion - internal problems would be a better description
Approver	Cathie ALLEN
Approval/Rejection Date	25/04/2008
Approval/Rejection Comment	25/04/2008 12:00:00 AM Catherine ALLEN: No comment was recorded

Associations

No Associations found

Records

No Records found

19330 No Title Provided

Report for QIS OQI as of 29/09/2022 12:16:18 PM

Report for QIS OQI -

19349 No Title Provided

OQI Details

Status	Closed Approved
Subject	Negative extraction control 346790262 from extraction batch CWIQEXT20080225_02 has shown a DNA profile below 75 RFU threshold.
Source of OQI	Internal Problems (QHPSS)
Date Identified	23/04/2008

OQI Creator Contact Details

Creator	Allan MCNEVIN
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigator/Actioner Contact Details

Actioner	Allan MCNEVIN
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigation Details

Investigation Completed 23/04/2008 **Root Cause Type** Procedure/Method/ProcessDuring the

Investigation Details

Genescan analysis of the negative extraction control (barcode [redacted]) of extraction batch CWIQEXT20080225_02, it was noted that there was the potential presence of a DNA profile, however peaks observed were below the 75RFU threshold. The batch was re-prepared and the negative extraction control was re-amplified immediately to confirm the presence of DNA within the sample. Both the original amplification and re-amplified samples were re-analysed with a 30RFU threshold. When analysed at the lowered threshold the partial DNA profiles contained within the negative extraction control were consistent with the positive extraction control used. All alleles within the positive extraction control were present in at least one of the amplifications of the negative extraction control. The two samples, positive and negative extraction controls, were processed in adjacent well positions at each stage of the process up to completion of the first amplification of the negative extraction control. Therefore contamination of one into the other may have occurred at any stage from use of the STORstar, through extraction on the MPII, to processing of the quantification batch (including decapping and recapping) up to the addition

	of DNA extracts during the preparation of the amplification batch on the MPII. At each of these stages, extensive validation and large numbers of routine samples have been processed with no problems detected. It is therefore not possible to determine the exact point where the contamination has occurred. In addition, the level of transference has been very low. When considering the DNA concentration of the DNA extract from positive extraction control (2.59ng/uL), a very small amount of this DNA extract (approximately 0.25uL) may have been sufficient to have been transferred to the DNA extract of the negative extraction control to display the low level of DNA profile observed.
Preformed By	Quality Information System

Action Details

Action Complete	23/04/2008	**Action Fix Type**	Changed ProcessOnce the
Title		**Action Description**	presence of the low-level contamination was confirmed, specimen notes and batch audit entries were made in AUSLAB against the extraction batch and all of the samples contained within the extraction batch. The team leaders of the teams in major crime and volume crime that had samples on the extraction batch were also notified of the presence of the low-level contaminant. The issue was discussed at the next available Analytical team meeting and will be re-visited at the next Analytical team meeting A review of the MPII extraction procedure is currently under way, in addition current processing does not involve the STORstar instrument and involves the use of two positive and two negative extraction controls per extraction batch.

Task Details

No Tasks found

Follow-up And Approval

Follow-up Status	Accepted
Follow-up Status Comment	23/04/2008 3:37:17 PM Allan MCNEVIN: No comment was recorded
Approver	Cathie ALLEN
Approval/Rejection Date	25/04/2008
Approval/Rejection Comment	25/04/2008 12:00:00 AM Catherine ALLEN: No comment was recorded

Associations

No Associations found

Records

No Records found

19349 No Title Provided

Report for QIS OQI as of 12/10/2022 11:40:58 PM

Report for QIS OQI -

19703 No Title Provided

OQI Details

Status	Closed Approved
Subject	During Genescan Analysis of batch CEPCW20080527_01 (to become batch GEN9CW20080604_01) it was noted that Negative Extraction Control 346797616 contained a DNA profile. This profile matches to the Positive Amplification Control.
Source of OQI	Internal Problems (QHPSS)
Date Identified	04/06/2008

OQI Creator Contact Details

Creator	Allan MCNEVIN
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigator/Actioner Contact Details

Actioner	Allan MCNEVIN
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigation Details

Investigation Completed	24/06/2008 **Root Cause Type** Procedure/Method/Process
Investigation Details	Negative extraciton control [redacted] was from microcon batch MCCW20080523_01. This sample consisted of Nanopure water passed through a microcon column concurrently with casework samples. Re-preparation of capillary electrophoresis batch CEPCW20080527_01 confirmed the presence of the profile consistent with the positive amplification control. Re-amplification of Negative extraciton control [redacted] failed to reproduce the DNA profile. This then indicates that the PCR reaction / PCR product was contaminated and not the DNA extract. Therefore microcon batch MCCW20080523_01 has not been adversely affected. Batch audit entries for amplification batch 9AMPC20080526_03 (to become capillary electrophoresis batch CEPCW20080527_01, to become Genotyper batch GEN9CW20080604_01) shows that master mix was manually loaded to positions 4 and 92 of the amplification plate. The positive amplification control and negative extraction control 346797616 were in positions 1 and 10 respectively. Upon review of the amplification plate 9AMPC20080526_03, visually less PCR product remained in position

	1 when compared with other wells. The conclusion then is that splashing from well 1 (A1) to well 10 (B2) was the likely cause for the cross-contamination. This is most likely to have occured during the removal of the adhesive tape seal that is used to seal the plate. This may have occured at one of two stages: a) if the adhesive seal was applied, then removed in order to add additional mastermix to wells 4 and 92 during the preparation of amplification batch 9AMPC20080526_03 or, b) during the removal of the adhesive seal during the preparation of capillary electrophoresis batch CEPCW20080527_01 Current protocol is to centrifuge the plate prior to removal of the adhesive seal, however the possibility of sample / PCR product remaining attached to the adhesive surface remains.
Preformed By	Quality Information System

Action Details

Action Complete	30/06/2008	**Action Fix Type**	Other
Title	Relevant AUSLAB specimen		
Action Description	notes and batch audit entries have been made This OQI will be discussed in the next Analytical team meeting. Areas to be highlighted for disussion are: - Check all volumes of all samples on plate before sealing plate. - Ensure plates are sealed correctly. - Ensure that individuals follow strict protocols during the removal of any seals for the preparation of capillary electrophoresis plates and that the corresponding amplification plates have been centrifuged for the nominated time and speed before removing them. - Investigation into the use of heat sealing films for sealing PCR plates rather than adhesive seals to prevent the retention of sample / PCR product on the adhesive surface post centrifugation A detailed report has been written and retained in I:\AAA Analytical Section\Adverse event investigations\		

Task Details

No Tasks found

Follow-up And Approval

Follow-up Status	Accepted
Follow-up Status Comment	30/06/2008 4:51:03 PM Allan MCNEVIN: No comment was recorded
Approver	Vanessa IENTILE
Approval/Rejection Date	28/07/2008
Approval/Rejection Comment	28/07/2008 12:00:00 AM Vanessa IENTILE: No comment was recorded

Associations

No Associations found

Records

No Records found

19703 No Title Provided

Report for QIS OQI as of 29/09/2022 12:40:35 PM

Report for QIS OQI -

20351 No Title Provided

OQI Details

Status	Closed Approved
Subject	CWIQEXT20080402_01 was found to have a partial minor DNA profile present in the extraction positive control (346792908). This partial minor DNA profile matches alleles present in samples [redacted] These samples are from a sexual assault case and the profile from these samples is the same. This profile also matches profiles from four separate volume crime cases located on this extraction batch.It appears as though the contaminating profiles have not only contaminated from right to left across the plate but also from left to right. Part of the investigation into this event has been researched by the Extraction Audit Team and a word document with all the details is being drafted and sent to the receiver of this OQI so that it can be included in the investigation part of the OQI process.
Source of OQI	Audit
Date Identified	08/08/2008

OQI Creator Contact Details

Creator	Kylie RIKA
Organisational Unit/s	Intelligence
Service/s	
Site Location/s	Coopers Plains

Investigator/Actioner Contact Details

Actioner	Allan MCNEVIN
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigation Details

Investigation Completed	09/12/2008 **Root Cause Type** | Procedure/Method/ProcessThis OQI
Investigation Details	relates to samples [redacted] Additionally sample 209439271 was investigated. All of the above samples were extracted on the same extraction batch CWIQEXT20080402_01. During the

investigation, the stored lysate (i.e. lysed material retained after removal from the para-magnetic resin during the automated DNA IQ extraction process) and the stored substrates (i.e. the material originally submitted for DNA extraction processed through the initial off-deck lysis steps of the initial extraction process) for all 13 samples were re-extracted. These results were analysed using GeneMapper ID-X software with a peak detection threshold of 20RFU to gain the most information. The extraction of the stored lysate for each of the 13 samples showed results consistent with that obtained from the initial extraction process. From these results, it can be concluded that the contamination of samples [redacted] by one or more of samples [redacted] must have occurred prior to or during the separation of the lysis solution and the para-magnetic resin. The re-extraction of the stored substrate gave differing results for samples [redacted]. These samples showed single source profiles consistent with one of the contributors to the two-person mixtures observed after the original extraction. The other contributor was consistent with the profiles obtained from samples [redacted] (the same profile for these samples was obtained from the original extraction, the stored lysate and re-extraction of the stored substrate). The results show that for samples [redacted], there was no contamination of the substrate during the manual processing on initial extraction (off-deck lysis procedure), and that contamination by one or more of samples [redacted] (due the 10 to 100 fold higher quantification values observed for these samples) has occurred between this step in the procedure and the lysis removal step noted above. The re-extraction of the stored substrates for samples 209066674 & 259718144 showed no DNA profile, so no further conclusions could be drawn. The re-extraction of sample [redacted] yielded a different DNA profile to that obtained from the original extraction and the stored lysate. However when the original profile was re-analysed at 20RFU threshold, peaks consistent with the re-extracted sample were observed, indicating that the re-extracted substrate has yielded the true profile and the original extraction was contaminated between the same steps as sample [redacted] noted above. For sample [redacted] extraction of the stored lysate and re-extraction of the stored substrate yielded the same DNA profile as the initial extraction. This shows that a contamination event of this sample is unlikely and common alleles with the contaminating profile is con-incidental. The potential steps at which contamination may have occurred are: 1. During the transfer of the lystate obtained from manual lysis into the deep-well plate via the use of the STORstar. However this is unlikely as staff are trained to perform such pipetting steps with due diligence and care. This is also unlikely in the case of samples [redacted] as there is at least a full column physical separation between the sample wells and the proposed contaminating samples. Due to character space limitations, investigation is continued in Action below.

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Action Details

Action Complete 09/12/2008 **Action Fix Type** Changed Process **Investigation Title**

Action Description cont'd 2. Seepage of sample into an adjacent well during cold storage of the lysed material in the deep-well plate. After the cessation of processing and the carrying out of investigations, it was noted in one instance that a heavily blood-stained lysate had condensed on the underside of the adhesive seal used

to seal the stored plate. This had seeped across into an adjacent well. This was possibly due to insufficient application of the adhesive seal to the interstitial barrier. This mechanism is only considered likely for the contamination of sample [redacted] by sample [redacted]. 3. During the removal of the adhesive seal. It was noted during Audit 8227 that condensation on the underside of the adhesive seal was not removed after centrifugation. This mechanism is considered most likely due the varied multiple-well nature of the contamination events investigated. 4. Operator error during the manual addition of DNA IQ para-magnetic resin during the start of the automated extraction procedure (i.e. incorrect pipetting procedure), however this is unlikely as staff are trained to perform such pipetting steps with due diligence and care. This mechanism is only considered possible for the contamination of sample [redacted] by sample [redacted]. 5. During the mixing of the deep-well plate (containing 1.5mL of buffers and resin within a 2.2mL well) on the extraction platform DPC shaker. This had not been observed, however has been proposed a possible mechanism for adjacent well contamination during close scrutiny of the automated procedure. This mechanism is only considered possible for the contamination of sample [redacted] by sample [redacted] During the lysis removal from the para-magentic resin to the storage plate. This procedure occurs twice during the automated extraction protocol (fresh disposable tip for each step). If there was dripping of the lysate containing unbound DNA and this was to drip from one well to another well this may account for the contamination event. A similar mechanism may occur if a bubble forms at the end of the pipette tip and bursts whilst in the vicinity of another well. This mechanism is only considered possible for the contamination of samples 346792908 & [redacted] due to the directional movement of the 8-tip arm. Contamination of samples [redacted] via this mechanism is unlikely. Action: As a result of previous OQI's raised and concerns identified around the automated DNA IQ extraction process, the extraction of samples using the automated DNA IQ procedure was halted on the 28-7-2008. Prior to this, Audit 8227 had been commissioned and carried out. A number of areas for improvement were identified through the audit, and these have been implemented or are under investigation as outlined in OQI's 20367, 20368 and 20369. After the cessation of the automated DNA IQ extraction protocol, a review of all batches processed through this protocol was carried out by a specially commissioned team. A number of potential contamination events were identified and each is to be investigated on batch-by-batch basis. Additionally, careful review of results obtained from samples processed through the automated DNA IQ extraction procedure prior to reporting will be carried out. Every DNA result obtained from these samples will be interpreted with caution. Modifications have been made to the automated DNA IQ extraction procedure (including the use of an alternative to the adhesive seal and an alternative resin mixing procedure). This modified procedure is undergoing extensive verification and approval from the DNA Analysis management team must be obtained prior to re-introduction. The contamination events and concerns and improvements etc. that surround the automated DNA IQ extraction procedure have been discussed at various departmental and team meetings.

Task Details

No Tasks found

Follow-up And Approval

Follow-up Status	Accepted

Follow-up Status Comment	14/04/2009 10:42:25 AM Helen GREGG: Hi Helen I was wondering if you, or delegate, could accept the follow-up on the above OQI. Kylie Rika is away on mat leave and I need it to be closed out to print for a casefile. Thanks Justin Howes
Approver	
Approval/Rejection Date	14/04/2009
Approval/Rejection Comment	14/04/2009 11:20:48 AM Paula TAYLOR: All changes to the automated process and validation of the new set-up will be detailed in a final report. Acceptance of results is made by reviewing the results and assessing a number of factors as per the Forensic Reporting and Intelligence Team checklist, which includes, but is not limited to, the comparison of all other results from samples processed alongside these results, to detect whether the integrity of each sample can be verified. Retesting can be conducted on identified samples which may confirm information in the original results. Where the integrity of the results cannot be confirmed, the results will be reported as a quality control failure.

Associations

No Associations found

Records

No Records found

20351 No Title Provided

Report for QIS OQI as of 12/10/2022 11:38:43 PM

Report for QIS OQI -

20925 No Title Provided

OQI Details

Status	Closed Approved
Subject	A partial profile in sample [redacted] (QPS070063831- position 16) on CWIQEXT20080403_01 matched at the above threshold and sub-threshold peaks (manually designated NR peaks) to the profiles of 334721596 (QP800012453-posn 24) and 334721585 (QP800012453- posn 23) from the same extraction batch. These are apparently unrelated cases.
Source of OQI	Internal Problems (QHPSS)
Date Identified	06/10/2008

OQI Creator Contact Details

Creator	Justin HOWES
Organisational Unit/s	Forensic Reporting and Intelligence
Service/s	
Site Location/s	Coopers Plains

Investigator/Actioner Contact Details

Actioner	Allan MCNEVIN
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigation Details

Investigation Completed	04/12/2008 **Root Cause Type** Procedure/Method/ProcessThis OQI
Investigation Details	relates to sample [redacted] to have likely been contaminated by one or more of samples [redacted] as outlined above. Additionally, sample [redacted] has been possibly contaminated by one or more of samples [redacted]. This event was raised as OQI#21050, but investigation and actions are covered in this OQI. All of the above samples were extracted on the same extraction batch CWIQEXT20080403_01. During the investigation, the stored lysate for all eight samples were re-extracted as well as the stored substrates. Throughout the investigation, the results from the original extraction and subsequent re-extractions were analysed using GeneMapper ID-X software with a peak detection threshold of 20RFU to gain the most information. The re-extraction of the stored lysate (i.e. lysed material that was retained after removal from the para-magnetic resin during the automated DNA IQ extraction process) for each of the eight samples showed results consistent

with that obtained from the initial extaction process. From these results, it can be concluded that the contamination of sample [redacted] by samples [redacted] must have occurred prior to or during the separation of the lysis solution and the para-magnetic resin. Additionally, contamination of sample 334230253 by one of samples [redacted], [redacted] must also have occurred prior to or during the separation of the lysis solution and the para-magnetic resin. The re-extraction of the stored substrate (i.e. the material originally submitted for DNA extraction that had passed through the initial off-deck lysis step of the original extraction process) gave a differing results for sample[redacted]. A partial single source DNA profile inconsistent with the profile observed from intial extraction was obtained. This profile was also inconsistent with the contaminating profiles obtained from extraction & re-extraction of samples [redacted]. When re-analysed at lowered peak detection thresholds, the original profile obtained from the original extraction showed alleles consistent with both the profile obtained from re-extraction of the substrate and the contaminating profile. This indicates that there was no contamination of the substrate during the manual processing on initial extraction (off-deck lysis procedure), and that contamination has occurred between this step in the procedure and the step noted above. The re-extraction of the stored substrate for sample [redacted] showed no DNA profile, so no conclusions could be drawn. Re-extraction of samples [redacted] all yielded alleles consistent with the original extraction profiles. The potential steps at which contamination may have occurred are: 1. During the transfer of the lystate obtained from manual lysis into the deep-well plate via the use of the STORstar. However this is unlikely as all staff are trained to perform such pipetting steps with due diligence and care. This is also unlikely in the case of sample [redacted] as there is a full column physically between the sample wells of this sample and the proposed contaminating sample. 2. Seepage of sample into well 16 [redacted] from one or other of the adjacent wells 23 or 24 [redacted]) during cold storage of the lysed material in the deep-well plate. After the cessation of processing and the carrying out of investigations, it was noted in one instance that a heavily blood-stained lysate had condensed on the underside of the adhesive seal used to seal the stored plate. This had seeped across into an adjacent well. This was possibly due to insufficient application of the adhesive seal to the interstitial barrier. This mechanism is unlikely for sample [redacted] due to the wells not being adjacent. Further Investigation included in Action below due to number of character limitations.

Preformed By Quality Information System

Action Details

Action Complete 04/12/2008 **Action Fix Type** Changed ProcessInvestigations

Title

Action Description cont'd: 3. During the removal of the adhesive seal. It was noted during Audit 8227 that condensation on the underside of the adhesive seal was not removed after centrifugation. 4. Operator error during the manual addition of DNA IQ para-magnetic resin during the start of the automated extraction procedure (i.e. incorrect pipetting procedure), however this is unlikely as staff are trained to perform such pipetting steps with due diligence and care. This mechanism is unlikely for sample[redacted] due to the wells not being adjacent. 5. During the mixing of the deep-well plate (containing 1.5mL of buffers and resin within a 2.2mL well) on the extraction platform DPC shaker. This had not been observed, however has been proposed a possible mechanism for adjacent well contamination during close scrutiny of the automated procedure. This mechanism is unlikely for sample

[redacted] due to the wells not being adjacent. 6. During the lysis removal from the para-magentic resin to the storage plate. This procedure occurs twice during the automated extraction protocol (fresh disposable tip for each step). If there was drippage of the lysate containing unbound DNA and this was to drip from one well to another well this may account for the contamination event. The same mechanism may occur where a bubble forms at the end of the pipette tip and bursts whilst still in the vicinity of another well. The likely source of the contamination of sample [redacted] is sample [redacted] due the higher quantification values obtained for this sample. The likely source of the contamination of sample [redacted] is either sample [redacted] due the higher quantification values obtained for these samples. Additionally, it must be noted that it could not be ruled out that the result obtained from sample 334230253 was the true result as there is the possibility of the cases being related. Action: As a result of previous OQI's raised and concerns identified around the automated DNA IQ extraction process, the extraction of samples using the automated DNA IQ procedure was halted on the 28-7-2008. Prior to this, Audit 8227 had been commissioned and carried out. A number of areas for improvement were identified through the audit, and these have been implemented or are under investigation as outlined in OQI's 20367, 20368 and 20369. After the cessation of the automated DNA IQ extraction protocol, a review of all batches processed through this protocol was carried out by a specially commissioned team. A number of potential contamination events were identified and each is to be investigated on batch-by-batch basis. Additionally, careful review of results obtained from samples processed through the automated DNA IQ extraction procedure prior to reporting will be carried out. Every DNA result obtained from these samples will be interpreted with caution. Modifications have been made to the automated DNA IQ extraction procedure (including the use of an alternative to the adhesive seal and an alternative resin mixing procedure). This modified procedure is undergoing extensive verification and approval from the DNA Analysis management team must be obtained prior to re-introduction. The contamination events and concerns and improvements etc. that surround the automated DNA IQ extraction procedure have been discussed at various departmental and team meetings.

Task Details

No Tasks found

Follow-up And Approval

Follow-up Status	Accepted
Follow-up Status Comment	17/12/2008 3:16:21 PM Justin HOWES: Checklists incorporated into DNAIQ case reporting, external assessors have visited the lab and produced report. Automated platforms will not be reimplemented until significant re-testing and evaluating of the new programs and equipment. I am satisfied with the above investigation.
Approver	Paula BRISOTTO
Approval/Rejection Date	08/01/2009
Approval/Rejection Comment	8/01/2009 12:00:00 AM Paula TAYLOR: No comment was recorded

Associations

No Associations found

Records

No Records found

20925 No Title Provided

Report for QIS OQI as of 12/10/2022 11:36:45 PM

Report for QIS OQI -

20615 No Title Provided

OQI Details

Status	Closed Approved
Subject	As part of the extraction audit, batch CWIQEXT20080409_01 results were checked for possible cross-contamination; matches were accounted for (stringency >12) and the plate released. During peer review of results for case 0800150167, using the audit checklist, components of a mixed DNA profile (s/sample# 320111781 - front left pocket) were found to possibly match to the same profile of 4 samples in a separate case (QP0800074065: [redacted]), also processed on this extraction batch. Initial results for sample #320111781 gave 6 alleles/18 NR's, while after reworking this became 27 alleles/6 NR's (GEN9CW20080710_02). The close proximity of affected samples, as reflected on the plate map, indicate the potential for this situation to occur. A Word document with further details is being prepared to be sent to the receiver of this OQI for inclusion in any subsequent investigations
Source of OQI	Audit
Date Identified	04/09/2008

OQI Creator Contact Details

Creator	Shannon MERRICK
Organisational Unit/s	Evidence Recovery and Quality
Service/s	
Site Location/s	Coopers Plains

Investigator/Actioner Contact Details

Actioner	Allan MCNEVIN
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigation Details

Investigation Completed	17/10/2008 **Root Cause Type** Procedure/Method/ProcessSample
Investigation Details	320111781 was extracted on extraction batch CWIQEXT20080409_01 in position 22. This sample gave a mixed DNA profile that was shown to be reproducible after concentration and re-amplification of the DNA extract. This result was expected due to the nature of the sample and the case. It was not possible to separate this profile into major and minor components and there were no reference samples were submitted for this case. Other

sub-samples from the same item gave the same partial and full unknown male 1 profile which was observed in the mixed DNA profile. During the peer review of this result, a potential for contamination of this sample from other samples on the same extraction batch was indicated and this was investigated. The samples identified as potential sources of the contamination were [redacted] from positions 28-31 respectively on the same extraction batch. During the investigation, the stored lysate for all five samples we re-extracted, as well as the stored substrate. Throughout this investigation, both the results from the initial extraction and any re-extracted material was analysed using Genemapper-IDX software with a peak detection threshold of 20RFU in order to gain the most information. The re-extraction of the stored lysate (i.e. material retained after removal from para-magnetic resin during the automated DNA IQ extraction process) for each of the five samples gave results consistent with that obtained from the initial extraction process. From these results, it can be concluded that the contamination of sample [redacted] from either sample [redacted] must have occured prior to the separation of the lysis solution and the para-magnetic resin. The re-extraction of the stored substrate (i.e. the material originally submitted for DNA extraction that passed through the initial stages of the original extraction process) gave differing results. The profile obtained from the substrate of 320111781 was consistent across almost all alleles contained within the mixture, however single alleles at the FGA, D18, D5 and D13 loci that were consistent with the potential source profiles were not found. The profiles obtained from re-extraction of substrates from samples [redacted] yielded profiles consistent to that obtained from the original extraction (sample 342340174 only yielded 4 alleles, however all were consistent with the original profile obtained). When assessing the results obtained from the original extraction and re-extraction of the substrate from sample [redacted] (with the assistance of the Reporting and Intelligence team leader), it can be seen that the mixture ratios are not conserved. When viewed in combination with the additional alleles obtained, it can be seen that contamination of sample 320111781 with a profile consistent with that obtained from samples [redacted] has occurred. Since sample [redacted] has a ten-fold higher level of DNA concentration than either of the other four samples under investigation (in all samples tested, including the original DNA extract, stored lysate and re-extracted substrate) and lies in the adjacent well to the contaminated sample (320111781) it can therefore be concluded that there has been contamination of sample [redacted] with sample [redacted] The results obtained from the investigation therefore indicate that contamination of sample [redacted] from sample [redacted] has occurred after the manual lysis of the substrates (off-deck lysis procedure), but prior to completion of the removal of lysed material from para-magnetic resin during the automated portion of the extraction procedure. The potential steps at which contamination may have occurred are outlined in Actions below.

Preformed By Quality Information System

Action Details

Action Complete 17/10/2008 **Action Fix Type** Changed Process**Title** **Action Description** The potential steps at which contamination may have occurred are: 1. During the transfer of the lysate obtained from manual lysis into the deep-well plate via the use of the Storstar. This however is considered unlikely. This is because lysate was added to well 22 prior to the addition of wells 28-31 and the nature of the pipetting process whereby individual care is taken whilst performing

the process. 2. During the removal of the adhesive seal used to seal the deep-well plate containing stored lysates awaiting automated DNA IQ extraction. This is the most likely as it was noted during Audit 8227 that condensation on the seal that could not be removed by centrifugation was a contamination risk. Additionally, it was observed in one instance that seepage across the adhesive seal (from a positive to a negative control in adjacent wells) appeared to have occurred. Samples [redacted] and [redacted] were extracted in adjacent wells (22 and 30 respectively). 3. There may have been operator error during the manual addition of DNA IQ para-magnetic resin during the start of the automated extraction procedure (i.e. incorrect pipetting procedure), however this is unlikely as staff are trained to perform such pipetting steps with due diligence and care. 4. During the mixing of the deep-well plate (containing 1.5mL of buffers and resin within a 2.2mL well) on the extraction platform DPC shaker. This had not been observed, however has been proposed as a possible mechanism during close scrutiny of the automated procedure. 5. During the 1st stage of removal of lysate from the para-magnetic resin to the storage plate. This procedure occurs twice during the automated extraction protocol (fresh disposable tips for each step). If there was drippage of the lysate containing unbound DNA and this was to drip from either well 30 into well 22 (or bubble burst), this may account for the contamination event. However, the physical movement of the 8-tip arm during this liquid transfer makes this an unlikely proposition (i.e. once the tips retract from well 30, it does not move directly over well 22). Actions As a result of previous OQI's raised and concerns identified around the automated DNA IQ extraction process, the extraction of samples using the automated DNA IQ procedure was halted on the 28-7-2008. Prior to this Audit 8227 had been commissioned and carried out. A number of areas for improvement were identified through the audit, and these have been implemented or are under investigation as outlined in OQI's 20367, 20368 and 20369. After the cessation of the automated DNA IQ extraction protocol, a review of all batches processed through this protocol was carried out by a specially commissioned team. A number of potential contamination events were identified and each is to be investigated on batch-by-batch basis. Additionally, careful review of results obtained from samples processed through the automated DNA IQ extraction procedure prior to reporting will be carried out. Every DNA result obtained from these samples will be interpreted with caution. Modifications have been made to the automated DNA IQ extraction procedure (including the use of an alternative to the adhesive seal and an alternative resin mixing procedure). This modified procedure will undergo extensive verification and approval from the DNA Analysis management team prior to re-introduction. The contamination events and concerns and improvements etc. that surround the automated DNA IQ extraction procedure have been discussed at various departmental and team meetings.

Task Details

No Tasks found

Follow-up And Approval

Follow-up Status	Accepted
Follow-up Status Comment	28/10/2008 3:41:28 PM Shannon MERRICK: No comment was recorded
Approver	Paula BRISOTTO
Approval/Rejection Date	06/11/2008

Approval/Rejection Comment	6/11/2008 12:00:00 AM Paula TAYLOR: No comment was recorded

Associations

No Associations found

Records

No Records found

20615 No Title Provided

Report for QIS OQI as of 29/09/2022 12:38:44 PM

Report for QIS OQI -

20231 No Title Provided

OQI Details

Status	Closed Approved
Subject	During review of extraction negative controls it was noted that the negative extraction control 346796064 contained peaks above threshold. The sample was extracted on batch CWIQEXT20080417_01 on the extraction platform B.
Source of OQI	Internal Problems (QHPSS)
Date Identified	24/07/2008

OQI Creator Contact Details

Creator	Chiron WEBER
Organisational Unit/s	DNA Analysis
Service/s	
Site Location/s	Coopers Plains

Investigator/Actioner Contact Details

Actioner	Allan MCNEVIN
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigation Details

Investigation Completed	29/07/2008 **Root Cause Type** Procedure/Method/ProcessThe correct
Investigation Details	negative extraction control lab number is [redacted] (the wrong lab number has been added to description). In July 2008, concern was raised as the quality of the automated DNA IQ extraction process, with a number of contamination events having occurred leading to evidence of well to well contamination during the automated DNA IQ extraction (using the MPII platform) process. A review of all negative extraction controls from all batches performed through this process was conducted. It was noted that negative extraction control sample [redacted] showed the presence of a partial DNA profile in AUSLAB. The DNA extract from [redacted] was then concentrated by Microcon concentration. The concentrated DNA extract was re-quantified, amplified and analysed. Two peaks were present above peak detection threshold. Both the original amplification result and the microcon amplification result were analysed at reduced peak height detection threshold (30RFU) and a number of alleles were determined. A combined profile was then obtained and this was searched against profiles

	obtained from the same extraction batch. A match was found to 346802502, an environmental monitoring sample that was submitted as a blind positive control (consisted of a staff members buccal swab). Further analysis of the extraction batch was conducted at reduced peak detection threshold and environmental monitoring samples [redacted] were found to contain consistent alleles. After reviewing the quantification values for the samples it was determined that sample 346802502 was most likely the source of the contamination as this sample had the highest quantification value (1.62ng/uL). Negative extraction control 346794568 was quantified and amplified on seperate batches to environmental sample [redacted] and the additional samples [redacted]. Therefore the contamination can be defined to have occured during the extraction procedure (either during off-deck lysis, or during automated extraction). This would be consistent with the prelimanry findings of Audit 8227 and previous OQI's raised (particularly 19330, 19349, 19477, 19767, and 19768).
Preformed By	Quality Information System

Action Details

Action Complete	08/08/2008	**Action Fix Type**	Other
Title	This OQI was raised as part of previously initiated actions relating to the investigation into the automated DNA IQ procedure (see Audits and OQI's listed in Investigation), A full investigation report surrounding this OQI can be located in I:\AAA Analytical Section\Adverse event investigations\2008 Events Appropriate specimen notes, UR notes and batch audit entries have been made in AUSLAB. This event will be discussed in the next available Analytical team meeting. Particular attention will additionally be placed on the initial acceptance of the Negative control (i.e. the control passing through the quality process). The need for due care when reviewing control results will be highlighted.		
Action Description			

Task Details

No Tasks found

Follow-up And Approval

Follow-up Status	Accepted
Follow-up Status Comment	15/08/2008 7:17:05 AM Chiron WEBER: No comment was recorded
Approver	Cathie ALLEN
Approval/Rejection Date	18/08/2008
Approval/Rejection Comment	18/08/2008 12:00:00 AM Catherine ALLEN: No comment was recorded

Associations

No Associations found

Records

No Records found

20231 No Title Provided

Report for QIS OQI as of 29/09/2022 12:31:00 PM

Report for QIS OQI -

19477 No Title Provided

OQI Details

Status	Closed Approved
Subject	A Negative extraction control sample [redacted] was extracted on CWIQLYS20080429_01/CWIQEXT20080430_01 had been profiled twice confirming a partial profile in the DNA extract.
Source of OQI	Internal Problems (QHPSS)
Date Identified	12/05/2008

OQI Creator Contact Details

Creator	Amy CHENG
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigator/Actioner Contact Details

Actioner	Allan MCNEVIN
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigation Details

Investigation Completed	22/07/2008 **Root Cause Type** | Procedure/Method/Process
Investigation Details	Negative extraction control [redacted] showed a single peak above 75RFU at Amelogenin after extraction. This was confirmed after re-amplification. The control was then concentrated via microcon and both the original results and the microcon results were analysed at 30RFU values. A full 9-loci profile was able to be elucidated from the concentrated sample. This was then searched against all profiles from the same extraction batch (CWIQEXT20080430_01). A match was found with samples [redacted] which are all from the same case. Further matches were found to a mixture 333810182 and a match was found to sample 288908564 after it had undergone a clean up procedure with the NucleoSpin Tissue Kit. Samples [redacted](5.61ng/uL), ([redacted]8.8ng/uL) & [redacted] (10.53ng/uL) had quite high quantification values and one or more were the most likely source(s) of the contamination. During the course of the investigation two further examples of potential well-to-well contamination have been identified and taken in conjunction with two previously documented events, these events build a picture of potential systematic

	problems. This other events have been documented as OQI's 19330, 19349, 19767, & 19768.
Preformed By	Quality Information System

Action Details

Action Complete	22/07/2008	**Action Fix Type**	Changed ProcessA full process
Title		**Action Description**	audit (audit #8227 ? DNA IQ) has been commissioned to thoroughly review all facets of the automated extraction process. This had been planned as a post implementation review but has been brought forward in view of events mentioned above. A investigation report has been written and stored in I:\AAA Analytical Section\Adverse event investigations\ An extra-ordinary meeting of the DNA Analysis management meeting was held 14/07/2008 and the following actions were agreed upon: i) Processing of Reference samples only on Extraction platform A (initial investigations indicated events were likely related to platform A) ii) Processing of Casework samples on Extraction platform B in a checkerboard pattern with extraction reagent blanks iii) Urgent progression of audit mentioned above and investigation into findings iv) A full information review of results from automated extractions with documented quality events and extractions without documented events to gain further information This OQI has been discussed in the Analytical team meeting. Staff have additionally been individually approached with questions re: concerns, possible solutions etc. by the audit team for audit #8227

Task Details

No Tasks found

Follow-up And Approval

Follow-up Status	Accepted
Follow-up Status Comment	8/08/2008 3:28:01 PM Amy CHENG: Accepted as this OQI will be addressed in the Process Audit 8227
Approver	Cathie ALLEN
Approval/Rejection Date	18/08/2008
Approval/Rejection Comment	18/08/2008 12:00:00 AM Catherine ALLEN: Part of a larger investigation and with Audit 8227.

Associations

No Associations found

Records

No Records found

19477 No Title Provided

Report for QIS OQI as of 29/09/2022 12:34:14 PM

Report for QIS OQI -

19768 No Title Provided

OQI Details

Status	Closed Approved
Subject	During the uploading of results for genotyper batch GEN9CW20080513_02, it was noted by the scientist that sample 34679 6064 (which was the negative control for extraction batch CWIQLYS20080502_02 and CWIQEXT20080506_01), was found to have a partial profile result which instead should have resulted in `NSD? which is expected for any negative control. The folder was reviewed through Genescan (version 3.7.2) and Genotyper (version 3.7.1) software, and confirmed that there were peaks visible which were overseen during the initial genescanning of that sample.
Source of OQI	Internal Problems (QHPSS)
Date Identified	14/06/2008

OQI Creator Contact Details

Creator	Maria AGUILERA
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigator/Actioner Contact Details

Actioner	Allan MCNEVIN
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigation Details

Investigation Completed	30/07/2008 **Root Cause Type** Procedure/Method/Process
Investigation Details	Initially, negative extraction control sample [redacted] was extracted as outlined above on batches CWIQLYS20080502_02 and CWIQEXT20080506_01. The DNA extract was then quantified with a quant value 0.00544 ng/uL. This value is above the limit of detection (0.00426ng/uL) but below the limit of reporting (0.0128ng/uL). The DNA extract was then amplified at 20uL. The sample was analysed on CE batch CEPCW20080509_01 and Genotyper batch GEN9CW20080513_02. A single peak at the Amelogenin locus was observed above the peak detection threshold (75RFU) but below the reporting threshold (150RFU) for casework samples. The DNA extract was re-amplified at 20uL with no peaks visible above peak detection threshold, however potential peaks

were visible below threshold. The DNA extract was then concentrated using the standard Microcon procedure. The DNA extract was reduced from approximately 50-60uL in volume to approximately 5uL in volume. This extract was then amplified and no DNA profile was observed. This was thought to be erroneous and the NUNC tube containing the DNA extract was visually reviewed. 3uL of DNA extract was shown to be remaining. This was most likely due to a failure of the MPII to pipette small volumes (see OQI 20113). The DNA extract was re-amplified and a partial DNA profile was observed. The partial DNA profiles obtained from the original amplification, the re-amplification and the repeat amplification of the concentrated extract were then re-analysed using a lowered peak detection threshold of 30RFU. A profile of 15 discernable alleles was then elucidated. This profile was then searched against all profiles obtained from samples on the same extraction batch (CWIQEXT20080506_01). Matches were made to two different samples, 342270241 and [redacted]. Further investigation was then carried out to determine at what processing step the contamination was likely to have occurred. The AUSLAB audit trails for negative extraction control [redacted] and samples [redacted] and [redacted] were reviewed. The quantification and amplification batches samples [redacted]0230 were processed on after extraction were found to be different to the batches negative extraction control [redacted] was processed on. Thus the only stage the samples and the control were processed together was during the extraction process (off -deck lysis and automated extraction). The most likely cause of contamination was during the MPII processing of the extraction batch, however the off-deck lysis component cannot be excluded. The off-deck lysis component is least likely as this is a manual process, during which only one tube is opened at a time and samples are processed sequentially, as negative extraction control [redacted] was the first sample on the lysis batch (position 1) and samples [redacted] were in positions 31 & 32 respectively, a large number of samples were processed in between.

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Action Details

Action Complete 30/07/2008 **Action Fix Type** Other This event has been discussed in an Analytical team meeting and will constitute part of the ongoing investigations and discussions around the automated extraction procedure. AUSLAB audit entries for extraction batch CWIQEXT20080506_01, and AUSLAB specimen notes, and notation in the comments section of the 9PLEX page to refer to specimen notes were made for all samples on this extraction batch. Additionally this OQI was also entered into the UR notes of all samples. A full process audit (Audit #8227 ? DNA IQ) has been commissioned to thoroughly review all facets of the automated extraction process. This had been planned as a post implementation review but was brought forward in view of this OQI and OQI's 19330, 19349, 19477, & 19767. An extra-ordinary meeting of the DNA Analysis management team was held 14/07/2008 and the following actions were agreed upon: Processing of Reference samples only on Extraction platform A (initial investigations indicated events were likely related to platform A) Processing of Casework samples on Extraction platform B in a checkerboard pattern with extraction reagent blanks Urgent progression of audit mentioned above and investigation into findings A full information review of results from automated extractions with documented quality events and extractions without documented quality events to gain further information Initial findings from Audit #8227 have highlighted some pipetting steps within the automated extraction process as being of

Action Description Title

particular concern. A second extra-ordinary meeting of the DNA Analysis management team was held on 28/07/2008 and a decision was made to cease processing of samples through the automated extraction process until problems identified could be rectified to the satisfaction of the management team.

Task Details

No Tasks found

Follow-up And Approval

Follow-up Status	Accepted
Follow-up Status Comment	31/07/2008 1:53:19 PM Maria AGUILERA: Note: The full report can be found in I:\AAA Analytical Section\Adverse event investigations\2008 Events
Approver	Cathie ALLEN
Approval/Rejection Date	18/08/2008
Approval/Rejection Comment	18/08/2008 12:00:00 AM Catherine ALLEN: No comment was recorded

Associations

No Associations found

Records

No Records found

19768 No Title Provided

Report for QIS OQI as of 29/09/2022 12:42:18 PM

Report for QIS OQI -

20422 No Title Provided

OQI Details

Status	Closed Approved
Subject	An unexpected mixed DNA profile was obtained for lab no. [redacted] (QP800235382) that had a major DNA profile matching the deceased in the matter, and a minor DNA profile matching a DNA profile obtained from a complainant in an apparently unrelated sexual assault matter (QP800088413).
Source of OQI	Internal Problems (QHPSS)
Date Identified	20/08/2008

OQI Creator Contact Details

Creator	Justin HOWES
Organisational Unit/s	Forensic Reporting and Intelligence
Service/s	
Site Location/s	Coopers Plains

Investigator/Actioner Contact Details

Actioner	Allan MCNEVIN
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigation Details

Investigation Completed	14/10/2008 **Root Cause Type** | Procedure/Method/ProcessSample
Investigation Details	365296308 was extracted on extraction batch CWIQEXT20080506_02 in position 7. This sample gave a mixed DNA profile that was shown to be reproducible from the DNA extract on re-amplification. This result was not expected due to the nature of the sample and the case. The sample was able to separated into major and minor mixture components. It was found that the minor contributor matched to samples [redacted]. These samples were extracted from positions 23 & 24 of the same extraction batch (CWIQEXT20080506_02). The profiles obtained from [redacted] & [redacted] are consistent with type of samples and the nature of the case. During the investigation, the stored lysate for all three samples we re-extracted, as well as the stored substrate. The re-extraction of the stored lysate (i.e. material retained after removal from para-magnetic resin during the automated DNA IQ extraction process) for each of the three samples gave results consistent with that obtained from the initial extraction process. From these results, it can be concluded that the

contamination of sample [redacted] from either [redacted] must have occured prior to the separation of the lysis solution and the para-magnetic resin. The re-extraction of the stored substrate (i.e. the material originally submitted for DNA extraction that passed through the initial stages of the original extraction process) gave different results. In this instance the profile obtained from the substrate of [redacted] was consistent only with the major component obtained from the original extraction. The profiles obtained from re-extraction of substrates from samples [redacted] gave profiles consistent to that obtained from the original extraction. These two sets of results therefore indicate that contamination of sample [redacted] from either [redacted] & 320124503 has occured after the manual lysis of the substrates (off-deck lysis procedure), but prior to completion of the removal of lysed material from para-magnetic resin during the automated portion of the extraction procedure. The potential steps at which contamination may have occured are: 1. During the transfer of the lysate obtained from manual lysis into the deep-well plate via the use of the Storstar. This however is least likely. This is because lysate was added to well 7 prior to the addition of wells 23 or 24, and well 7 is physically covered and not adjacent to wells 23 & 24 during the storage process. 2. During the removal of the adhesive seal used to seal the deep-well plate containing stored lysates awaiting automated DNA IQ extraction. This is the most likely as it was noted during Audit 8227 that condensation on the seal that could not be removed by centrifugation was a contamination risk. Additionally, the random nature of potential aerosol formation and the physical positioning of samples also makes this scenario more likely. 3. There may have been operator error during the manual addition of DNA IQ para-magnetic resin during the start of the automated extraction procedure (i.e. incorrect pipetting procedure), however this is unlikely as staff are trained to perform such pipetting steps with due diligence and care. 4. During the 1st stage of removal of lysate from the para-magnetic resin to the storage plate. This procedure occurs twice during the automated extraction protocol (fresh disposable tips for each step). If there was drippage of the lysate containing unbound DNA and this was to drip from either wells 23 & 24 into well 7 (or bubble burst), this may account for the contamination event. However, the physical movement of the 8-tip arm during this liquid transfer makes this an unlikely proposition (i.e. once the tips retract from wells 23 & 24, it does not move directly over well 7).

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Action Details

Action Complete 14/10/2008 **Action Fix Type** Changed Process

Title As a result of

Action Description previous OQI's raised and concerns identified around the automated DNA IQ extraction process, the extraction of samples using the automated DNA IQ procedure was halted on the 28-7-2008. Prior to this Audit 8227 had been comissioned and carried out. A number of areas for improvement were identified through the audit, and these have been implemented or are under investigation as outlined in OQI's 20367, 20368 and 20369. After the cessation of the automated DNA IQ extraction protocol, a review of all batches processed through this protocol was carried out by a specially comissioned team. A number of potential contamination events were identified and each is to be investigated on batch-by-batch basis. Additionally, careful review of results obtained from samples processed through the automated DNA IQ extraction procedure prior to reporting will be carried out. Every DNA result obtained from these samples will be interpreted with caution. Modifications have been made to the automated DNA IQ extraction procedure (including the use of an alternative to the

adhesive seal). This modified procedure will undergo extensive verification and approval from the DNA Analysis management team prior to re-introduction. The contamination events and concerns and improvements etc. that surround the automated DNA IQ extraction procedure have been discussed at various departmental and team meetings.

Task Details

No Tasks found

Follow-up And Approval

Follow-up Status	Accepted
Follow-up Status Comment	22/10/2008 8:52:59 AM Justin HOWES: I accept the findings and agree that results from automated DNAIQ extractions need to be interpretted with caution, including using Eactraction Batch macros and checklists devised by DNA Analysis.
Approver	
Approval/Rejection Date	22/10/2008
Approval/Rejection Comment	22/10/2008 8:52:59 AM Paula TAYLOR: Checklists for interpretation of results are included in the case files. All investigations, findings and outcomes of these events will be compiled in a report.

Associations

No Associations found

Records

No Records found

20422 No Title Provided

Report for QIS OQI as of 29/09/2022 12:32:41 PM

Report for QIS OQI -

19767 No Title Provided

OQI Details

Status	Closed Approved
Subject	During the genescan of batch CEPRF20080521_01 (to become GEN9REF20080526_01), it was noted that a mixture was found in a FTA sample (barcode [redacted]). Initially FTA sample [redacted] was processed through routine FTA processing procedures: on batch FTA20080207_01 (to become GEN9REF20080225_03), and due to the initial `NSD? profile obtained on this original process, the sample was re-punched and processed on FTARUN20080318_02 (to become GEN9REF20080423_04). The results after being re-punched resulted in a partial DNA profile of 12 alleles plus Amelogenin. This partial DNA profile necessitated an automated extraction of the FTA card. The FTA sample was again re-punched in batch FTAEXT20080515_01 and was extracted on batch FFIQEXT20080515_01 on the MPII extraction platform. After this extraction process, the sample progressed through to routine quantification and amplification processes. The DNA extract resulted in a quantitation value of 0.0558ng/?L, in which 20?L of the DNA extract was then amplified. The PCR product was then prepared and run through the Applied Biosystems 3130xl Prism Genetic Analyser and analysed using Genescan (version 3.7.2). It was at this stage that the mixture on this sample was noted.
Source of OQI	Internal Problems (QHPSS)
Date Identified	14/06/2008

OQI Creator Contact Details

Creator	Maria AGUILERA
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigator/Actioner Contact Details

Actioner	Allan MCNEVIN
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigation Details

Investigation Completed	22/07/2008	**Root Cause Type**	Procedure/Method/ProcessA
Investigation Details	mixture was located in reference sample 184858899 from extraction batch		

RFIQEXT20080515_01 during the genescan analysis of capillary electrophoresis batch CEPRF20080521_01. The sample was re-prepared and re-analysed and the mixture was still present. The sample was then re-amplified and the the mixture was still present. The mixture contained alleles in common with all alleles seen from a partial profile obtained when the same sample had been processed on FTA batch FTARUN20080318_02. Sample [redacted] was re-extracted under barcode [redacted] and this gave a full single source profile consistent with previously obtained partial profile. The mixture obtained from the original extraction of [redacted] was then separated into the true profile and the second mixture contributor. The mixture contributor was then searched against all profiles obtained from extraction batch RFIQEXT20080515_01 and a match was found with sample [redacted] The presence of a reproducible DNA profile from within the extract and with both samples ([redacted] being both processed alongside each other on initial quantification and amplification batches indicates that contamination has occurred at the point of the first Amplification set-up or earlier (e.g. quantification or extraction). During the course of investigation information has been gained that when viewed alongside other previous and subsequent quality events (namely OQI?s #19330, 19349, 19477, & 19768) a picture of potential systematic quality failure of the quality processes from the automated extraction processes is present. Further information is required to further elucidate and rectify if necessary any problems.

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Action Details

Action Complete 22/07/2008 **Action Fix Type** Other

Title A full process audit (audit

Action Description #8227 ? DNA IQ) has been commissioned to thoroughly review all facets of the automated extraction process. This had been planned as a post implementation review but has been brought forward in view of events mentioned above. A investigation report has been written and stored in I:\AAA Analytical Section\Adverse event investigations\ An extra-ordinary meeting of the DNA Analysis management meeting was held 14/07/2008 and the following actions were agreed upon: i) Processing of Reference samples only on Extraction platform A (initial investigations indicated events were likely related to platform A) ii) Processing of Casework samples on Extraction platform B in a checkerboard pattern with extraction reagent blanks iii) Urgent progression of audit mentioned above and investigation into findings iv) A full information review of results from automated extractions with documented quality events and extractions without documented events to gain further information This OQI has been discussed in the Analytical team meeting. Staff have additionally been individually approached with questions re: concerns, possible solutions etc. by the audit team for audit #8227.

Task Details

No Tasks found

Follow-up And Approval

Follow-up Status Accepted

Follow-up Status Comment	24/07/2008 1:51:03 PM Maria AGUILERA: No comment was recorded
Approver	Cathie ALLEN
Approval/Rejection Date	15/09/2008
Approval/Rejection Comment	15/09/2008 12:00:00 AM Catherine ALLEN: As this sample was processed with the other sample on extraction, quant and amp, it is difficult to pinpoint the possible root cause. The sample was also processed on the BSD machine with the other sample, so this is a possible source too. A large investigation is being undertaken into this and other OQIs and will be documented.

Associations

No Associations found

Records

No Records found

19767 No Title Provided

Report for QIS OQI as of 12/10/2022 11:34:09 PM

Report for QIS OQI -

21309 No Title Provided

OQI Details

Status	Closed Approved
Subject	When performing a quality check of batch CWIQEXT20080531_01 for case QP0800214797 (blue case), it was found that the 9 loci profile from sample [redacted] could not be excluded from contributing to the profile for sample [redacted] from unrelated case QP0800232528; and this profile was also found to be a below threshold match to the minor profile in sample 302265397 from a second unrelated volume case.
Source of OQI	Internal Problems (QHPSS)
Date Identified	06/11/2008

OQI Creator Contact Details

Creator	Thomas NURTHEN
Organisational Unit/s	Reporting 3
Service/s	
Site Location/s	Coopers Plains

Investigator/Actioner Contact Details

Actioner	Allan MCNEVIN
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigation Details

Investigation Completed	04/12/2008 **Root Cause Type** Procedure/Method/Process
Investigation Details	Samples [redacted] both contain mixed DNA profiles from apparently unrelated cases, additionally the single source DNA profile obtained sample 333810771 could have contributed to the mixture in both samples. This sample is also from an apparently unrelated case. As part of the investigation, the stored lysate and the stored substrate for all three samples were re-extracted. Throughout the investigation, the results from the original extraction and subsequent re-extractions were analysed using GeneMapper ID-X software with a peak detection threshold of 20RFU to gain the most information. The re-extraction of the stored lysate (i.e. lysed material that was retained after removal from the para-magnetic resin during the automated DNA IQ extraction process) for each of the three samples showed results consistent with that obtained from the initial extraction process. From these results, it can be concluded that the contamination of samples [redacted]

must have occurred prior to or during the separation of the lysis solution and the para-magnetic resin. The re-extraction of the stored substrate (i.e. the material originally submitted for DNA extraction that had passed through the initial off-deck lysis step of the original extraction process) gave a differing results for samples [redacted]. Both gave single source DNA profiles that were consistent with the alternate contributor of the initial mixed DNA profiles when conditioned against contaminating sample [redacted]. Sample [redacted] yielded the same single source profile as obtained initially. This indicates that there was no contamination of the substrate during the manual processing on initial extraction (off-deck lysis procedure), and that contamination has occurred between this step in the procedure and the step noted above. The potential steps at which contamination may have occurred are: 1. During the transfer of the lystate obtained from manual lysis into the deep-well plate via the use of the STORstar. However this is unlikely as all staff are trained to perform such pipetting steps with due diligence and care. 2. Seepage of sample [redacted] (well 88) into adjacent wells 87 [redacted] and 96 [redacted] during cold storage of the lysed material in the deep-well plate. After the cessation of processing and the carrying out of investigations, it was noted in one instance that a heavily blood-stained lysate had condensed on the underside of the adhesive seal used to seal the stored plate. This had seeped across into an adjacent well. This was possibly due to insufficient application of the adhesive seal to the interstitial barrier. 3. During the removal of the adhesive seal. It was noted during Audit 8227 that condensation on the underside of the adhesive seal was not removed after centrifugation. 4. Operator error during the manual addition of DNA IQ para-magnetic resin during the start of the automated extraction procedure (i.e. incorrect pipetting procedure), however this is unlikely as staff are trained to perform such pipetting steps with due diligence and care. 5. During the mixing of the deep-well plate (containing 1.5mL of buffers and resin within a 2.2mL well) on the extraction platform DPC shaker. This had not been observed, however has been proposed a possible mechanism for adjacent well contamination during close scrutiny of the automated procedure. 6. During the lysis removal from the para-magentic resin to the storage plate. This procedure occurs twice during the automated extraction protocol (fresh disposable tip for each step). If there was drippage of the lysate containing unbound DNA and this was to drip from one well to another well this may account for the contamination event. The same mechanism may occur where a bubble forms at the end of the pipette tip and bursts whilst still in the vicinity of another well.

Preformed By Quality Information System

Action Details

Action Complete 04/12/2008 **Action Fix Type** Changed Process

Title As sample [redacted] contained significantly higher levels of DNA (quantification values of 3.59ng/ul, 14.0ng/ul and 7.91ng/ul were obtained from the initial DNA extract, re-extraction of the lysate and re-extraction of the substrate respectively) when compared to the affected samples, only a small amount of lysate would have been required to have transferred to an adjacent well to have caused the contamination. Given the single source profiles obtained from samples [redacted] after re-extraction of the stored substrate, contamination has occurred. As a result of previous OQI's raised and concerns identified around the automated DNA IQ extraction process, the extraction of samples using the automated DNA IQ procedure was halted on the 28-7-2008. Prior to this, Audit 8227 had been commissioned and carried out. A number of areas for improvement were identified through the audit, and these have been implemented or are

under investigation as outlined in OQI's 20367, 20368 and 20369. After the cessation of the automated DNA IQ extraction protocol, a review of all batches processed through this protocol was carried out by a specially commissioned team. A number of potential contamination events were identified and each is to be investigated on batch-by-batch basis. Additionally, careful review of results obtained from samples processed through the automated DNA IQ extraction procedure prior to reporting will be carried out. Every DNA result obtained from these samples will be interpreted with caution. Modifications have been made to the automated DNA IQ extraction procedure (including the use of an alternative to the adhesive seal and an alternative resin mixing procedure). This modified procedure is undergoing extensive verification and approval from the DNA Analysis management team must be obtained prior to re-introduction. The contamination events and concerns and improvements etc. that surround the automated DNA IQ extraction procedure have been discussed at various departmental and team meetings.

Task Details

No Tasks found

Follow-up And Approval

Follow-up Status	Accepted
Follow-up Status Comment	5/11/2013 4:15:42 PM Thomas NURTHEN: Agree with actions taken
Approver	Paula BRISOTTO
Approval/Rejection Date	14/11/2013
Approval/Rejection Comment	14/11/2013 10:49:51 AM Paula BRISOTTO: Nil

Associations

No Associations found

Records

No Records found

21309 No Title Provided

Report for QIS OQI as of 12/10/2022 11:28:28 PM

Report for QIS OQI -

20617 No Title Provided

OQI Details

Status	Closed Approved
Subject	A match was found between [redacted] (Pos. 47 - single 9L profile) and two other profiles; [redacted] Pos. 55 - mixture)and [redacted] Pos. 46 - mixture) on CWIQEXT20080614_02. After conditioning of these mixtures a match to 3[redacted] can be obtained. The initial match was found to the profile 320106592 whilst performing an extraction batch search (using autofilter elimination on the extraction batch excel file) on alleles observed on an UKM1 profile in mixture 289030178. Subsequent searching revealed the match with 320106705.
Source of OQI	Internal Problems (QHPSS)
Date Identified	05/09/2008

OQI Creator Contact Details

Creator	Rhys PARRY
Organisational Unit/s	Reporting 3
Service/s	
Site Location/s	Coopers Plains

Investigator/Actioner Contact Details

Actioner	Allan MCNEVIN
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigation Details

Investigation Completed	02/12/2008 **Root Cause Type** Procedure/Method/Process
Investigation Details	Samples [redacted] were all extracted on the same extraction batch CWIQEXT20080614_02. Samples [redacted] (from positions 55 & 46 respectively) contained mixed DNA profiles, and upon analysis of the results were deemed to have been contaminated by a profile matching that of sample [redacted] (from position 47) as outlined above. During the investigation, the stored lysate for all three samples were re-extracted as well as the stored substrate. Throughout the investigation, the results from the original extraction and subsequent re-extractions were analysed using GeneMapper-IDX software with a peak detection threshold of 20RFU to gain the most information. The re-extraction of the stored lysate (i.e. lysed material that was retained

after removal from the para-magnetic resin during the automated DNA IQ extraction process) for each of the three samples gave results consistent with that obtained from the initial extaction process. From these results, it can be concluded that the contamination of samples [redacted] & [redacted] by sample [redacted] must have occured prior to or during the separation of the lysis solution and the para-magnetic resin. The re-extraction of the stored substrate (i.e. the material originally submitted for DNA extraction that had passed through the initial off-deck lysis step of the original extraction process) gave differing results for samples [redacted]. Both of these samples only yielded single source profiles (compared to the mixed DNA profiles obtained from the original extraction). Sample [redacted] showed the same single source profile as originally obtained. These results show that the obsreved contamination has occured after the processing of the stored substrate (i.e. after the manual off-deck lysis procedure). The potential steps at which contamination may have occurred are: 1. During the transfer of the lystate obtained from manual lysis into the deep-well plate via the use of the STORstar. However this is unlikely as staff are trained to perform such pipetting steps with due diligence and care. 2. Seepage of sample from well 47 to adjacent wells 46 and 55 during cold storage of the lysed material in the deep-well plate. After the cessation of processing and the carrying out of investigations, it was noted in one instance that a heavily blood-stained lysate had condensed on the underside of the adhesive seal used to seal the stored plate. This had seeped accross into an adjacent well. This was possibly due to insufficient application of the adhesive seal to the interstitial barrier. 3. During the removal of the adhesive seal. It was noted during Audit 8227 that condensation on the underside of the adhsive seal was not removed after centrifugation. 4. Operator error during the manual addition of DNA IQ para-magnetic resin during the start of the automated extraction procedure (i.e. incorrect pipetting procedure), however this is unlikely as staff are trained to perform such pipetting steps with due diligence and care. 5. During the mixing of the deep-well plate (containing 1.5mL of buffers and resin within a 2.2mL well) on the extraction platform DPC shaker. This had not been observed, however has been proposed a possible mechanism for adjacent well contamination during close scrutiny of the automated procedure. 6. During the lysis removal from the para-magentic resin to the storage plate. This procedure occurs twice during the automated extraction protocol (fresh disposable tip for each step). If there was drippage of the lysate containing unbound DNA and this was to drip from well 47 to adjacent wells (either 46 or 55) this may account for the contamination event. The same mechanism may occur where a bubble forms at the end of the pipette tip and bursts whilst still in the vicinity of the adjacent wells.

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Action Details

Action Complete 02/12/2008 **Action Fix Type** Changed Process

Title As a result of

Action Description previous OQI's raised and concerns identified around the automated DNA IQ extraction process, the extraction of samples using the automated DNA IQ procedure was halted on the 28-7-2008. Prior to this, Audit 8227 had been commissioned and carried out. A number of areas for improvement were identified through the audit, and these have been implemented or are under investigation as outlined in OQI's 20367, 20368 and 20369. After the cessation of the automated DNA IQ extraction protocol, a review of all batches processed through this protocol was carried out by a specially commissioned team. A number of potential contamination events were identified and each is to be investigated on batch-by-batch basis.

Additionally, careful review of results obtained from samples processed through the automated DNA IQ extraction procedure prior to reporting will be carried out. Every DNA result obtained from these samples will be interpreted with caution. Modifications have been made to the automated DNA IQ extraction procedure (including the use of an alternative to the adhesive seal and an alternative resin mixing procedure). This modified procedure is undergoing extensive verification and approval from the DNA Analysis management team must be obtained prior to re-introduction. The contamination events and concerns and improvements etc. that surround the automated DNA IQ extraction procedure have been discussed at various departmental and team meetings.

Task Details

No Tasks found

Follow-up And Approval

Follow-up Status	Accepted
Follow-up Status Comment	12/12/2008 12:45:37 PM Rhys PARRY: No comment was recorded
Approver	Paula BRISOTTO
Approval/Rejection Date	08/01/2009
Approval/Rejection Comment	8/01/2009 12:00:00 AM Paula TAYLOR: No comment was recorded

Associations

No Associations found

Records

No Records found

20617 No Title Provided

Report for QIS OQI as of 12/10/2022 11:26:12 PM

Report for QIS OQI -

21222 CWIQEXT20080620_02 DNA IQ investigation

OQI Details

Status	Closed Approved
Subject	When doing IQ extraction batch checks for QT0800122035 (sexual assault case in Rockhampton), it was found that sample [redacted] from case QT0800353430 (voume crime from Lawnton) showed below threshold similarities to intimate samples and complainant profile in QT0800122035. Below threshold analysis (using Genemapper) on sample [redacted] supported the match.
Source of OQI	Internal Problem
Date Identified	28/10/2008

OQI Creator Contact Details

Creator	Julie CONNELL
Organisational Unit/s	Reporting 3
Service/s	
Site Location/s	Coopers Plains

Investigator/Actioner Contact Details

Actioner	Thomas NURTHEN
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigation Details

Investigation Completed	14/05/2009	**Root Cause Type**	Procedure/Method/Process
Investigation Details	Original		

[redacted]	81	89
	82	90
	83	91
	84	92
	85	93
	365654129	94
	87	95

[redacted]

88	96

81	89
82	90
83	91
84	92
85	93
364910110	94
87	95
88	96

81	89
82	90
83	91
84	92
85	93
364910051	94
87	95
88	96

This batch of casework samples appears to have a single contamination event.

- Position F11 (86) contains a mixture. The mixture was consistent with the DNA profile found in 5 positions (B10/74, C10/75, F10/78, G10/79, and H10/80).
- Re-extraction of the stored lysate of the contaminated sample (F11/86) yielded a DNA profile consistent with the original contamination.
- Re-extraction of the original substrate (F11/86) yielded an NSD profile with an undetermined Quant value.

- Direction of contamination – left to right.

A contamination of the samples has occurred at a point during the extraction process before the removal of the lysate but after removal of the substrate, as some of the lysate profiles are contaminated (consistent with original 9PLEX contamination). The right to left direction of contaminations are consistent with robotic movement. Contamination may have occurred during storage, during plate agitation on the robot, by robotic dripping, or by the removal of plate seals.

Preformed By Thomas NURTHEN

Investigation Completed 11/12/2008 **Root Cause Type** Procedure/Method/Process

Investigation Details Re-extraction of the stored lysates of the potential contributors and of the affected sample confirms the contributing profile. Analysis of the affected sample with low threshold GeneMapper software reveals a mixture. Re-extraction of the substrate from the affected sample failed to yield any

DNA profile. The presence of the mixture in the stored lysate and the apparent movement of the DNA from a well or wells left to right indicate the event occurred after the removal of the substrate but prior to the removal of the lysate. The event may have been caused by seepage of the plate seal, removal of the plate seal, agitation on the instrument or during storage. The most likey cause is belived to be seepage from position 78 (Quantitation value of around 11ng/uL) into position 86. Seepage between wells has been observed previously -OQI XXXX, and can be attributed to the plate seal not fully sealing between adjacent wells. this is becuase the plate seal used for this procedure was not designed to seal deep well plates.

Preformed By Quality Information System

Action Details

Action Complete 26/05/2009 **Action Fix Type** Changed ProcessIntroduction of new process
Title

Action Description A new process for reporting DNA IQ cases has been implemented within the Forensic Reporting and Intelligence team, no result is released without being checked to see if results are reportable

Please note in the investigation reference was made to OQI xxxx. This actually refers to all OQIs raised for DNA IQ adverse events. Please see list below

19330 FTAEXT20080205_01
19349 CWIQEXT20080225_02
19477 CWIQEXT20080430_01
19767 FTAEXT20080515_01
19768 CWIQEXT20080506_01
20231 CWIQEXT20080417_01
20351 CWIQEXT20080402_01
20422 CWIQEXT20080506_02
20437 CWIQEXT20080630_01
20615 CWIQEXT20080409_01
20617 CWIQEXT20080614_02
20690 CWIQEXT20080628_01
20925 CWIQEXT20080403_01
21222 CWIQEXT20080620_02
21309 CWIQEXT20080531_01
22880 CWIQEXT20080614_02
22882 CWIQEXT20080507_01

Action Complete 26/05/2009 **Action Fix Type** Changed ProcessModifications to Automated DNA IQ protocol
Title

Action Description

- Modifications have been made to the automated DNA IQ extraction procedure (including the use of an alternative to the adhesive seal and an alternative resin mixing procedure).
- This modified procedure will undergo extensive verification and approval from the DNA Analysis management team will be obtained prior to re-introduction. A report for this re-verification will be written and made available to all staff

Action Complete 15/05/2009 **Action Fix Type** No Fix RequiredNil action
Title **Action Description** Nil

Task Details

No Tasks found

Follow-up And Approval

Follow-up Status	Accepted
Follow-up Status Comment	26/05/2009 2:04:47 PM Thomas NURTHEN: Actions added as per Approver 26/05/2009 1:59:13 PM Thomas NURTHEN: Add details of new plate checking process for all DNA IQ cases 25/05/2009 7:45:30 PM Thomas NURTHEN: On Behalf of Julie Connell with permission from Justin Howes
Approver	Paula BRISOTTO
Approval/Rejection Date	26/05/2009
Approval/Rejection Comment	26/05/2009 2:12:28 PM Paula TAYLOR: Agree with all actions and proposed actions 26/05/2009 12:24:32 PM Paula TAYLOR: Can you please add the same actions as from the other OQI's raised in relation to the automation contamination events

Associations

No Associations found

Records

No Records found

21222 CWIQEXT20080620_02 DNA IQ investigation

Report for QIS OQI as of 12/10/2022 11:22:43 PM

Report for QIS OQI -

20690 No Title Provided

OQI Details

Status	Closed Approved
Subject	Sample 333846478 from sexual assault case QT800069474 (Gladstone)run on extraction batch CWIQEXT20080628_01 posn 65 displays a minor DNA profile matching sample [redacted] from sexual assault case QT800361528 (Nobby's Beach) posn 50. This sample [redacted] also appears to have matching components present in blue case QT800316025 sample posns 53,55,56,57 [redacted] which were pooled and reported under [redacted]
Source of OQI	Internal Problems (QHPSS)
Date Identified	15/09/2008

OQI Creator Contact Details

Creator	Jacqui WILSON
Organisational Unit/s	Forensic Reporting and Intelligence
Service/s	
Site Location/s	Coopers Plains

Investigator/Actioner Contact Details

Actioner	Allan MCNEVIN
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigation Details

Investigation Completed	09/12/2008 **Root Cause Type** | Procedure/Method/ProcessThis OQI relates to potential contamination of the following samples [redacted] by one of samples [redacted] during the processing of extraction batch CWIQEXT20080628_01. Samples [redacted] were subsequently pooled after initial extraction and processed under lab-number [redacted]. It is this lab-number that was identified as being contaminated. During the investigation, the stored lysate and stored substrate for all ten samples were re-extracted. The results from the original extraction and subsequent re-extractions were analysed using GeneMapper ID-X software with a peak detection threshold of 20RFU to gain the most information. The re-extraction of the stored lysate (i.e. lysed material retained after removal
Investigation Details	

from the para-magnetic resin during the automated DNA IQ extraction process) for sample [redacted] showed the same profile as that obtained from the profiling of sample 334655219 (the sample containing the pooled extracts). The lysate re-extractions for samples [redacted] & [redacted] showed DNA profiles consistent with the profile obtained from pooled sample 334655219 with the contaminating alleles removed. The re-extraction of the stored substrate (i.e. the material originally submitted for DNA extraction that had passed through the initial off-deck lysis step of the original extraction process) for each of the four samples all gave a mixed DNA profile matching the lysate profiles of [redacted] & [redacted], again consistent with the profile obtained from pooled sample [redacted] with the contaminating alleles removed. The re-extraction of the stored lysate & stored substrate for samples [redacted] all gave profiles consistent with the original extraction results. This then indicates that contamination of [redacted] has occurred at some step of the process between the manual processing of the substrate during off-deck lysis and prior to or during the removal of the lysed supernatant from the para-magnetic resin during the automated extraction procedure. Contamination of the pooled DNA extract sample [redacted] has occurred by combining the contaminated DNA extract [redacted] with uncontaminated [redacted] & [redacted] The re-extraction of the stored lysate & stored substrate for sample 333846478 gave differing results. The stored lysate yielded a mixed DNA profile consistent with that obtained from the original extraction. The stored substrate yielded a single source profile consistent with one of the contributors to the mixed profile initially obtained. The other contributor to the mixture was consistent with profiles obtained from samples [redacted]. This then indicates that contamination of [redacted] has occurred at some step of the process between the manual processing of the substrate during off-deck lysis and prior to or during the removal of the lysed supernatant from the para-magnetic resin during the automated extraction procedure. The re-extraction of the stored lysate for sample [redacted] gave 6 alleles, these were consistent with the two alleles obtained from the initial extraction. Neither of these alleles were consistent with the contaminating profiles. The re-extraction of the stored substrate gave no DNA. This would indicate that no contamination of sample [redacted] has occurred. The potential steps at which contamination may have occurred are: 1. During the transfer of the lysate obtained from manual lysis into the deep-well plate via the use of the STORstar. This is unlikely as staff are trained to perform such steps with due diligence and care. This is also unlikely in the case of sample 333846478 as there is a full column separating this sample and the proposed contaminating sample. Due to character space limitations, the investigation is continued in Action below.

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Action Details

Action Complete 09/12/2008 **Action Fix Type** Other **Title** Investigation Cont'd: 2.

Action Description Seepage of sample into an adjacent well (e.g. contamination of sample [redacted] by sample [redacted] during cold storage of the lysed material in the deep-well plate. After the cessation of processing and the carrying out of investigations, it was noted in one instance that a heavily blood-stained lysate had condensed on the underside of the adhesive seal used to seal the stored plate. This had seeped across into an adjacent well. This was possibly due to insufficient application of the adhesive seal to the interstitial barrier. This mechanism is unlikely for sample [redacted] due to the wells not being adjacent. 3. During the removal of the adhesive

seal. It was noted during Audit 8227 that condensation on the underside of the adhesive seal was not removed after centrifugation. Given that two contamination events have occurred (most likely contamination of samples [redacted] by sample [redacted] in a left-to-right manner, this scenario seems most likely. 4. Operator error during the manual addition of DNA IQ para-magnetic resin during the start of the automated extraction procedure (i.e. incorrect pipetting procedure), however this is unlikely as staff are trained to perform such pipetting steps with due diligence and care. This mechanism is unlikely for sample [redacted] due to the wells not being adjacent. 5. During the mixing of the deep-well plate (containing 1.5mL of buffers and resin within a 2.2mL well) on the extraction platform DPC shaker. This had not been observed, however has been proposed a possible mechanism for adjacent well contamination during close scrutiny of the automated procedure. This mechanism is unlikely for sample 333846478 due to the wells not being adjacent. 6. During the lysis removal from the para-magentic resin to the storage plate. This procedure occurs twice during the automated extraction protocol (fresh disposable tip for each step). If there was drippage of the lysate containing unbound DNA and this was to drip from one well to another well this may account for the contamination event. The same mechanism may occur where a bubble forms at the end of the pipette tip and bursts whilst still in the vicinity of another well. Action: As a result of previous OQI's raised and concerns identified around the automated DNA IQ extraction process, the extraction of samples using the automated DNA IQ procedure was halted on the 28-7-2008. Prior to this, Audit 8227 had been commissioned and carried out. A number of areas for improvement were identified through the audit, and these have been implemented or are under investigation as outlined in OQI's 20367, 20368 and 20369. After the cessation of the automated DNA IQ extraction protocol, a review of all batches processed through this protocol was carried out by a specially commissioned team. A number of potential contamination events were identified and each is to be investigated on batch-by-batch basis. Additionally, careful review of results obtained from samples processed through the automated DNA IQ extraction procedure prior to reporting will be carried out. Every DNA result obtained from these samples will be interpreted with caution. Modifications have been made to the automated DNA IQ extraction procedure (including the use of an alternative to the adhesive seal and an alternative resin mixing procedure). This modified procedure is undergoing extensive verification and approval from the DNA Analysis management team must be obtained prior to re-introduction. The contamination events and concerns and improvements etc. that surround the automated DNA IQ extraction procedure have been discussed at various departmental and team meetings.

Task Details

No Tasks found

Follow-up And Approval

Follow-up Status	Accepted
Follow-up Status Comment	10/12/2008 1:45:27 PM Jacqui WILSON: Agree with investigations and findings
Approver	Paula BRISOTTO
Approval/Rejection Date	12/12/2008 12/12/2008 12:00:00 AM Paula TAYLOR:

Approval/Rejection Comment	No comment was recorded

Associations

No Associations found

Records

No Records found

20690 No Title Provided

Report for QIS OQI as of 29/09/2022 12:44:48 PM

Report for QIS OQI -

20437 No Title Provided

OQI Details

Status	Closed Approved
Subject	CWIQEXT20080630_01 : Whilst this process was running on the robot the operator noted that the plate was misaligned on the deck, resulting in the 8tip arm contacting the lysate plate. The operator noted that the disposable conductive tip at position 6 was bent and appeared to have made contact with the well of position 14 (adjacent). Position 6 did not result in a DNA profile. (QT700518732, UUMV, Greenslopes) Position 14 resulted in an incomplete male DNA profile. (QT800323269, burglary, Noosaville) During further checks of this extraction batch, it was discovered that the DNA profile in position 5 matched the DNA profile in position 7. Position 5 contained extract from the oral swab of a SAIK (QT800131925, Townsville), and this DNA profile matches to the DNA profiles obtained from the other intimate samples from the SAIK of that case, so it appears to be a true result. Position 7, however, was supposed to contain extract from a swab from the right throttle of a motorbike involved in the UUMV case mentioned above (QT700518732). The other sample for this case was in position 6, which did not result in a profile, and no reference samples were received in relation to this matter for further comparison. Summary : position 5 (sample [redacted] from a sexual assault case has matched to position 7 (sample [redacted] from an apparently unrelated unlawful use of motor vehicle case in another part of the State.
Source of OQI	Internal Problems (QHPSS)
Date Identified	21/08/2008

OQI Creator Contact Details

Creator	Amanda REEVES
Organisational Unit/s	Reporting 3
Service/s	
Site Location/s	Coopers Plains

Investigator/Actioner Contact Details

Actioner	Allan MCNEVIN
Organisational Unit/s	Analytical

Service/s
Site Location/s Coopers Plains

Investigation Details

Investigation Completed 23/10/2008 **Root Cause Type** Procedure/Method/Process

Investigation Details Samples [redacted] were extracted on extraction batch CWIQEXT200800630_01 in positions 7, 6 & 14 respectively. During the processing of this extraction batch there were some instrument errors encountered. Whilst removing the lysate supernatant (during the second step of removal) the probe of the instrument intended to access position 6 (sample of [redacted]) crashed into position 14 (sample [redacted] thereby contaminating sample 365366424 with the DNA from [redacted]. During the investigation, the stored lysate and the stored substrate for both samples was re-extracted. Throughout this investigation, both the results from the initial extraction and any re-extracted material was analysed using Genemapper-IDX software with a peak detection threshold of 20RFU in order to gain the most information. Profiles obtained from the stored lysate for both samples yielded the same DNA profile, however no DNA profile could be obtained from the stored substrate. Therefore the true source of the DNA profile obtained could not be truly ascertained (although sample [redacted] in position 6 is the most likely). During a review of all results obtained from samples extracted using the automated DNA IQ extraction procedure it was noted that sample [redacted] from extraction batch CWIQEXT200800630_01 position 7 matched to sample [redacted] from position 5 on the same extraction batch. Given the nature of the samples and the nature & other results obtained from the two different cases involved, it was thought that sample [redacted] (position 7) was contaminated by sample [redacted] (position 5). During the investigation, the stored lysate for both samples were re-extracted, as well as the stored substrate. Throughout this investigation, both the results from the initial extraction and any re-extracted material was analysed using Genemapper-IDX software with a peak detection threshold of 20RFU in order to gain the most information. The re-extraction of the stored lysate (i.e. material retained after removal from para-magnetic resin during the automated DNA IQ extraction process) for both samples gave results consistent with that obtained from the initial extraction process. From these results, it can be concluded that the contamination of sample [redacted] from sample [redacted] must have occurred prior to the separation of the lysis solution and the para-magnetic resin. The re-extraction of the stored substrate (i.e. the material originally submitted for DNA extraction that passed through the initial stages of the original extraction process) gave differing results. No DNA profile could be obtained from the substrate of [redacted]. However the substrate of sample [redacted] yielded a DNA profile that that obtained from the initial extraction process. This indicates that the DNA profile from this sample truly originated from that sample. This profile was also consistent with that obtained from other samples of a similar intimate nature from the same case. The results obtained from the investigation therefore indicate that contamination of sample [redacted] from sample [redacted] has occurred after the manual lysis of the substrates (off-deck lysis procedure), but prior to completion of the removal of lysed material from para-magnetic resin during the automated portion of the extraction procedure. The potential steps at which contamination may have occurred are outlined in Actions below.

Preformed By Quality Information System

Action Details

Action Complete	23/10/2008	**Action Fix Type**	Changed Process
Title	The potential		
Action Description	steps at which contamination may have occurred are: 1. During the transfer of the lysate obtained from manual lysis into the deep-well plate via the use of the Storstar. This however is considered unlikely. This is because lysate was added to well 5 prior to the addition of well 7 and with the two wells separated by well 6 (that failed to show evidence of contamination from the same profile) and the nature of the pipetting process whereby individual care is taken whilst performing the process. 2. During the removal of the adhesive seal used to seal the deep-well plate containing stored lysates awaiting automated DNA IQ extraction. This is the most likely as it was noted during Audit 8227 that condensation on the seal that could not be removed by centrifugation was a contamination risk. 3. There may have been operator error during the manual addition of DNA IQ para-magnetic resin during the start of the automated extraction procedure (i.e. incorrect pipetting procedure), however this is unlikely as staff are trained to perform such pipetting steps with due diligence and care. 4. During the 1st stage of removal of lysate from the para-magnetic resin to the storage plate. This procedure occurs twice during the automated extraction protocol (fresh disposable tips for each step). If there was bubbling of the lysate containing unbound DNA and this was to burst, contamination from well 5 into well 7 may account for the contamination event. However, the physical movement of the 8-tip arm during this liquid transfer makes this an unlikely proposition (i.e. once the tip retracts from well 5, it does not move directly over well 7). Actions As a result of previous OQI's raised and concerns identified around the automated DNA IQ extraction process, the extraction of samples using the automated DNA IQ procedure was halted on the 28-7-2008. Prior to this Audit 8227 had been commissioned and carried out. A number of areas for improvement were identified through the audit, and these have been implemented or are under investigation as outlined in OQI's 20367, 20368 and 20369. After the cessation of the automated DNA IQ extraction protocol, a review of all batches processed through this protocol was carried out by a specially commissioned team. A number of potential contamination events were identified and each is to be investigated on batch-by-batch basis. Additionally, careful review of results obtained from samples processed through the automated DNA IQ extraction procedure prior to reporting will be carried out. Every DNA result obtained from these samples will be interpreted with caution. Modifications have been made to the automated DNA IQ extraction procedure (including the use of an alternative to the adhesive seal and an alternative resin mixing procedure). This modified procedure will undergo extensive verification and approval from the DNA Analysis management team prior to re-introduction. The contamination events and concerns and improvements etc. that surround the automated DNA IQ extraction procedure have been discussed at various departmental and team meetings.		

Task Details

No Tasks found

Follow-up And Approval

Follow-up Status	Accepted
Follow-up Status Comment	27/11/2008 2:48:15 PM Amanda REEVES:

	No comment was recorded
Approver	
Approval/Rejection Date	01/12/2008
Approval/Rejection Comment	1/12/2008 12:00:00 AM Paula TAYLOR: No comment was recorded

Associations

No Associations found

Records

No Records found

20437 No Title Provided

Report for QIS OQI as of 12/10/2022 11:20:37 PM

Report for QIS OQI -

21589 No Title Provided

OQI Details

Status	Closed Approved
Subject	Sample [redacted] was a negative control on batch RFIQLYS20080627_01 and RFIQEXT20080630_01. These batches only contained project samples. During the Genemapper ID-X validation it was noted that this sample contained a below threshold profile. Additionally this sample did not contain a 9FTAR Processing Comment to indicate this sample was a negative extraction control.
Source of OQI	Internal Problems (QHPSS)
Date Identified	05/12/2008

OQI Creator Contact Details

Creator	Chiron WEBER
Organisational Unit/s	DNA Analysis
Service/s	
Site Location/s	Coopers Plains

Investigator/Actioner Contact Details

Actioner	Allan MCNEVIN
Organisational Unit/s	Analytical
Service/s	
Site Location/s	Coopers Plains

Investigation Details

Investigation Completed	27/03/2009 **Root Cause Type** Procedure/Method/Process
Investigation Details	Extraction batch RFIQEXT20080630_01 consisted of a positive and a negative extraction control and 15 samples. The 15 samples were samples used in the assessment of a new swab type (Copan 4N6 swab). Each sample consisted of 30ul of buccal suspension from the same volunteer (previously profiled staff member) spotted onto various swabs. There were three swab types under test and each was done five times (therefore 15 samples).

After initial processing and profiling, all samples and controls yielded expected results and no further action was taken.

At a later stage, during the validation of a new software package for the analysis of DNA profiles, GeneMapper-IDX, it was noted that the negative extraction control (barcode 357280480) from extraction batch RFIQEXT20080630_01 contained a partial DNA profile that were not previously detected. This was due to the reduced peak detection threshold used in GeneMapper-IDX. The negative extraction control was concentrated using microcon filter centrifugation. All alleles obtained from initial processing and after concentration were consistent with the DNA profile obtained from all 15 samples. It is notable, however, that at the time of processing, there was an expectation of staff to observe potential peaks below peak detection threshold in negative extraction controls during sample analysis.

At the time of processing extraction batch RFIQEXT20080630_01, the process in use at the time was a two-stage automated extraction method using promega DNA IQ technology. This process involved a manual tube lysis of samples, storage of the lysed sample in a deep-well plate (that was potentially sealed and stored at 4oC prior to processing), then DNA extraction on the MPII liquid handling platform. During the automated extraction, the lysate containing the lysis buffers and any potentially un-bound DNA (i.e. DNA not bound to the para-magnetic resin) was removed and stored. This stored lysate was re-extracted for all samples and controls on the extraction batch. DNA profiles obtained from the stored lysates were all consistent with those obtained from initial processing.

Therefore, contamination of the negative extraction control had occurred prior to or during the removal of the lysate during the automated extraction process. As noted in audit 8227 and a number of OQi's prior to and

subsequent to the audit, a number of steps within the automated extraction and associated processes were potentially the cause of the contamination event. These are:
1. During the processing of the manual in-tube lysis procedure or transfer of the lysate into the deep-well plate via the use of the Storstar. This however is considered unlikely as the lysate of the negative extraction control was processed at all stages prior to any samples and the nature of the processing whereby individual care is during the process to prevent contamination.
2. During the removal of the adhesive seal used to seal the deep-well plate containing stored lysates awaiting automated DNA IQ extraction. This is the most likely as it was noted during Audit 8227 that condensation on the seal that could not be removed by centrifugation was a contamination risk. Additionally, it was observed in one instance that seepage across the adhesive seal (from a positive to a negative control in adjacent wells) appeared to have occurred. The negative extraction control was in an adjacent well and diagonal from samples.
3. There may have been operator error during the manual addition of DNA IQ para-magnetic resin during the start of the automated extraction procedure (i.e. incorrect pipetting procedure), however this is unlikely as staff are trained to perform such pipetting steps with due diligence and care.
4. During the mixing of the deep-well plate (containing 1.5mL of buffers and resin within a 2.2mL well) on the extraction platform DPC shaker. This had not been observed, however has been proposed as a possible mechanism during close scrutiny of the automated procedure.
5. During the 1st stage of removal of lysate from the para-magnetic resin to the storage plate. This procedure occurs twice during the automated extraction protocol (fresh disposable tips for each step). If there was dripping of the lysate containing unbound DNA and this was to drip from from a tip containing sample (or a bubble of liquid burst), this may account for the

contamination event.

Preformed By Allan MCNEVIN

Action Details

Action Complete 27/03/2009 **Action Fix Type** Expenditure of Resources

Title Improvements to automated DNA IQ protocol & implementation of GeneMapper-IDX

Action Description As a result of previous OQI's raised and concerns identified around the automated DNA IQ extraction process, the extraction of samples using the automated DNA IQ procedure was halted on the 28-7-2008.

Prior to this, Audit 8227 had been commissioned and carried out. A number of areas for improvement were identified through the audit, and these have been implemented as outlined in OQI's 20367, 20368 and 20369.

After the cessation of the automated DNA IQ extraction protocol, a review of all batches processed through this protocol was carried out by a specially commissioned team. A number of potential contamination events were identified and each investigated on a batch-by-batch basis. Careful review of results obtained from samples processed through the automated DNA IQ extraction procedure prior to reporting will be carried out. Every DNA result obtained from these samples will be interpreted with caution.

Modifications have been made to the automated DNA IQ extraction procedure. This modified procedure is undergoing extensive verification and will require approval from the DNA Analysis management team prior to re-introduction. The contamination events and concerns and improvements etc. that surround the automated DNA IQ extraction procedure have been discussed at various departmental and team meetings.

Lastly, with the introduction of GeneMapper-IDX software, a procedure whereby all extraction negative controls are checked with a peak detection threshold of 20RFU (compared with 50RFU for samples) is performed in order to locate any potential below threshold profiles.

Task Details

No Tasks found

Follow-up And Approval

Follow-up Status Accepted

Follow-up Status Comment 22/04/2009 7:45:01 AM Chiron WEBER:

Investigation and findings OK.

Approver Paula BRISOTTO

Approval/Rejection Date 05/05/2009

Approval/Rejection Comment 5/05/2009 8:27:11 AM Paula TAYLOR:

Agreed

Associations

No Associations found

Records

No Records found

21589 No Title Provided

TN-10

Thomas Nurthen

From: Allan McNevin [redacted]
Sent: Friday, 18 April 2008 11:48 AM
Subject: Contamination of a reference sample - feedback please
Attachments: Reference Mixture Investigation - draft.doc

Hello guys,

I have written up a report on the investigation I have done where one FTA contaminated another somehow,

please carefully read the attached draft, I haven't yet created an OQI (I intend on doing so), and I am also at a loss to fully explain it,

I would like feedback on what you guys think about this event

thank you
Al

Allan McNevin
Senior Scientist - Analytical Section
DNA Analysis (Forensic Biology)
Forensic & Scientific Services
Queensland Health

[redacted].au

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[redacted] Mixture located in a Reference sample

[redacted] nior Scientist, DNA Analysis, Queensland Health

Investiga[redacted]

During the [redacted] GEN9REF20080211_05, it was noted that lab number 333993604 [redacted] DNA profile. R[redacted]ples should naturally be single sourc[redacted]act of 333993604 was then re-amplified to confirm presence of [redacted]ime, previous run results for 333993604 were reviewed. It was shown that 333993604 had been processed through the FTA punching process and given a sing[redacted]yper batch GEN9REF20080128_02). The result from GEN9REF2[redacted]m an extraction of the FTA sample. When re-amplified the DNA ext[redacted] presence of a mixture. The FTA sample 333993604 was then re-extracted under barcode 333802806 and yielded a single source profile cons[redacted]e contributors and the original FTA processing profile obtained fro[redacted]s are summarised in table 1 below.

T[redacted]93604

SAMPL[redacted]	EX	D8	D21	D18	D5	D13	D7
[redacted]	,Y	13,15	30,NR	NSD	12,NR	NSD	NSD
[redacted]	,Y	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	NSD
[redacted]	[redacted]	[redacted]	[redacted]3.2	14,16,17	11,12,13	8,9,11,12	8,11
[redacted]	[redacted]	[redacted]	[redacted]	14,17	12,13	8,12	8,11

The o[redacted]ssing for sample 333993604 (Genotyper batch GEN9[redacted] at lowered peak height RFU thresholds, with no evider[redacted]ure present. An analysis of results obtained after re-extraction (barcode 33380[redacted] lowered peak height RFU thresholds also showed no evidence of a mixtur[redacted]

The p[redacted]erefo[redacted]there h[redacted]contamination event of DNA extract 33399[redacted]econd contributor to the mixed DNA profile was then worked out (shown in table [redacted] searched against the staff database. No matches were found. When searc[redacted]other profiles obtained from the same extraction batch (FTAE[redacted]5_01) a match was found with sample 333941730.

Table [redacted]ofile, contributors & matches

SAMPLE ID	[redacted]	vWA	FGA	SEX	D8	D21	D18	D5	D13	D7
[redacted]	16,18	16,17	20,21,24	X,Y	12,13,15	29,30,33.2	14,16,17	11,12,13	8,9,11,12	8,11
[redacted]	16,16	16,17	20,24	X,Y	13,15	30,33.2	14,17	12,13	8,12	8,11
[redacted]	14,18	16,17	21,24	X,X	12,13	29,30	16,17	11,12	9,11	8,9
[redacted]	14,18	16,17	21,24	X,X	12,13	29,30	16,17	11,12	9,11	8,9

Figures 1 and 2 below shows profiles obtained from the original FTA processing (when re-anlaysed at lowered peak height RFU threshold) and re-amplification of the contaminated DNA extract of 333993604. F[redacted]ws the profile obtained from the re-extra[redacted] 333993604 (under lab number 333802806) and figure 4 shows profile obtained from the contaminating sample 333941730.

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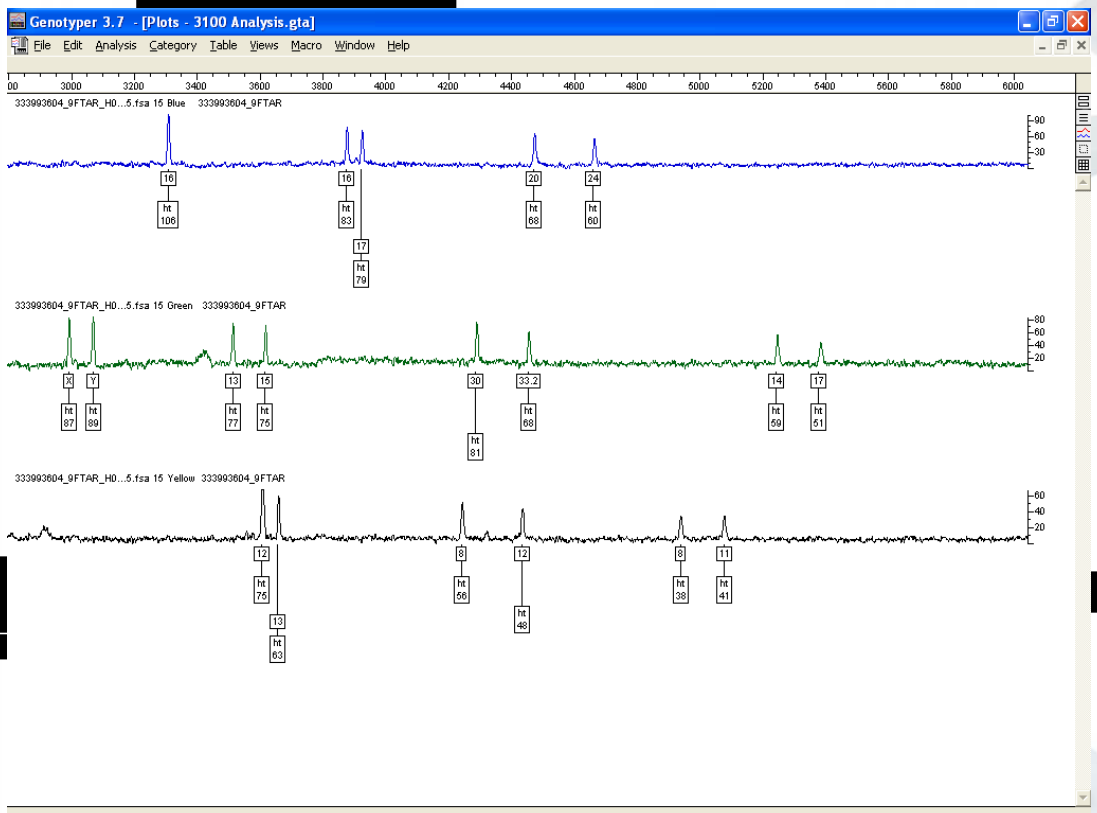
[redacted]



[redacted]

Figure [redacted] rofile from 333993604 from FTA processing

[redacted]

[redacted]

[redacted]

[redacted]

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[redacted]

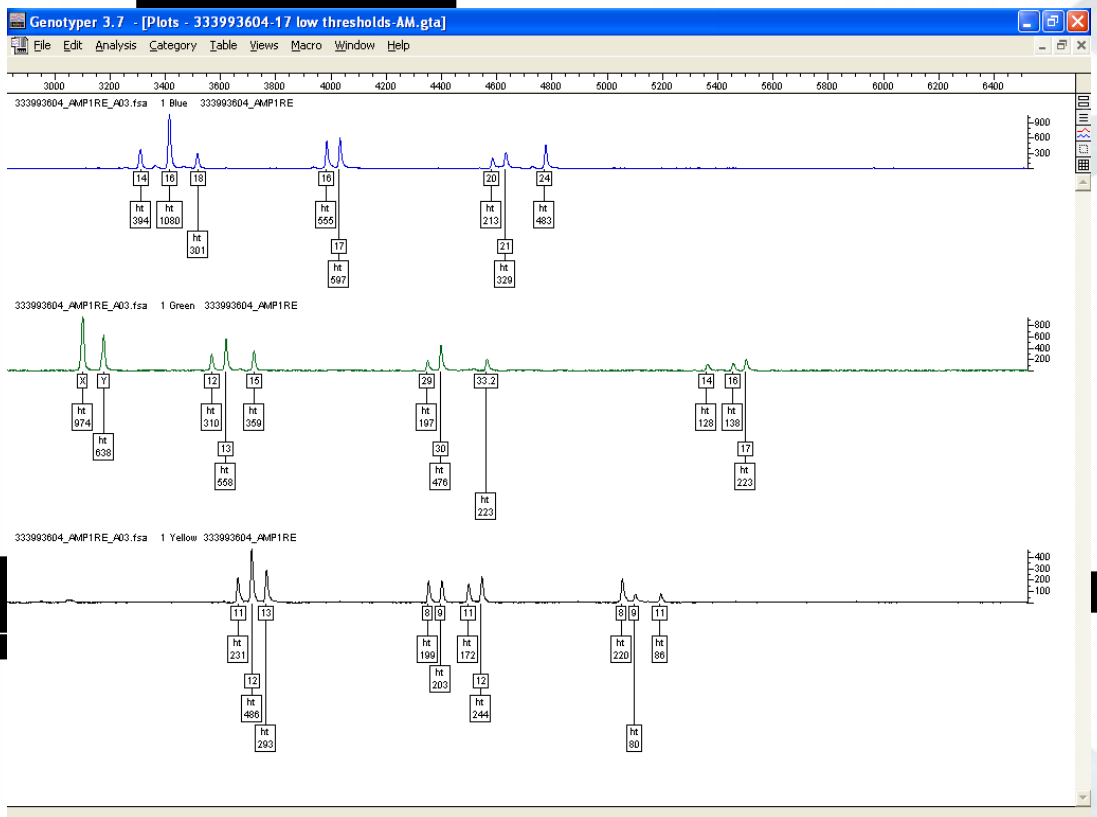

Figure [redacted] cation of contaminated DNA extract 333993604

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

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[redacted]

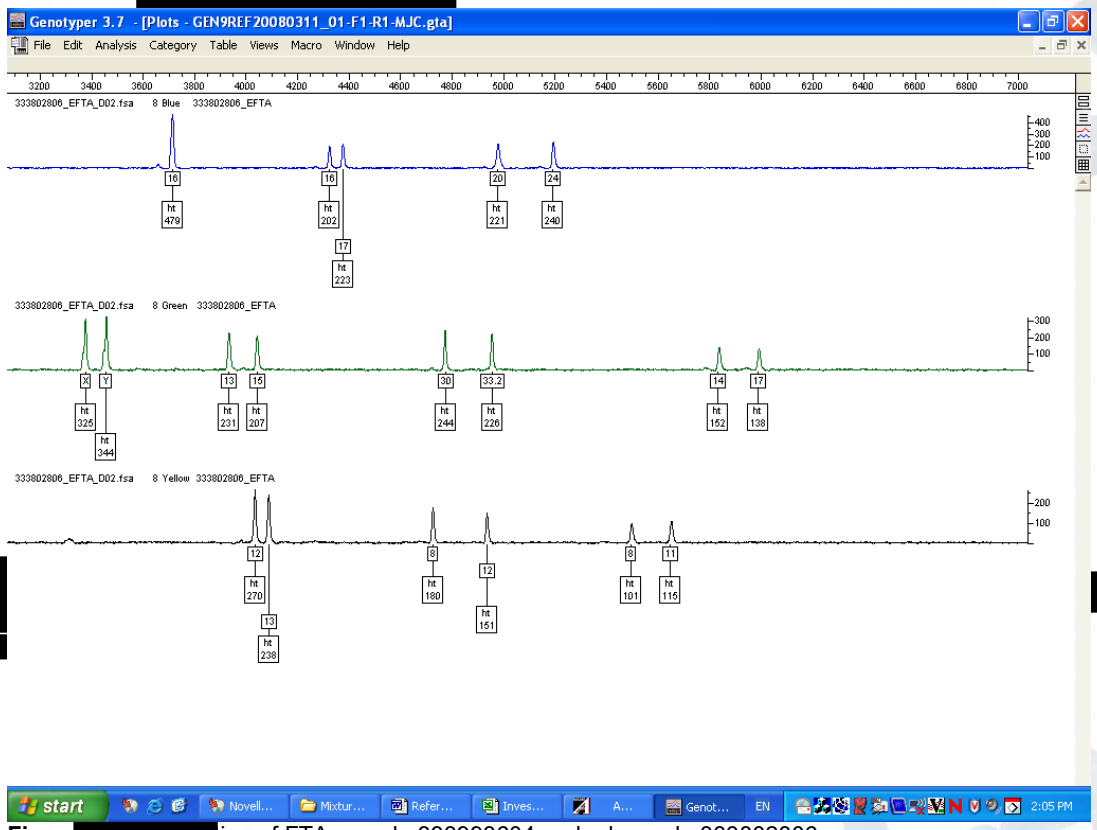

Figure [redacted]tion of FTA sample 333993604 under barcode 333802806

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

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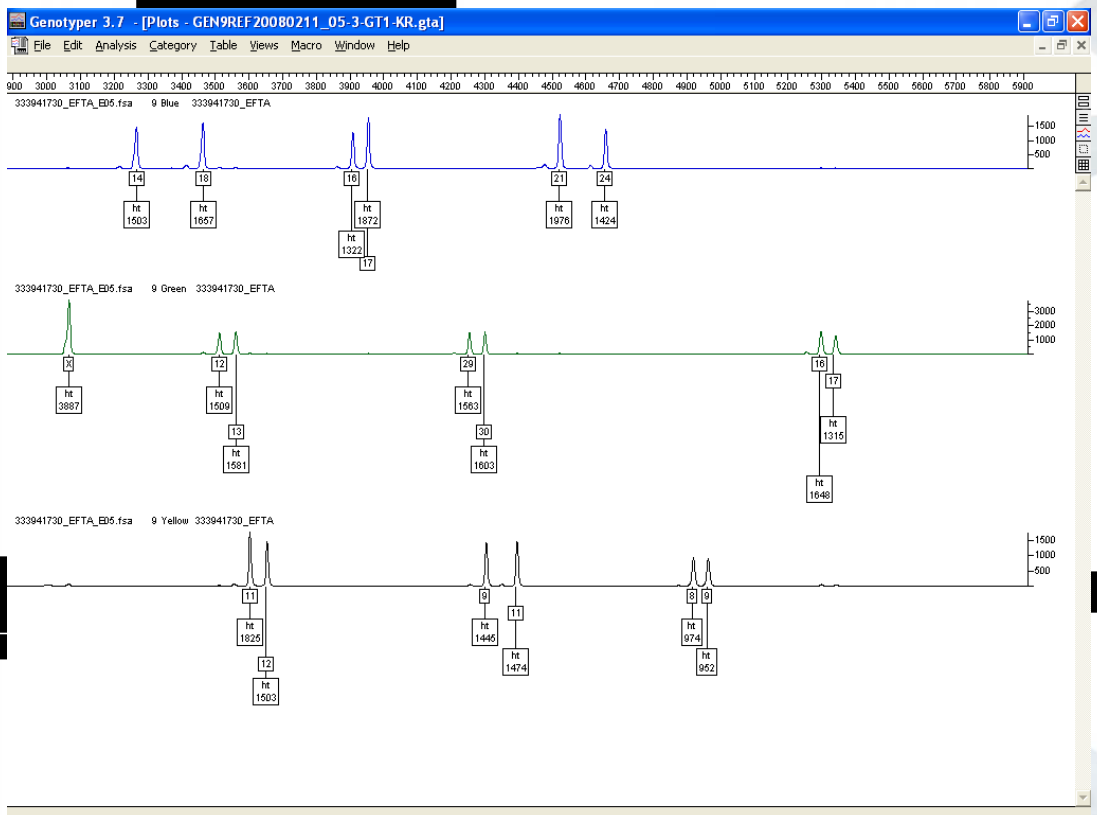
[redacted]



[redacted]

Figure [redacted] ture contributor 333941730

As bo[redacted]les [redacted] and [redacted] were extracted, quantified and amplified on the[redacted]es, further investigation was carried out. FTA samples requiring extraction are ha[redacted]m punches put into an appropriate well of a Slicprep™ 96 device (Prom[redacted] BSD 600 DUET. Well positions are designated according to a well map gener[redacted]he punching process (i.e. as each sample is processed, the barcode is scann[redacted]ll map is created). The well map is then imported into AUSLAB software and a[redacted]ated to mirror the well map. In the example under examination, the batch [redacted]_01 was the batch that the samples were punched into the Slicprep™ 96 [redacted] this batch is completed in AUSLAB, another batch is then created in exactly the [redacted]ion etc. for the DNA extraction process (utlising the Promega DNA IQ kit in [redacted]ith a PerkinElmer MultiPROBE® II PLUS HT EX with Gripper™ Integration liquid handling platform dedicated to DNA extraction). In the example under investigation, the extraction batch was RFIQEXT20080205_03.

On extraction batch, sample [redacted] was processed in position 12 (D2) and [redacted] was processed in position 25 (A4). These positions are not adjacent and therefore any potential contamination from one sample to the next during either the BSD punching process or the automated extraction process very unlikely. A review of the spin basket portion of the Slicprep™ 96 device showed 4 spots in each of the well positions, as would be expected, therefore there has been no “jumping” of spots.

After completion of extraction batch RFIQEXT20080205_03, both DNA extracts are sent to the outstanding quantification batch allocation list for DNA quantification (using Applied Biosytems Quantifiler™ Human DNA Quantification kit in conjunction with a PerkinElmer MultiPROBE® II PLUS HT EX with Gripper™ Integration liquid handling platform dedicated to PCR set up). Both samples were then allocated to the same quantification batch

(QUAREF2 ell positions, 333993604 in position 47 (G6) and 333941730 NA quantification, results are imported into AUSLAB and a calcu ftware determine the volume of DNA extract to be added to th plied Biosystems AMPFℓSTR Profiler Plus® PCR Amplificatio onsequently amplified on the same amplification batch (9AMPR200 ell positions, position 38 (F5) and 333941730 mmary of this information, in conjunction with quantificatio ble 3 below.

Table 3. batch ID's and quantification values of affected samples

Sample ID		333941730
Batch ID	0205_03	FTAEXT20080205_03 pos 25 (A4)
	0205_03	RFIQEXT20080205_03 pos 25 (A4)
position (well	0206_01 pos 47 (G6)	QUAREF20080206_01 pos 46 (F6)
	1	9AMPR20080207_01 pos 37 (E5)
		1.7100ng/ul

ies or notations made on utlined in table 3 to suggest any unusual these batches.

As ou ation is unlikely to have occurred at the extraction or ext aration (BSD processing) steps. Contamination must have occurred prior to ampli he mixture was detected after the first amplification of the extract). There s between completion of extraction and amplification must be considered. These

a. f the es du and retrieval after extraction, prior to ion, after quantification, prior to amplification.
b. uncapping or recapping of extract tubes in the quantification procedure or of tubes at the start of the amplification procedure
c. pipetting of DNA extracts during the quantification procedure

The p NA extracts during the amplification procedure can not be considered as DNA 41730 required a dilution of the extract prior to addition to the amplification o the pipetting order and tip movements on the Multiprobe II, DNA extract ll have been added to the PCR reaction prior to any pipetting of DNA extract

During preparation of DNA extracts for quantification and amplification the caps of all DNA extract tubes are removed simultaneously using the LifeTool™ RECAP 96M. DNA extracts are vortexed then centrifuged prior to being placed within the RECAP 96 for decapping. During pipetting of DNA extracts when preparing quantification batches, the Multiprobe II tips move along the X axis of motion, not the Y axis, therefore if contamination were to occur the most logical expectation is for a sample located in E5 to contaminate well E6, not F5.

When considering whether any of these steps are likely to be when contamination has occurred, is worth considering how much of DNA extract 333941730 would be needed to transferred into 333993604 to cause the level of contamination that was visualised. With the help of senior scientist Justin Howes, the separation of mixture components and an assessment of the mixture ratio was made. The approximate mixture ratio is 1:1, or at the most conservative value (i.e. the least amount of DNA extract 333941730), 2:1 where the mixture would consist of twice the amount of DNA from 333993604 as from 333941730. The DNA extract 333941730 was quantified at 1.71ng/ul, this is approximately 20x the

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concentrati[redacted]he method of dual elution that is done with the routine method of [redacted]d at FSS DNA Analysis yields a final extract volume of 100µL, to d[redacted]ewhere between 1:1 and 2:1, approximately 2.5-5µL of DNA extrac[redacted] have been transferred to DNA extract 333993604. This is a ve[redacted]ally if considering droplet or aerosol formation.

At current (1[redacted]n raised

[redacted]

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Testing of samples with different detergents in the extraction lysis buffer

Dr. Vojtech Hlinka
11th of April, 2008

Background:
Previously we have found that SDS does cause a precipitate to form during DNA IQ extractions. This suspends the beads and leads to bead and therefore DNA loss. On the other hand, Sarcosyl and Triton X-100 did not form these precipitates. At this stage it was unknown how the detergents would affect DNA yield and profiling results.

Aim:
To determine the effect of different detergents on DNA yield and profile quality using DNA IQ.

Method:
1. 30µL of blood samples were spotted onto each of twelve swabs (QC blood swab VKI Lot GSL02112007_01).
2. Buccal cell samples were created. One cytobrush was utilised per each cheek. One cheek side was brushed with one cytobrush for 1 minute. Then the cytobrush was whirled in 2mL saline. The other cheek side was brushed with a second cytobrush for 1 minute. Then the cytobrush was whirled in 2mL saline.
3. 10µL of buccal cell samples (Cell donor CJA) were spotted onto each of twelve swabs using the following method.
4. One negative control was extracted per detergent set.

The extraction batch for this experiment was VALB20071129_01, while the Quantifiler batch was QUAVAL20071203_01.

		Sample No	26
		µL/sample	µL
SDS buffer	TNE	462.5	12025
	Proteinase K	25	650
	SDS (20% w/v)	12.5	325
Sarcosyl buffer	TNE	462.5	12025
	Proteinase K	25	650
	Sarcosyl (40% w/v)	12.5	325
Triton X-100 buffer	TNE	470	12220
	Proteinase K	25	650
	Triton X-100 (100%)	5	130

Results and Discussion:

Quantifiler concentration values for the samples extracted with different detergents

Quant values	SDS	Sarcosyl	Triton X-100
Negative Control	0.000	0.000	0.000
30uL QC blood swab	0.503	0.546	0.181
30uL QC blood swab	0.565	0.532	0.179
30uL QC blood swab	0.624	0.490	0.161
30uL QC blood swab	0.000	0.511	0.169
Mean	**0.423**	**0.520**	**0.173**
StdDev	**0.286**	**0.025**	**0.009**
10uL cell swab	0.0552	0.0390	0.0916
10uL cell swab	0.0430	0.0638	0.0776
10uL cell swab	0.0531	0.0525	0.0607
10uL cell swab	0.0711	0.0471	0.0641
Mean	**0.0556**	**0.0506**	**0.0735**
StdDev	**0.0116**	**0.0104**	**0.0141**

Profiling results for the samples extracted with different detergents

Samples	**SDS**	**Sarcosyl**	**Triton X-100**
Negative Control	NSD OK	NSD OK	NSD OK
30uL QC blood swab	OK	OK	OK
30uL QC blood swab	OK	OK	OK
30uL QC blood swab	OK	OK	OK
30uL QC blood swab	XS	OK	OK
10uL cell swab	PA	OK	OK
10uL cell swab	PA Bad injection	PA	OK
10uL cell swab	OK	OK	PA AI@D5 ~57.6%
10uL cell swab	OK	OK	PA

Quantifiler values were lower for the Triton X-100 blood extractions and highest for the Triton X-100 cell extractions. Sarcosyl was comparable to SDS in approximate Quantifiler value. Profiling results were comparable across all the detergents.

Conclusion:

Sarcosyl-extracted samples appeared to be the best overall because they produced similar results to those from SDS but did not result in forming the gel-like substance often encountered with SDS. Triton X-100-extracted samples produced low Quantifiler concentrations for blood samples and therefore this detergent is not recommended for use as a detergent in the TNE extraction buffer for samples of various origins when utilised with DNA IQ. Sarcosyl is recommended as a superior substitute for SDS in the TNE extraction buffer.

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Investig[redacted]ubstance in extraction buffer

Dr. Vojte[redacted]
11th of Ap[redacted]

1. Abstra[redacted]

The modified DNA IQ™ protocol utilises Sodium Dodecyl Sulfate (SDS) is an ionic detergent to aid in th[redacted]r membrane. SDS is known to precipitate at low temperature[redacted]gths nad has been observed in our manual and au[redacted]tion protocols. In this experiment SDS was compared with two other de[redacted]osyl was found to perform as well as SDS, Triton X[redacted]

[redacted]

[redacted]e to form during DNA IQ e[redacted] leads to bead and therefore DNA loss. On the other [redacted] not form these precipitates. At this stage it was unkno[redacted]NA yield and profiling results.

3. A[redacted]

To de[redacted]effect of different detergents on DNA yield and profile quality using DNA IQ.

4. M[redacted]

4.[redacted] of blood samples were spotted onto each of twelve swabs (QC blood swab [redacted]ot GSL02112007_01).

4.[redacted]al cell samples were created. One cytobrush was utilised per each cheek. [redacted] cheek side was brushed with one cytobrush for 1 minute. Then the [redacted]rush was whirled in 2mL saline. The other cheek side was brushed with a [redacted]nd cytobrush for 1 minute. Then the cytobrush was whirled in 2mL saline.

[redacted]µL of buccal cell samples (Cell donor CJA) were spotted onto each of twelve [redacted]wabs using the following method.

[redacted]One negative control was extracted per detergent set.

[redacted]

5. Viscosity experiment

1 swab, 2 swabs, blank

Initial results

All +SDS formed viscous liquid
-SDS was not viscous

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After weel

+ SDS +D

Most visco iscous and be ed even in blank.

-SDS +DT

Very visco ank.

+SDS –DT

Moderatel n blank

-

S			Sample	Gel rating	
Cotto		ut	Cotton wooden swab No blood Cut	50.00%	gel
Cotto		ncut	Cotton wooden swab No blood Uncut	50.00%	gel
Cotto	wab	Blood Cut	Cotton wooden swab Blood Cut	0.00%	no gel
Cotto	wab	Blood Uncut	Cotton wooden swab Blood Uncut	0.00%	no gel
Cotto	b	No blood Cut	Cotton paper swab No blood Cut	75.00%	gel
Cotto	b	No blood Uncut	Cotton paper swab No blood Uncut	80.00%	gel
Cotto	b	od	Cotton paper swab Blood Cut	0.00%	no gel
Cotto	b	Blood Uncut	Cotton paper swab Blood Uncut	0.00%	no gel
Starc	oden swab	Blood	Starch Cotton wooden swab Blood	0.00%	no gel
Starc	oden swab	No blood	Starch Cotton wooden swab No blood	80.00%	gel
Starc	er swab	Blood	Starch Cotton paper swab Blood	0.00%	no gel
Starc	er swab	No blood	Starch Cotton paper swab No blood	80.00%	gel
FTA	nches	No blood	FTA 3.2mm 0 punches No blood	20.00%	slight gel-li
	punch	No blood	FTA 3.2mm 1 punch No blood	20.00%	slight gel-li
	2 punches	No blood	FTA 3.2mm 2 punches No blood	20.00%	slight gel-li
	3 punches	No blood	FTA 3.2mm 3 punches No blood	20.00%	slight gel-li
FTA 3.2mm 4 punches		No blood	FTA 3.2mm 4 punches No blood	20.00%	slight gel-li
FTA 3.2mm 5 punches		No blood	FTA 3.2mm 5 punches No blood	20.00%	slight gel-li
Starch		Blood	Starch Blood	0.00%	no gel
Starch			Starch No blood	80.00%	gel-like
FTA starch		Blood	FTA starch Blood	0.00%	no gel
FTA starch		No blood	FTA starch No blood	67.00%	gel
FTA 3.2mm 0 punches		Blood	FTA 3.2mm 0 punches Blood	0.00%	no gel
FTA 3.2mm 1 punch		Blood	FTA 3.2mm 1 punch Blood	0.00%	no gel
FTA 3.2mm 2 punches		Blood	FTA 3.2mm 2 punches Blood	0.00%	no gel
FTA 3.2mm 3 punches		Blood	FTA 3.2mm 3 punches Blood	0.00%	no gel
FTA 3.2mm 4 punches		Blood	FTA 3.2mm 4 punches Blood	0.00%	no gel
FTA 3.2mm 5 punches		Blood	FTA 3.2mm 5 punches Blood	0.00%	no gel

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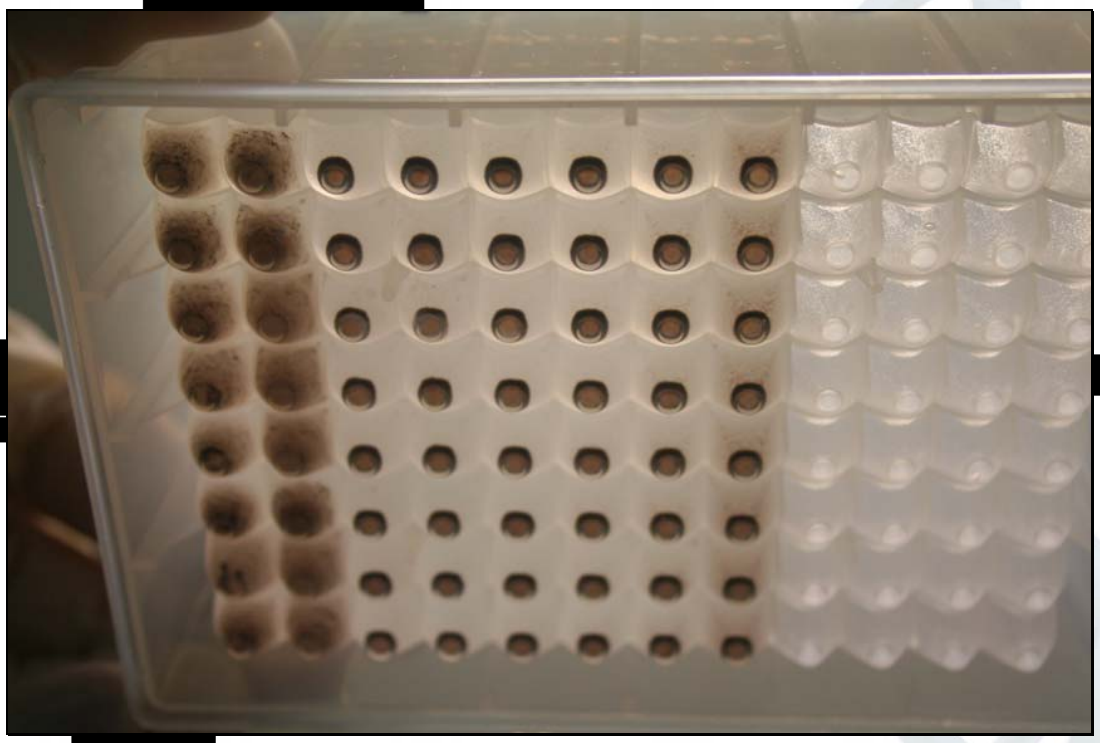
[redacted]

6. Quan[redacted]



Figur[redacted]

[redacted]

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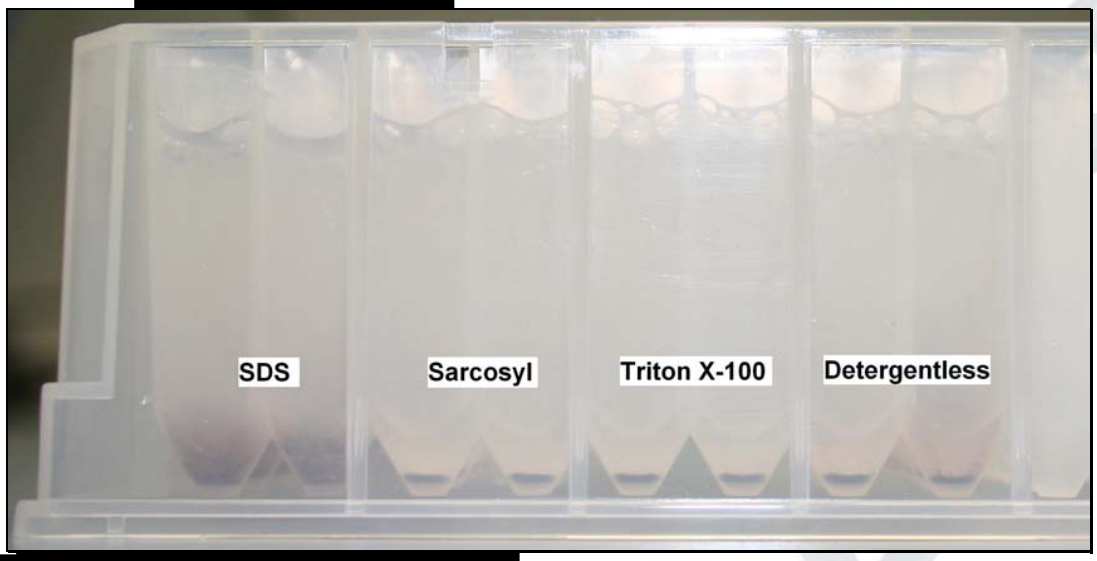
[redacted]



[redacted]

		µL/sample	µL
		462.5	12025
SDS		25	650
	SDS (20% w/v)	12.5	325
	TNE	462.5	12025
Sarc	Proteinase K	25	650
	S[redacted] w/v)[redacted]		325
	TNE	470	12220
Triton [redacted]er	Proteinase K	25	650
	Triton X-100 (100%)	5	130

[redacted] **and Discussion:**

Quantifiler concentration values for the samples extracted with different detergents

[redacted]

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		Sarcosyl Quant (ng/µL)	Triton X-100 Quant (ng/µL)
Negative c		0.000	0.000
QC Swab_		0.546	0.181
QC Swab_		0.532	0.179
QC Swab_3		0.490	0.161
QC Swab_4		0.511	0.169
Mean	**0.423**	**0.520**	**0.173**
Std Dev	**0.286**	**0.025**	**0.009**
Cell Swab_		0.0390	0.0916
Cell Swab_		0.0638	0.0776
Cell Swab_	0.0551	0.0525	0.0607
Cell Swab_		0.0471	0.0641
Mean	**0.0556**	**0.0506**	**0.0735**
Std Dev	**0.0116**	**0.0104**	**0.0141**

gure 3

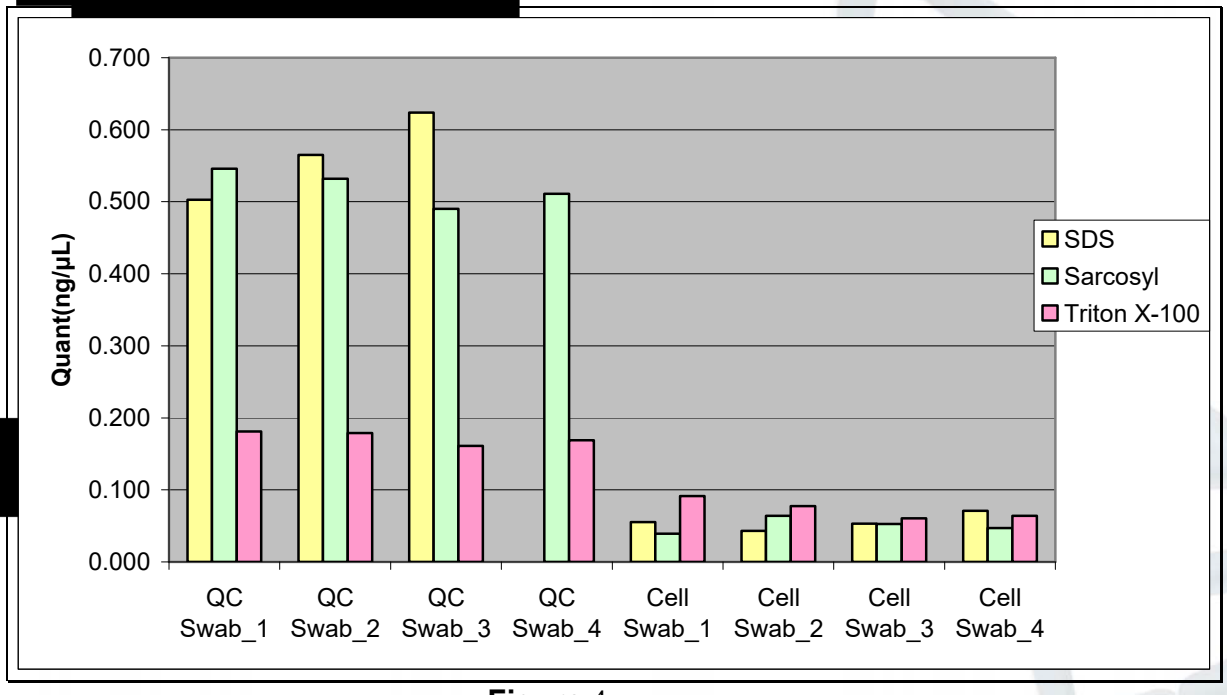

Figure 4

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		Sarcosyl	Triton X-100
Negative c		NSD OK	NSD OK
QC Swab_		OK	OK
QC Swab_		OK	OK
QC Swab_3		OK	OK
QC Swab_4		OK	OK
Cell Swab_1	PA	OK	OK
Cell Swab_2	PA Bad injection	PA	OK
Cell Swab_		OK	PA AI@D5 ~57.6%
Cell Swab_		OK	PA

Figur e samples extracted with different detergents

C -100 blood extractions and highest for the Triton X arable to SDS in approximate Quantifiler value. the d

Sarco the best overall because they produced similar result from SDS but did not result in forming the gel-like substance often encou n SDS. Triton X-100-extracted samples produced low Quantifiler conce blood samples and therefore this detergent is not recommended for use as a dete TNE extraction buffer for samples of various origins when utilised with DNA IQ. Sa ommended as a superior substitute for SDS in the TNE extraction buffer.

9. R s:

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DNA Analysis - Management Team

AGENDA

Chairperson:	JAH	**Date and Time:**	14 July 2008 2pm – 3.45pm
Venue:	FSS Conference Room 102	**Contact:**	
Attendees:	VKI, JAH, AAP, AM, AJS, EJC, KDR, PT, PAC, RS, TEN, WH		
Apologies:	CJA		
Guests:	Ingrid Moeller, Julie Connell		

1.0 PREVIOUS MINUTES ENDORSED

No Previous meeting held

2.0 NEW BUSINESS

OQIs and actions around reporting and investigation OQI trends.

- OQIs 19768, 19477, and 19349 refer. Positive control profiles found in negative controls on two separate occasions. Case work profile appears to have contaminated 3 wells on another plate, including the negative control.

  Appears that well to well contamination has occurred during extraction.

- How are we going to report these cases affected? i.e "Quality Failure has been detected".

  Need to establish words for inclusion in statements.

  Need to be prepared to answer questions in court.

- Agreed to not report on all samples affected in the OQIs mentioned.

Where EXRSs have been released, need to send an Intell letter to FIRMU to explain the quality failure and the changes to results.

New EXR to be created to use for results not yet reported and appropriate wording to be developed for statements.

Agreed that DNA Analysis should be conservative; do not report on samples affected.

Full disclosure is a must. Reporting Scientist should be comfortable and confident in their reporting.

- For future cases of contamination – an EXR line (specific wording) will need to be created.

- Need to determine the source, to prevent reoccurrence. Amy, Iman and Peter Clausen are currently reviewing processes. Chiron is collating information on equipment / trends etc.

  All but one of the issues appeared to be attributed to Platform A.

- An audit is currently being conducted on all processes. Appears that the contamination is sporadic.

- Vanessa to communicate to DNA Analysis regarding OQI processes; what we are doing to identify the source.

- Agreed to commence from tomorrow (15 July 2008) to have –
  - 1 pos control
  - 1 neg control
  - 47 blank
  - 47 samples

per plate. Working in a diagonal design. Blanks will be amped to see if any problems.

Robyn to advise Operational Officers of the need to register all 47 blank spaces on the batch.

- Time required to manually extract - 4 hrs for 24 samples.

- During Audit Process - Platform A to be only used for Reference samples.
- Platform B to be used for casework.

- All Analytical OQIs are detailed on the Analytical Issues Log – adverse events log tab.

- Agreed that a discussion needs to be held at action/approval stage of OQIs.
- Robyn to flag these occurrences at management team meetings.

This is in addition to current email correspondence about OQI.

- Allan to update Minor Change Register regarding change in checkerboard pattern for extractions.

3.0 CLOSURE

Queensland Government
Queensland Health

MEMORANDUM

To: DNA Analysis

Copies to:

From: Vanessa Ientile, Managing Scientist [redacted]

Subject: DNA IQ Extractions

File Ref: 140708

An extraordinary management team meeting was held on the 14th July to discuss what actions should be taken to address concerns within DNA Analysis in relation to reporting samples on extraction batches referred to in a number of OQIs. The purpose of this memo is to outline the agreed decisions and actions as a result of the meeting.

Concerns have been raised specifically in relation to OQIs # 19477, 19768 and 19349. Initial investigations appear to indicate some form of well to well cross contamination during the extraction process. The investigations undertaken to date have not been able to identify the cause of this contamination. The three events have occurred on or around the following dates:

OQI#19477 – 29/4/08
OQI#19768 – 2/5/08
OQI#19349 – 25/2/08

As each of these events was discovered a thorough investigation commenced to determine the possible causes. This process involves attempting to identifying the point at which the contamination may have occurred by repeating the samples (i.e. extraction, quantitation, amplification, 3130), determining where the contamination came from (well to well, automated or manual process, operator error etc) and working out whether additional controls or changes to procedures are required to prevent the event from reoccurring.

Automated DNA IQ extractions were introduced in October 2007, after an extensive validation process. The results of various tests undertaken during this validation phase demonstrated no well to well contamination or transfer. This process was approved and implementation was agreed to by the management team with the understanding that ongoing optimisation would continue as part of the normal continuous improvement process.

Given the issues cannot be easily identified or reproduced, the following actions were agreed to by the Management team:

Reporting the results from the affected extraction batches

All samples on the affected extraction batches will be reported as a "quality system failure" and therefore the profiles or matches will not be reported. It was agreed that full disclosure is required.
A new EXR line will be created to cover this.
Appropriate statement wording is being developed.
If any of these samples have been previously reported via EXRs, then an Intelligence type letter to FIRMU will be drafted explaining the changes to the results reported.

Further investigations

An audit of the entire automated extraction process is underway to attempt to identify any potential causes or areas of improvement. This audit is being conducted by Amy Cheng, Iman Muhuram and Pete Clausen.
Further testing may be required to rule out or confirm potential causes identified through this process.

The details of all the events are being reviewed to see if any common factors can be identified. For example at this early stage, it appears that all but one event has occurred on Platform A. No confirmed trends have yet been identified.

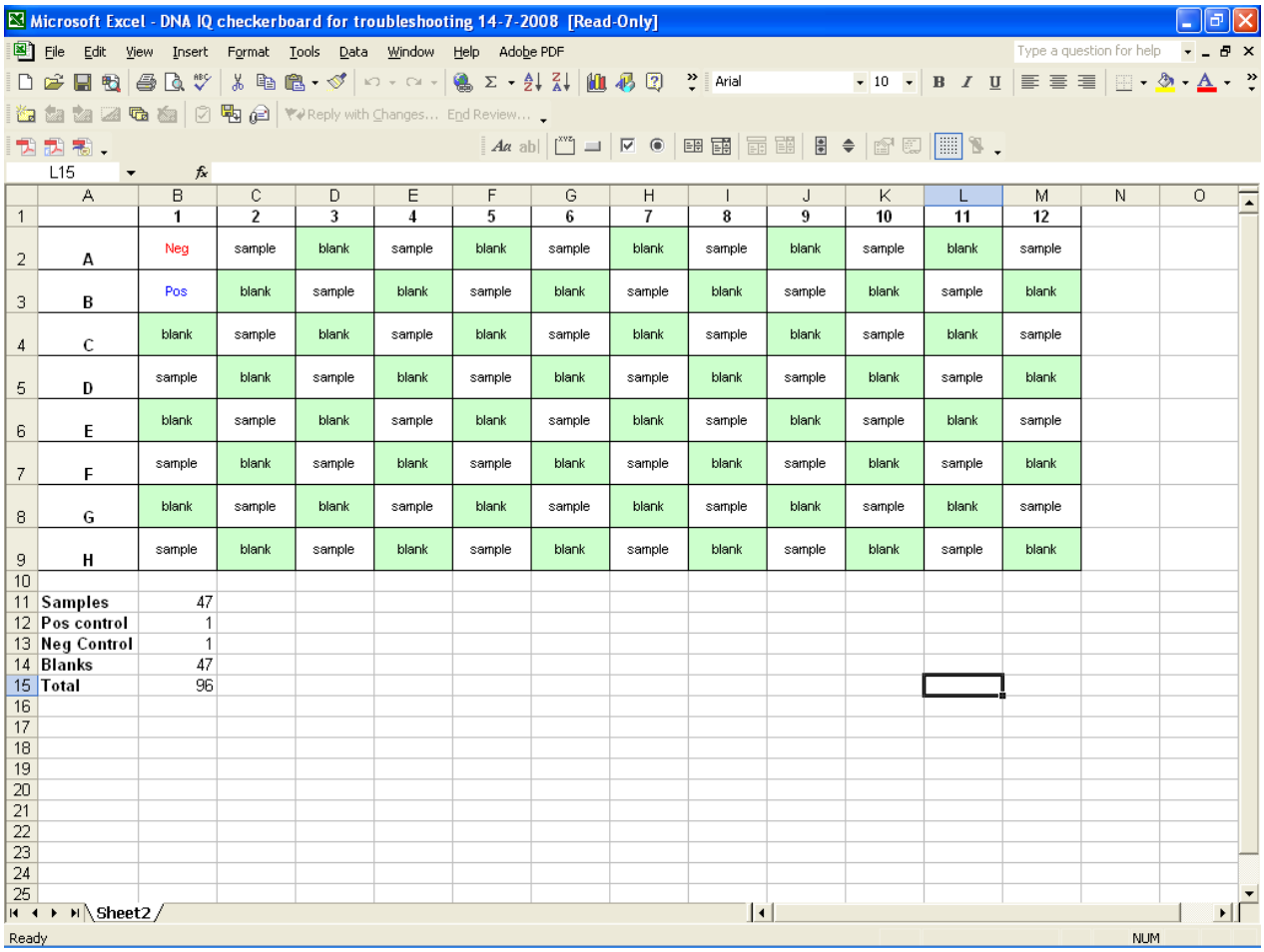
Interim changes to procedures

The decision has been made that processing will need to continue during the investigation phase. It is not feasible to stop all extractions. It is also not feasible at this stage to move to completely manual processing. The interim changes decided upon are designed to increase the number of controls used on each plate in an effort to identify any additional issues and minimise the chances of any further occurrences. As the findings from the audit become available, these interim changes will be reviewed as required.

From the 15th July 2008, the following changes will be implemented:

1. Platform A will not be used for casework extractions. It was agreed that reference samples could be extracted on platform A, as these can be repeated.
2. Case work extractions will continue on platform B with a new checkerboard sample arrangement (see example below). This will half the number of samples per batch but will allow us to easily identify through analysis of the blanks whether any additional well to well contamination has occurred.

These changes have been added to the Minor Change Register.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1		1	2	3	4	5	6	7	8	9	10	11	12		
2	A	Neg	sample	blank	sample	blank	sample	blank	sample	blank	sample	blank	sample		
3	B	Pos	blank	sample	blank	sample	blank	sample	blank	sample	blank	sample	blank		
4	C	blank	sample	blank	sample	blank	sample	blank	sample	blank	sample	blank	sample		
5	D	sample	blank	sample	blank	sample	blank	sample	blank	sample	blank	sample	blank		
6	E	blank	sample	blank	sample	blank	sample	blank	sample	blank	sample	blank	sample		
7	F	sample	blank	sample	blank	sample	blank	sample	blank	sample	blank	sample	blank		
8	G	blank	sample	blank	sample	blank	sample	blank	sample	blank	sample	blank	sample		
9	H	sample	blank	sample	blank	sample	blank	sample	blank	sample	blank	sample	blank		
10															
11	**Samples**	47													
12	**Pos control**	1													
13	**Neg Control**	1													
14	**Blanks**	47													
15	**Total**	96													

Additional updates will be provided to all staff as the investigation progresses.

Vanessa Ientile
Managing Scientist
14/7/2008

TN-15

Report for QIS Audit as of 12/10/2022 10:20:28 PM

Report for QIS Audit -

8227 DNA Extraction Process (DNA IQ)

Audit Contact Details

Contact	Thomas NURTHEN
Organisational Unit/s	Analytical
Site/Locations	Coopers Plains

Audit (Lead/Internal) Contact Details

Auditor (Internal/Lead)	Iman MUHARAM
Organisational Unit/s	DNA Analysis
Site/Locations	Coopers Plains

Other Auditors

Auditors (Other)	

Audit Details

Date Audit Performed	15/07/2008
Audit Type	Process
Audit Status	Closed
Audit Subject	DNA Extraction Process (DNA IQ)
Audit Objective	No Objective
Audit Scope	No Scope
Audit Criteria	No Criteria

Audit Outcome

Audit Findings	This audit was unable to determine the exact source of contamination as reported in OQI's 19477, 19768 and 19349. Although some risks for mislabelling, contamination or cross contamination exist in the procedure, there are appropriate and sufficient quality control measures in place to minimise these risks. Although we observed bubbles and droplets forming at the end of disposable tips during the automated DNA IQ protocol, these were not observed to have dripped into any wells and were discarded in the tip chute. Bubble formation can be reduced and eliminated by further optimising the pipetting parameters within the protocol. A follow-up of samples processed in checkerboard format on batch CWIQLYS20080714_02 did not show any instances of well-to-well cross contamination, as evidenced by the absence of DNA profiles in all of the water blanks. We commend the department for actively engaging in a continual methods improvement process (either to improve QA/QC or increase ease-of-use and efficiency of a procedure).

	The auditors have identified 28 points that may improve the automated DNA IQ extraction process, and have made the recommendations as outlined in the 18-page Audit 8227 report (Cheng, Clausen & Muharam, 2008) located in Quality Management, DNA Analysis. Three OQI's were raised as an outcome of the audit, in order to address the 28 points of recommendation: OQI 20367 - Automated DNA IQ process, including documentation. OQI 20368 - Enhancement of the MP II extraction platforms, including environment. OQI 20369 - Training and personnel related to the DNA IQ process. Please refer to the associated OQI's for the specific points addressed by each OQI.
Contact Comments	

Associations

Module	Document			
QIS Record	Automated DNA IQ Method of Extracting DNA from Reference and Casework samples			
QIS Record Number	24897	**Associated Version**	4.0Migrated Data from QIS	
Status	Superseded	**Current Version**	**Association Description**	
	version 1			
Module	OQI			
QIS Record	No Title Provided			
QIS Record Number	20368	**Associated Version**		
Status	Closed Approved	**Current Version**	**Association Description**	1
Module	OQI			
QIS Record	No Title Provided			
QIS Record Number	20368	**Associated Version**		
Status	Closed Approved	**Current Version**	**Association Description**	1
Module	OQI			
QIS Record	No Title Provided			
QIS Record Number	20367	**Associated Version**		
Status	Closed Approved	**Current Version**	**Association Description**	1
Module	Audit			
QIS Record	DNA IQ follow up audit			
QIS Record Number	9642	**Associated Version**		
Status	Closed	**Current Version**	**Association Description**	
	DNA IQ System (including Off-Deck Lysis)			

Records

No Records found

TN-16

From: Allan McNevin
To: Analytical Staff; Biology Management Team
Date: 23/07/2008 4:14 pm
Subject: Use of Extraction platforms

Hello all,

Further information has come to light bringing a total of 6 batches where there is observed cross-contamination most likely occurring on the extraction platforms. Investigation has shown that 3 of these were processed on platform A and 3 on platform B. Therefore initial assumptions that platform A may have been the problem have been eliminated.

Cathie and Vanessa have therefore agreed that processing of casework samples can occur on either platform (continuing to use the checkerboard pattern as previously discussed).

For Analytical staff - please leave platform A free for Chiron and Jenny to continue trialing the new magnet tomorrow (24/7)

cheers
Al

Allan McNevin
Senior Scientist - Analytical Section
DNA Analysis (Forensic Biology)
Forensic & Scientific Services
Queensland Health

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DNA Analysis - Management Team

AGENDA

Chairperson:	VKI	Date and Time:	28 July 2008 11.00am – 12.30pm
Venue:	FSS Conference Room 611	Contact:	
Attendees:	VKI, JAH, AAP, AM, AJS, EJC, KDR, PAC, PT, TEN, WH		
Apologies:	CJA , RS		
Guests:			

Follow up meeting on preliminary DNA IQ audit report

- Audit report has been prepared – currently in draft. PAC agrees with comments made in the draft report. Audit report covers process from start to finish.

Summary found at -
I:\AAA Analytical Section\Adverse event investigations\IQ extraction evaluation July 2008\DNA IQ troubleshooting strategy 25-7-08.doc

Agreed with findings and actions. Steps to commence -

1. Troubleshooting – look at performance of pipettes and syringes
2. Review of methods, programs by PerkinElmer and other labs using multiprobe (not limited to those using platforms for forensic work). Look at enhancing existing program. Including review of deck positions.
3. Look at assessing current maintenance and ascertain whether additional maintenance steps are required.

All of the above actions can run concurrently.

- All extractions to stop on the platforms immediately. Return to chelex and nucleospin extraction* (*as required)
- Availability of bench space is an issue. Tom and Allan to manage extractions with current amount of staff and using current bench space.
- Currently approx 6 batches that have had off deck lysis completed will now have manual IQ performed. Notes must be included that detail what steps will be taken to do this.
- How & When to return to using platforms?
  Discuss regularly at Management Team meetings to identify a decision point.

- A Virtual Extraction plate (mini extraction plate) to be done of everything processed since implementation of platforms (October 2007). A team will be but together to review these results. (team = staff from the Reporting teams). Need to cross check all extraction plates – priority plates first (most plates will have a priority 1 sample on them).

- VKI will forward a briefing to executive.

- Separate correspondence to be prepared for QPS regarding manual extraction processes / audit findings / timeframe reduction.

- Susan Brady, Angelina Keller, Rebecca Gregory to obtain the required information from AUSLAB (approx 140 batches) for the checking to commence. TEN to prepare a brief procedure on the process.

- Team Leaders to meet with their teams this afternoon (briefly) to advise of the outcomes of this meeting, with a full team meeting planned for tomorrow (Tuesday 29 July).

- Follow up individual team meetings to occur after this whole team meeting to answer any queries.

- Reporting of samples from October until present - Case Scientists to decide on a case by case basis.

TN-18

From: Vanessa Ientile
To: Forensic Biology
Date: 28/07/2008 1:20 pm
Subject: DNA IQ Extraction Update

Afternoon everyone

The management team has met this morning to review the draft audit report prepared by Iman, Pete and Amy and to determine the next steps.

The audit has highlighted a number of areas throughout the extraction process that require further investigation and optimisation.

This email is to give you all a very brief update that will be followed by team meetings this afternoon and tomorrow to discuss the outcomes and actions in more detail.

The following decisions have been made:

1. From today, we will revert to using chelex and nucleospin extraction protocols for all samples.
2. Samples that have been prepared up to off-deck lysis stage will be completed using manual DNA IQ extraction protocol.
3. The extraction platforms will not be used until further work is completed and we are satisfied that the issues seen have been addressed appropriately.

A more detailed explanation of the audit, the findings, the actions required and the flow on decisions will be provided in the whole team meeting tomorrow and will be discussed by your line managers with you this afternoon.

The management team is committed to addressing and resolving the issues and have agreed that this is the top priority. They are working together to ensure that adequate resources are allocated across the teams to this to resolve the issues as soon as possible. It is acknowledged that the changes we have implemented will decrease the capacity within the Analytical section and may impact on reporting but this is necessary at this stage and will be communicated to both the FSS Executive and QPS.

At this stage, I would also like to acknowledge and thank Iman, Pete and Amy for their efforts in auditing the procedures and preparing such a detailed report. I understand that they are still finalising the report and that it will be made available to everyone once they have finished with it.

Regards

Vanessa

Vanessa Ientile
Managing Scientist
DNA Analysis
Queensland Health
Forensic and Scientific Services
[redacted]

TN-19

Report for QIS Audit as of 12/10/2022 10:54:48 PM

Report for QIS Audit -

8752 Audit of all extraction batches from

Audit Contact Details

Contact	Cathie ALLEN
Organisational Unit/s	Evidence Recovery and Quality
Site/Locations	Coopers Plains

Audit (Lead/Internal) Contact Details

Auditor (Internal/Lead)	Susan BRADY
Organisational Unit/s	Intelligence
Site/Locations	Coopers Plains

Other Auditors

Auditors (Other)	

Audit Details

Date Audit Performed	28/07/2008
Audit Type	Process
Audit Status	Closed
Audit Subject	Audit of all extraction batches from implementation of DNAIQ and use of robots (23.10.2007 to present)to identify any contamination events.
Audit Objective	No Objective
Audit Scope	No Scope
Audit Criteria	No Criteria

Audit Outcome

Audit Findings	All initial results obtained from casework samples processed with DNA IQ and the MultiPROBE II PLUS HT EX platforms between the 23 October 2007 and the 28 July 2008 were investigated, this included a total of 216 extraction batches. Of these, 202 batches were released (94%), six batches were placed on hold pending the release of results (3%) and five batches were placed on hold due to the involvement in previous OQIs (2%). An additional three batches were quarantined through the identification of new contamination events that resulted in three new OQIs (1%). The methodology applied in this audit provides scientists with a new tool to reinforce the existing quality system. It may also assist in strengthening the confidence scientists have in reporting results. This quality tool could have applications outside the scope of this audit and be applied to other types of batches. Possible recommendations from this audit are listed below:

Contact Comments	- The Batch Comparison Macro could be applied to all new extraction batches to assist in the identification of any adverse events prior to the release of results. - If an adverse event is identified, a streamlined process needs to be in place to address the issue effectively with efficient laboratory communication (eg. e-mail alert system). - Availability of AUSLAB functions to export all results from any batch type, facilitating other types of audits and quality measures. - A previously identified system fault in AUSLAB needs to be addressed to ensure that all samples progress from extraction to quantification.

Associations

No Associations found

Records

No Records found

8752 Audit of all extraction batches from

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Management Team Meeting Minutes

Adverse Events – relating to OQIs & further investigations

Date: 11.30am, 5th August 2008

Present: EJC, RS, AJS, TEN, AM, KDR, AAP, JAH, PT, CJA

Issue: Possible well to well contamination on a plate with an unaffected Negative control (Unconfirmed mixture of a Hit & Run Case with a sexual assault)

Well to well contamination fits with the other related OQIs – it appears that a sample from column 3 has contaminated a well in column 1. This was detected by the Plate Macro being implemented by the Extraction Audit Team. There are other mixtures on this plate that are still being investigated.

Outcome: to consider the following proposal

- Determined that all the batches processed on the extraction platforms should be deemed 'Interpret with Caution'
- All batches that 'pass' the Plate Macro (ie no well to well contamination is detected) should be assessed by the Case Scientist for within case contamination
- Batches which have been designated as 'failing' can still be reported upon if specific criteria are met. Examples of this criteria are: the profile on the batch is 'unique' to that batch; profile obtained is from a SAIK/intimate swab and matches with donor and this is not an unexpected result.
- A set of guidelines to be drafted which support reporting or non-reporting of results from affected batches. The guidelines would include the consideration of re-sampling of the item or the particular stain originally submitted and requesting additional items/swabs from QPS.

The management team agreed to consider the above proposal overnight, to meet again on Wednesday morning at 8.30am to discuss proposal and if changes are required and then arrange a meeting with the reporting scientists to discuss this proposal with them and seek feedback.

TN-21

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Audit 8227. Process Audit of the Automated DNA IQ™ System (including Off-Deck Lysis)

2008

Amy Cheng, Peter Clausen, Iman Muharam

Forensic And Scientific Services

Clinical and Scientific Services

Queensland Health

safe | sustainable | appropriate

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Audit 8227: Process Audit of the Automated DNA IQ™ System (including Off-Deck Lysis)

Amy Cheng[1], Peter Clausen[2], Iman Muharam[3]
[1] Analytical Section, DNA Analysis, Forensic and Scientific Services
[2] Senior Scientist, Scientific Skills Development Unit, Forensic and Scientific Services
[3] Automation / LIMS Implementation Project, DNA Analysis, Forensic and Scientific Services

8 August 2008

1. Background

Initial investigations relating to OQI's 19477, 19768 and 19349 (Table 1) indicate potential instances of well-to-well cross contamination during automated DNA IQ™ extraction on the MultiPROBE® II PLUS HT EX platforms. Investigations performed so far, including repeating samples through extraction, quantitation, amplification and capillary electrophoresis protocols, have not been able to identify the exact cause of the contamination. A memo was released on 15 July 2008 to communicate these findings to DNA Analysis staff members.

Table 1. List of OQI's that document possible recent instances of well-to-well cross contamination.

OQI	Description	Investigation Summary
19477	A negative extraction control (ID 346795477) generated a DNA profile that was confirmed after reprocessing.	A DNA concentration value of 0.008ng/µL was detected during quantitation. A peak at Amelogenin was discovered. After performing a Microcon concentration, amplification and analysis at 30RFU, a full 9-loci profile was observed. The profile matched to 8 other samples (all from the same case) on the extraction batch. Three of these samples were found to be possible sources of the contamination.
19768	A negative extraction control (ID 346796064) generated a DNA profile that was confirmed after reprocessing.	A DNA concentration value of 0.005ng/µL was detected during quantitation. A peak at Amelogenin was discovered. After Microcon concentration, amplification and analysis at 30RFU, 15 discernable alleles were elucidated and matched to 2 samples from the same off-deck lysis and extraction batches.
19349	A negative extraction control (ID 346790262) generated a DNA profile that was confirmed after reprocessing.	The negative control did not yield a DNA concentration value. The control generated peaks below the 75RFU threshold. After re-preparation and reamplification followed by analysis at 30RFU, the profile detected was found to match the DNA profile of the positive control. The exact point where the contamination occurred could not be elucidated.

An audit team consisting of the authors was formed in order to perform a process audit of the automated DNA IQ™ protocol that is performed on two MultiPROBE® II PLUS HT EX platforms. The aim of the audit team was to identify any steps in the protocol where a potential for quality breakdown was present, and also to identify areas of improvement that may benefit the protocol. This report documents the findings and recommendations of the audit team.

2. Findings and Observations

A series of process audits on the complete DNA IQ™ protocol (including off-deck lysis and use of the STORstar instrument) were performed by the authors between 15-28 July 2008. The batches that were audited by the authors are listed in Table 2 below.

Table 2. List of batches and protocols that were reviewed in this audit.

Batch ID	Protocol type	MP II Platform	Auditor[s]	Date
RSPNT20080714_02	Off-deck (retained supernatant)		IAM, AC	15 July 2008
CWIQ20080711_01	STORstar lysate		IAM	15 July 2008
CWIQLYS20080714_02	Off-deck (no retained supernatant)		IAM, AC	15 July 2008
RFIQEXT20080711_01	Automated DNA IQ (Reference)	MP II B	IAM	16 July 2008
LNIQEXT20080715_01	Automated DNA IQ (Reference)	MP II A	AC	16 July 2008
CWODL20080715_01	Off-deck (no retained supernatant)		PAC	16 July 2008
CWODL20080715_02	Off-deck (no retained supernatant)		PAC	16 July 2008
CWIQEXT20080710_01	Automated DNA IQ (Casework), elution	MP II B	IAM	17 July 2008
RFIQEXT20080724_02	Automated DNA IQ (Reference)	MP II B	PAC	28 July 2008

The training records of 16 staff members from the Analytical and Automation Project teams were also reviewed. This included three staff members who have left DNA Analysis.

2.1. *Off-deck lysis (with retained supernatant)*

IAM and AC both reviewed off-deck lysis batch RSPNT20080714_02. The protocol was performed by a trainee under supervision by a trainer (the trainer's *Competent to Train* statement for the DNA IQ™ protocol was submitted to SSDU on 27 June 2008). While performing the protocol, the trainee had access to a controlled copy of the most recent version of the SOP (QIS 24897 R3). When questioned about the SOP, the trainee said that the SOP was easy to follow, but suggested separating the off-deck lysis protocol from the remainder of the SOP, which was relatively large at 33 pages. It must be noted that the DNA IQ™ training module (QIS 24896) does not contain any KPC's relating to the off-deck lysis component. The protocol was performed in a sterilised fume hood with the appropriate PPE.

Observations that were made are listed below.

2.1.1. The worksheet used for the protocol is inappropriate because a Chelex® extraction worksheet is used, as configured in AUSLAB for the RSPNT batch type, which may lead to quality issues (e.g. forgetting to record the TNE incubation step). However, it is understood that configuration of the appropriate worksheets is in progress by LISS/PJAS. As a temporary measure, reagent lot numbers and operator details are recorded in a worksheet that forms part of the DNA IQ™ SOP: *Appendix 18.2. Reagent & Batch details recording tables*.

2.1.2. When adding Sarcosyl, the reagent should be decanted from the stock bottle into a smaller "working" container (e.g. Falcon tube or 10mL tube) to avoid potential contamination of the stock solution. This should be specified in the SOP. The Proteinase K is appropriately aliquoted into individual tubes.

2.1.3. The operators were concerned regarding inconsistencies in label types: sometimes they would be of the 3-part type, and other times they would be of the Nunc type. When requiring extra labels, the operator is often required to print a 3-part label, which they considered to be wasteful. The labelling on some tubes appeared to come off at the edges, but this was most likely due to inadequate pressure being applied during label application.

2.1.4. The top of tubes containing the retained supernatant is labelled with the text "sup" as appropriate to identify them as retained supernatant, as per the SOP.

2.1.5. Some details that should be added to the SOP include:
- Step 11, a pipette mix should be performed.
- Step 18, store lysates in a 4ºC fridge immediately after performing the incubation (and not stored on the bench).

Although some suggestions are made to improve the process, we did not observe any steps where a potential quality breakdown could occur and cause sample-to-sample cross contamination when the protocol is performed following the SOP.

2.2. *Off-deck lysis (without retained supernatant)*

IAM and AC both reviewed off-deck lysis batch CWIQLYS20080714_02. The protocol was performed by the same trainee and trainer as per RSPNT20080714_02. This off-deck lysis batch had a new configuration type as detailed in the memo of 15 July 2008. Briefly, the lysis batch contained 1 positive and 1 negative control, with 47 samples and 47 blanks (containing nanopure water) arranged in checkerboard format. This new batch format was chosen in order to better identify any occurrences of well-to-well cross contamination. As per 2.1., the DNA IQ™ training module does not contain KPC's related to this component of the process.

PAC reviewed off-deck lysis batches CWODL20080715_02 (performed by a trainer with trainee observing) and CWODL20080715_01 (steps 7 to 12).

Observations that were made are listed below.

2.2.1. Three steps where a possibility of either contamination or cross contamination can occur were identified:
- 2.2.1.1. Subsampling/re-processing of sample.
- 2.2.1.2. Incorrect use of a multistep pipettor.
- 2.2.1.3. Transferring substrate from lysis tube to spin basket.

These are described in more detail below.

2.2.2. Some swab samples retained excessive lengths of swab shaft. Out of 47 samples on CWIQLYS20080714_02, 6 swabs required re-sampling, and one swab head was accidentally flicked during reprocessing and landed on the floor of Room 6120. The appropriate corrective actions (e.g. adding Specimen Notes) were performed by the operator.

2.2.3. The procedure for preparing water blanks was reviewed and the following recommendations have been made:

2.2.3.1. The water should be decanted from the stock bottle into a Falcon tube to avoid contaminating the stock reagent.

2.2.3.2. If water is dispensed into individual tubes (labelled with the appropriate barcodes), different tubes (e.g. tubes with white caps) should be used in order to differentiate these from sample tubes and therefore removing the possibility of accidentally adding water to the samples instead of extraction buffer. The accidental addition of water to a sample may cause inefficient extraction of the affected sample.

2.2.3.3. As the water blanks do not actually undergo the off-deck lysis protocol, nanopure water could be added directly to the ABgene plate during STORstar of the lysates, with the appropriate barcodes being scanned from a roll/list. This removes any potential detection of contamination originating from the tubes, and enables the use of the water blanks to specifically diagnose and identify any well-to-well cross contamination events that may occur during automated DNA IQ™ processing. In this instance, only the positive/negative controls and 47 samples require sequence checking by another operator.

2.2.4. The operators for each of the batches reported difficulties using the 3-part labels that may lead to confusion during labelling and possible mislabelling events, as the barcodes contained extra information that crowds the label. However, sequence checking of samples ensures detection of any mislabelling events.

2.2.5. The use of a multistep pipettor to dispense aliquots of reagent may be a potential source of cross contamination from splashing or aerosolisation if the multistepper is not used correctly. As only one sample tube is opened and processed at any one time, the potential contamination can only occur if incorrect use of the multistepper has caused extract to splash on to the multistepper syringe, and subsequently carried-over to the next sample. This hypothetical event would only potentially contaminate downstream samples. Appropriate and adequate training in the use of a multistep pipettor is deemed sufficient to prevent this event from occurring.

2.2.6. Different methods for transferring substrate matrices to spin baskets exist in the laboratory:

2.2.6.1. Using stainless steel forceps that are sterilised in between samples using 10% bleach, 70% ethanol and flaming with a

Bunsen burner. Using forceps may cause cross-contamination in downstream samples if forceps are not sterilised correctly.

2.2.6.2. Using autoclaved twirling sticks to either:
- Assist in tipping the substrate into the spin basket.
- Pickup the substrate and transfer to spin basket using a "chopstick" method.

2.2.6.3. Some standardisation of the method for transferring substrates to spin baskets should be considered. The different methods should be assessed to determine which one is the best method with the least potential for contamination.

2.2.6.4. Cross contamination from sample-to-sample via droplets is unlikely because only one sample tube is open at any one time and each sample is processed individually.

2.2.7. It was noted that 2mL 4N6 tubes are designed with a wide bottom, and therefore 500µL of Extraction Buffer may not always immerse the sample substrate (e.g. a swab head). This may potentially cause inefficient extraction of DNA from the sample.

2.2.8. Feedback from questioned staff indicates that a high level of workload exists in the Analytical Section, coupled with frequent protocol changes, resulting in overworked and tired staff members that may result in a quality breakdown.

2.2.9. It was observed that newly trained staff members have access to the bench copy of SOP (QIS 24897 R3) when processing ODL batches but are not regularly referring to the SOP for guidance. A tick box checklist system is recommended for re-assurance that the process is followed methodically.

Although there are several steps where opportunities for contamination or mislabelling exist, staff consider that there are appropriate and sufficient quality control measures in place that minimise these risks. We recommend that the issue of overworked staff be investigated further.

2.3. ***STORstar of lysate***

The *automate.it* STORstar system is used as an automated sequence checking system to ensure that:
- A sample lysate is transferred from a sample tube to the correct well in a 96-deep well plate;
- The correct Nunc™ Bank-It DNA extract storage tube is positioned in the correct grid coordinates for any sample ID.

DNA Analysis FSS has purchased two STORstar units: one is located in the Workflow Area for sequence checking DNA extract tubes, while the other is located in the DNA Suite for both sequence checking of extract tubes and also transfer of sample lysate. The STORstar system utilises an electronic platemap generated by AUSLAB that contains a list of unique sample identifiers linked to a specific grid coordinate. The electronic file is imported into AUSLAB as a log file.

The DNA IQ™ training module does not contain any KPC's related to the STORstar process. The STORstar protocol for the DNA IQ™ process is described in QIS 24256 R1, separate to the DNA IQ™ SOP (QIS 24897 R3).
Observations that were made are listed below.

2.3.1. Lysate tubes are vortexed and centrifuged briefly for 30 sec – 1 min prior to transfer.

2.3.2. The instrument is sterilised appropriately using 10% bleach and 70% ethanol between uses. The sample aperture on top of the unit can also be sterilised between samples using the same reagents.

2.3.3. After scanning the barcode on each sample tube, the operator confirms that the sample ID displayed in the bottom right hand corner of the OVERLORD software displays the sample ID that is on the tube. This is a commendable QC measure to ensure that the correct sample lysate is transferred to the correct well.

2.3.4. Operator feedback indicates that they are satisfied with the performance and ease of use of the instrument. Operators also feel that the environment around the instrument is sufficient to maintain integrity of the process.

2.3.5. The operator suggested to decrease the diameter of the aperture to allow only one well to be exposed at any one time, but did not feel that the current design (coupled with the protocol) compromises sample integrity.

2.3.6. When transfer is complete, the deep well plate is sealed using adhesive film. It was noted in downstream processes that condensation can collect and stick on the adhesive film.

2.4. ***Automated DNA IQ™ Protocol (reference)***

From various reference batch types performed, the authors observed the following:

2.4.1. When preparing reagents for automated DNA IQ™ processing, the operator is required to constantly move from one room to another in order to access reagents. Reagent preparation occurs in the fume hood within Room 6122 of the DNA Suite, which is commonly used for FTA® washing preparation. Normally, the operator prepares reagents in the shared fumehood workspace, however TNE buffer, sarcosyl and reagents that require fridge/freezer storage are located in Room 6120, and therefore the operator is required to travel between the two rooms to fetch the required solutions. The reagent-making process cannot be combined in Room 6120 because it requires the use of a fumehood. When IAM was observing, the operator travelled back and forth for a total of 3 times. The Elution Buffer is stored in Room 6127, which is

acceptable because this reagent is not required until towards the end of the protocol.

2.4.2. As mentioned in point 2.1.1, the DNA IQ™ worksheet has not been configured in AUSLAB and therefore the operator was required to use the worksheet in Appendix 18.2 of QIS 24897 R3.

2.4.3. There is no controlled copy of the DNA IQ™ SOP (QIS 24897) in Room 6122. The operator normally calculates the required volumes in Room 6125 and brings their worksheet into Room 6122 prior to reagent preparation.

2.4.4. Two uncontrolled copies of Appendix 18.1 from QIS 24897 R3 were found on a shelf in Room 6122. One uncontrolled copy of page 5 from QIS 24897 R2 (archived document) containing handwritten changes with no initials were also found in Room 6122. IAM removed these.

2.4.5. It should be noted that prior to commencing the procedure, an operator mentioned that the last time they were rostered on to the Extraction MP II platform was around 3-4 months ago, and they had not been retrained since changes have been made to the DNA IQ™ protocol. The operator had never performed an automated REF FTA protocol before.

2.4.6. Appendix 18.1 of QIS 24897 R3 had a note underneath each table, instructing operators to calculate *n* to the nearest multiple of 8. An operator did not note this because the note was not clearly visible, and had to recalculate all reagent volumes before commencing to prepare reagents.

2.4.7. In Table 7 of Appendix 18.1 of QIS 24897 R3, the formula to calculate the volume of Elution Buffer is incorrect. There should be a minimum volume of 3mL in each channel if the batch size is small due to the large dead volume required for this type of labware. Having volumes less than 3mL will cause inaccurate pipetting of volumes. Although this has been identified as a mistake within the SOP, comments had not been entered into QIS.

2.4.8. The results from calculating reagent volumes are not checked by a different operator in order to confirm that the calculations are correct.

2.4.9. Reagent volumes are measured using gradations on a Falcon tube, which are not calibrated. This is acceptable for bulk reagents (e.g. Lysis Buffer, Wash Buffer, Elution Buffer), but should not be used for reagents that need to contain a certain concentration of a reagent (e.g. TNE Buffer, which is then mixed with Proteinase K to a validated concentration).

2.4.10. After completing the reagent-making process, the operator carries all the reagents (in Falcon tubes or Schott bottles) from Room 6122 into

Room 6125. When entering Room 6125, the operator does not have dedicated space to place the reagents while the operator prepares their PPE.

2.4.11. The operator adds reagents to specific troughs. A different operator does not check to ensure that the reagents have been poured into the correct troughs.

2.4.12. When IAM was observing, a new version of the MP II program was being used in order to enable shaking of plates without the use of a Wallac Isoplate support. This version is not in the current SOP but a comment has been made in QIS. The operator was able to show all the documentation and testing associated with the new version.

2.4.13. Observations associated specifically with the automated method are outlined below.

2.4.13.1. Automated scanning of barcodes on 96-well plates does not work 100% of the time on both MP II platforms, requiring operator intervention.

2.4.13.2. On MP II Platform B, heating tile #1 (45W) is broken on the right-hand side, and the operator is required to click the plate into place prior to commencing the incubation. This information is not present in the SOP.

2.4.13.3. The aluminium foil that is used needs to be properly sealed on to the plate to minimise the risk of cross contamination due to evaporation or condensation. Sealing should be performed using the supplied brown plastic tool and pressing gently to ensure a perfect seal.

2.4.13.4. There is not much room for the operator to move within the MP II hood when adding the DNA IQ™ resin manually which may become an OH&S hazard. When adding the resin to the deep-well plate, the resin should be dispensed onto the side of the plate without touching or mixing with the lysate in order to remove the potential for contamination.

2.4.13.5. When adding Wash Buffer to its specific trough, the operator is required to reach over the plate containing samples.

2.4.13.6. Operators report difficulties with the tip chute receptacle (the tip catcher). Because of rusting, tips can become stuck in the catcher and cause subsequent tips to flick out during ejection, and possibly cause contamination of plates that are in close vicinity to the tip chute. Tip loss may also cause contamination of the workspace.

2.4.13.7. Addition of Lysis Buffer to the sample lysate, followed by pipette-mixing using disposable tips, is a crucial step and takes approximately 1hr to perform for a full plate. The subsequent transfer and shaking on the DPC Shaker platform does not create a vortex of suitable intensity to mix the resin. Furthermore, because the volume within each well is considerably full at this stage, the shaking process may

increase the probability of a splash back and therefore increase the risk of cross contamination, especially if the volume of lysate exceeded 500µL. This step is redundant and should be removed.

2.4.13.8. Operators report that the ABgene plate that is currently used is not 100% identical to the Slicprep™ device, as it requires clicking into the magnet. Resin loss, and therefore sample loss, occurs if the plate is not clicked in properly.

2.4.13.9. When transferring supernatant to the storage plate, the first transfer is always performed efficiently but the second transfer exhibits bubbles that are possibly caused by aspirating large air gaps, however this problem may only potentially contaminate the storage plate (and not the sample plate). However, if bubbles pop towards column 12 of the storage plate, potential cross contamination may occur in columns 1 or 2 of the Nunc storage tubes that sit adjacently (note that extraction controls are located in column 1). A second set of tips should be used for the second transfer to remove this problem.

2.4.13.10. On MP II Platform B, a blockage of tip 2 was identified due to the consistent appearance of frothing. The operator noted this but did not inform the Supervising Scientist. Although not perceived to be a source of cross contamination, maintenance on this tip should be performed.

2.4.13.11. The rack that is used to store lids from decapped Nunc tubes is cleaned daily using bleach. Racks should be cleaned daily in Decon solution prior to washing. New, cleaned racks should be used daily: the operator can collect cleaned racks from the Workflow Area at the beginning of a run, and return the used rack into a Decon bucket at the conclusion of a run.

2.4.13.12. For the RFIQ worksheet, there are no fields to record positive, negative and blank control barcodes, which are instead affixed onto the general surface of the worksheet which causes inconsistencies. There are no fields to record lot numbers for Slicprep™ devices, etc.

2.4.13.13. After incubation at 65°C, the deep-well plates warp due to heating. The plate needs to cool down before the operator can click it on to the magnet appropriately. The waiting period is generally 1-2 minutes and the operator generally enters the hood about 2-3 times to check if the plate can click onto the magnet. The plate should not be forced onto the magnet as splash back can potentially occur. This process is not described in the SOP.

2.4.13.14. During this time, the eluate appears to be evaporating as evidenced by condensation forming on the tops of the wells. The risk of possible contamination should be evaluated. To avoid evaporation, aluminium foil can be applied onto the plate. To avoid causing cross contamination when peeling the film, pierceable foil can be used. Additionally, a septa may be appropriate.

2.4.13.15. During transfer of eluate from the plate to individual tubes, one bubble and one drop was observed, with both popping at the tip chute. The formation of bubbles may be attributed to either:
- Warm liquid is being transferred, causing the polypropylene tip to expand during movement and causing the movement of air into the spaces, which then form bubbles as the eluate is being dispensed.
- Inefficient programming of the pipetting step. In this case, the performance file for the tip should be examined, and perhaps reducing the volume of system air gap may remove the formation of bubbles.
- The use of non-conductive versus conductive tips.
- The use of tips with smaller aperture may decrease bubble formation and should be investigated.

2.4.13.16. When the procedure is finished, the operator applies aluminium foil to seal the Slicprep™ sample plate and the storage plate. Each plate is then placed into different Ziploc bags, and Nunc tubes are recapped manually. Plates are then transferred to the Workflow Area for storage.

2.4.13.17. There is insufficient storage space in the freezers.

2.4.13.18. Washing and decontamination of the labware is inconsistent and should be standardised in order to minimise the risk of contamination from the tip chute. The tip chute and tip catcher are washed and dried in a rack adjacent to reagent troughs and reagent bottles, or dried on positions in a rack that is not officially reserved for reagent troughs.

2.4.14. The MP II maintenance log for each MP II platform is well maintained and is used effectively to document maintenance schedules that are performed, including replacement of any components.

2.4.15. The syringe plungers on the MP II platforms appeared dirty, which may increase the likelihood of bubble formation that affects pipetting accuracy. Syringes are not normally replaced until they break down.

2.4.16. The PC hard drives for both instruments contain archived performance files and electronic plate maps that should be archived to disc on a monthly basis.

2.4.17. Although environmental cleaning is regularly performed monthly, the top of the MP II hood appeared to be quite dusty. An appropriate cleaning method for hard to reach areas should be investigated.

2.5. *Automated DNA IQ™ Protocol (casework)*

Most of the observations for the reference protocol also apply to the automated DNA IQ™ casework protocol.

Video footage of the entire double elution processes was captured and included with this report as appendices:

- Video 1: the entire elution process (Platform B extraction.mpeg).
- Video 2: detail into the formation of 2 bubbles during the elution process (bubbles.mpeg).

Refer to the appropriate timestamps in parentheses for footage of each observation.

From Video 1, we observed:

Elution 1

2.5.2. The robot was unable to automatically click sample plate into the magnet after 65°C incubation due to plate warping, therefore requiring operator intervention (0:06:41, 0:07:04, 0:07:29 – 0:07:45).
2.5.3. Aspiration of first eluate using non-conductive disposable tips did not identify any problems, with uniform volumes of aspirate (and transport air gap) in each tip (e.g. 0:09:02).
2.5.4. Tip 7 ejection problem was encountered (0:09:12), requiring operator intervention (0:09:24).
2.5.5. No resin was transferred to the elution tubes (except towards 0:13:00).
2.5.6. Bubble formation was observed at the end of a disposable tip during transfer into elution tube (0:11:15).
2.5.7. Nunc tubes were picked up by the tips after dispensing eluate (as the 8-tip arm retracted), causing the tubes to raise in the rack, requiring operator intervention (0:13:03). If the operator did not intervene, there was a risk of the 8-tip arm crashing into the Nunc rack.
2.5.8. Bubble formation was observed at the end of a disposable tip after dispensing eluate into elution tube (0:13:30).
2.5.9. Resin was transferred to elution tube (0:14:17).
2.5.10. Droplet formation was observed at the end of a disposable tip after dispensing eluate into elution tube (0:14:24). The droplet subsequently came off at the tip chute (0:14:38).

Elution 2

2.5.11. Shaking on DPC shaker appears to shake resin sufficiently in wells (0:15:31).
2.5.12. Condensation formed on the sides of each well as the plate was cooling on the magnet (0:18:14).
2.5.13. Operator intervention was required to click in the warm plate onto the magnet (0:18:34 – 0:19:09).
2.5.14. Aspiration of second eluate using non-conductive disposable tips did not identify any problems, with uniform volumes of aspirate (and transport air gap) in each tip (e.g. 0:20:44).
2.5.15. Droplet formation was observed at the end of a disposable tip after dispensing eluate into elution tube (0:23:48).
2.5.16. Resin was transferred into elution tube (0:24:07).
2.5.17. A Nunc tube was lifted off the rack after dispensing eluate into the elution tube, requiring operator intervention (0:24:38).

From Video 2, we observed some details from Video 1:

2.5.18. Bubble formation from 2.5.6 (0:00:06).
2.5.19. Droplet formation from 2.5.10 (0:00:22).
2.5.20. Also from 2.5.10, the droplet was caught onto the outer surface of the tip chute (0:00:35).

In addition, operators have identified that sample plates often display condensation at the top of the wells and underneath the adhesive film after prolonged storage in the fridge (Figure 1). The condensation was not removed after one cycle of centrifugation (Figure 2), and therefore may require further centrifugations. Incomplete removal of condensation may cause cross contamination when the adhesive film is removed.

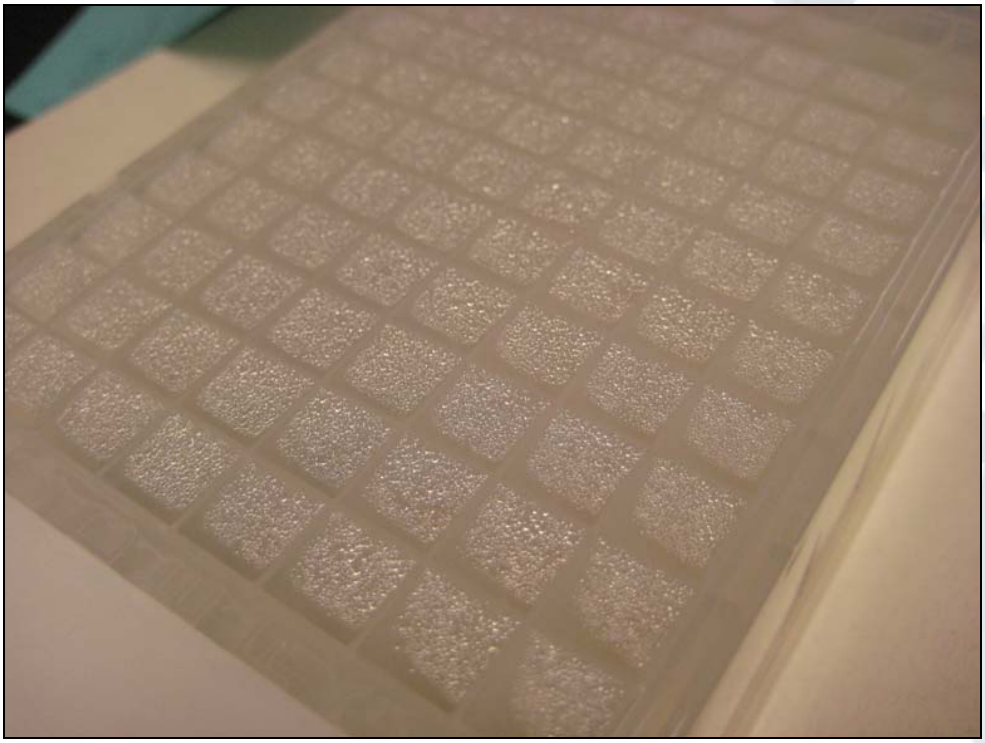

Figure 1. Condensation was visible underneath the adhesive film after removal from cold storage, before centrifugation.

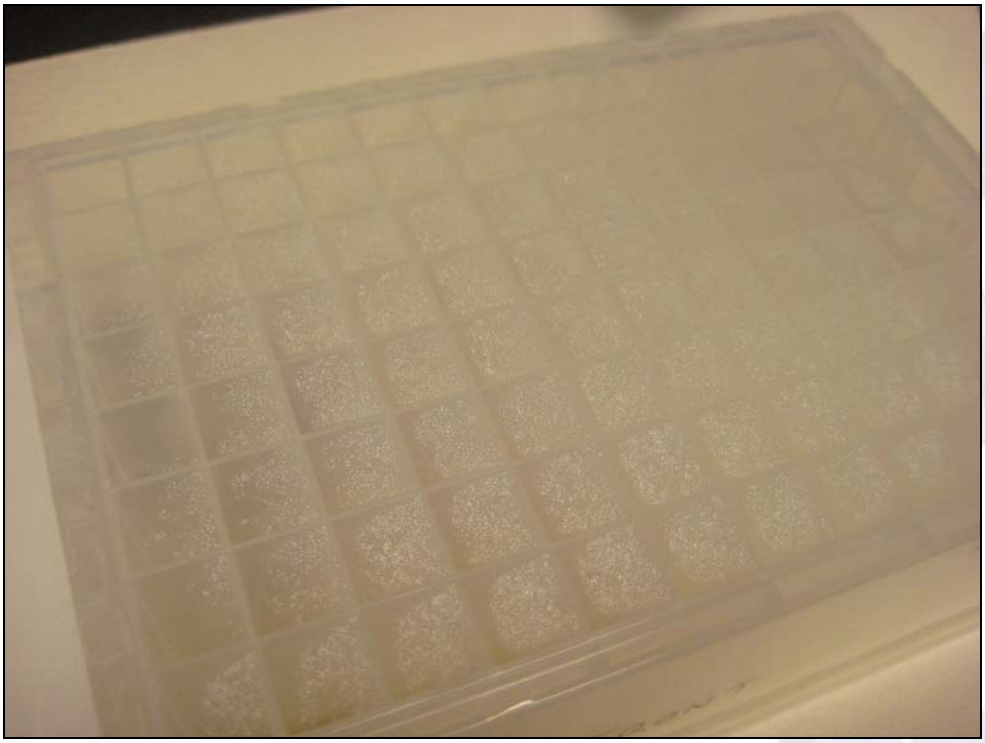

Figure 2. The same plate as per Figure 1, displaying incomplete removal of condensation after one cycle of centrifugation.

3. Trends

We identified various trends throughout the audit process.

3.1. KPC's for the off-deck lysis and STORstar components are not included in the DNA IQ™ training module (QIS 24896 R0), but are integral to the DNA IQ™ protocol.

3.2. The majority, but not all, training records (e.g. QIS 24450 Operation and Maintenance of the MultiPROBE® II PLUS HT EX Robotic Platform TM and QIS 24896 Automated DNA Extraction with the DNA IQ Kit TM) for staff members were either available in the QIS Professional Development module or ready for upload to QIS.

3.3. Staff in the Automation Project team, involved in the development of the SOP (QIS 24897) and Training module (QIS 24896), have either completed the training modules or possessed "Statements of Competence" records.

3.4. "Statement of Competence to Train" records were available for some but not all trainers. All trainers have previously attended Train the Trainer.

3.5. Staff members generally do not have a checklist system to ensure that they have performed a specific step within any particular protocol.

3.6. Staff members use different methods to transfer substrate matrices into spin baskets.

3.7. Volume calculations for DNA IQ™ reagents are not checked by a different operator to confirm calculation results. The worksheet to record calculations (Appendix 18.1 of QIS 24897 R3) is often not used or not included with the DNA IQ™ worksheet (Appendix 18.2 of QIS 24897 R3).

3.8. The volume of critical reagents (e.g. TNE buffer) is not measured using calibrated volumetric devices.

3.9. Some procedures within the automated DNA IQ™ protocol, e.g. (1) transfer of supernatant to the storage plate and (2) the double elution steps, require a review and optimisation due to apparent inefficient pipetting parameters.

3.10. Operators are consistently required to manually secure the 96-well plate on to the magnet when performing the automated DNA IQ™ protocol.

3.11. The MP II maintenance log for each MP II platform is used effectively to document maintenance schedules that are performed, including any work performed by the PerkinElmer engineer. Day-to-day work and observations is recorded appropriately in specific logs for each platform.

3.12. Some staff members that were questioned feel that they are frequently exposed to changes in protocols and methods, and are required to adapt quickly. Although some staff members were comfortable with this environment, others feel slightly overwhelmed.

4. Summary and Recommendations

This review was unable to determine the exact source of contamination as reported in OQI's 19477, 19768 and19349. Although some risks for mislabelling, contamination or cross contamination exist in the procedure, there are appropriate and sufficient quality control measures in place to minimise these risks. Although we observed bubbles and droplets forming at the end of disposable tips during the automated DNA IQ™ protocol, these were not observed to have dripped into any wells and were discarded in the tip chute. Bubble formation can be reduced and eliminated by further optimising the pipetting parameters within the protocol. A follow-up of samples processed in checkerboard format on batch CWIQLYS20080714_02 did not show any instances of well-to-well cross contamination, as evidenced by the absence of DNA profiles in all of the water blanks.

We commend the department for actively engaging in a continual methods improvement process (either to improve QA/QC or increase ease-of-use and efficiency of a procedure), whereby staff input on method changes are investigated and eventually implemented if appropriate. To alleviate the feeling of being overwhelmed by frequent changes, staff members may benefit from a formal handover period as new task rotations occur at the beginning of a week, i.e. the previous operator rostered on a task will convene with the new rostered operator to

describe any changes to protocols and methods. Changes to protocols should be disseminated generally to other staff either through meeting agendas (and is therefore recorded) and e-mail if appropriate, which is already the current procedure in place. Furthermore, the Minor Changes Register is used effectively to document specific changes within the laboratory, and can be accessed by all staff members. We noted that QIS also appears to be used appropriately for the purposes of recording suggestions and changes to protocols.

The authors have identified 28 points that may improve the automated DNA IQ™ extraction process, and have made the following recommendations:

4.1. Add KPC's for off-deck lysis and STORstar components into the DNA IQ training module (QIS 24896 R0).

4.2. As part of DNA IQ™ training delivery and the associated training module, incorporate more aspects into the background and theory of the system, including discussions on the composition and function of each buffer reagent.

4.3. A Training Delivery Plan needs to be developed for training in the automated DNA IQ™ extraction process. Note that a TDP already exists for training on the use of the MultiPROBE® II platforms (used in conjunction with QIS 24450).

4.4. "Statement of Competence to Train" records must be finalised for appropriate Automation Project team members.

4.5. Review the expected timeframes to complete training modules QIS 24450 (Operation and Maintenance of the MultiPROBE® II PLUS HT EX Robotic Platform) and QIS 24896 (Automated DNA Extraction with the DNA IQ Kit).

4.6. Trainers and supervisors need to progress the completion of training modules with staff. Consider adding progress reports as an agenda item in weekly team meetings or an appropriate alternative.

4.7. Apart from staff identified as trainers, it is recommended that all DNA Analysis staff attend *Trainer the Trainer*. This will assist with 1) trainer and trainee responsibilities, 2) adult learning styles, 3) introduces the FSS Learning and Development Manual (QIS 23651).

4.8. A re-evaluation of pipetting skills should be performed in order to benchmark and standardise techniques. The evaluation can incorporate demonstrations on differences in the pipetting behaviour of hot, warm and cool liquids; reagents containing a high proportion of solvents (e.g. ethanol), etc. A SOP and TM detailing and assessing pipetting techniques (e.g. forward versus reverse pipetting) should be created, if not yet available (e.g. see QIS 23899). The re-evaluation should also assess the use of multichannel and multistep pipettors in combination with various tip types.

4.9. The issue of overworked staff in the Analytical Section needs to be investigated further.

4.10. In-tube sample submissions to the Analytical Section must contain the appropriate amount/length of sample in the first instance, in order to eliminate the need for reprocessing and reduce the risk of contamination.

4.11. If proceeding with a checkerboard format for DNA extractions, the method for preparing the water blanks must be reviewed and standardised (see point 2.2.3).

4.12. Standardisation of the method for transferring substrates to spin baskets should be considered (see 2.2.6).

4.13. Investigate the isolation of all DNA IQ™ reagents and off-deck lysis protocols in one working area. The authors are aware, however, that the current physical design of the DNA Suite may not allow this.

4.14. Investigate the advantages of separating the DNA IQ™ SOP (QIS 24897) into two separate documents, e.g. off-deck lysis (including STORstar) and automated DNA IQ™, and implement as appropriate. The SOP needs to be updated to reflect changes and correct minor errors (e.g. see points 2.4.7, 2.4.13.2, 2.4.13.13).

4.15. Finalise configuration of the appropriate AUSLAB worksheets for use throughout the DNA IQ™ method, so that operators are using the correct worksheets and are able to record all of the necessary batch details in designated fields.

4.16. The automated DNA IQ™ protocols must be reviewed and further optimised to increase liquid handling performance (e.g. incorporate the use of different syringe sizes and tip types) with the assistance of a qualified PerkinElmer specialist (e.g. see points 2.4.13.7, 2.4.13.9, and resin transfer in points 2.5.9, 2.5.16). The optimised protocol should be tested and verified prior to routine use, as per current practice.

4.17. Further to 4.16, the applicability of a different magnet in order to minimise the need to manually secure the plate to the magnet should be investigated. Alternatively, a 96-deep well plate that is not prone to heat warping should be sourced.

4.18. The option for using pierceable film or septa on plates during the automated DNA IQ™ protocol should be investigated (see point 2.4.13.14).

4.19. A procedural checklist should be considered for each protocol so that individual operators can keep track of each specific step as they are performed. This checklist can be added as an appendix to SOP's in QIS that can be printed out by operators prior to performing the procedure.

Alternatively, the checklist can be configured in AUSLAB and printed out together with the batch worksheet.

4.20. Checking of calculation results for reagent volumes by a different operator should be introduced, as should the dispensing of reagents into the correct troughs.

4.21. The use of “working” containers and aliquots should be enforced where appropriate so that the possible contamination of stock solutions is minimised.

4.22. Appropriate calibrated volumetric devices should be sourced to measure the volume of critical reagents such as TNE buffer.

4.23. A process to change syringes more frequently at regular intervals should be implemented. Because of this, the process to calibrate or check new syringes will be time consuming and therefore alternative calibration or pipetting verification systems should be sourced (e.g. Artel MVS).

4.24. The BSD Duet 600 instrument can be moved to a different location in order to decrease human traffic and increase the amount of working space available around the MP II extraction platforms. A portable biohazard hood can be introduced into Room 6125 to enable some sample processing outside of the MP II hoods (e.g. manual addition of DNA IQ™ resin).

4.25. Investigate the use of a tip catcher that is made of a material not prone to rusting (e.g. plastic).

4.26. The procedure for washing and drying the MP II tip chutes must be reviewed (see point 2.4.13.18). Designate a rack position or location for drying of the tip chute and tip catcher, separate from the rack used for reagent troughs. Furthermore, a spare tip chute can be made available for each MP II, therefore used tip chutes can be allowed to decontaminate in a Decon bucket to fully decontaminate the tip chute, without compromising throughput of the MP II.

4.27. The cleaning regime of the MP II, including surroundings and enclosure (e.g. top of MP II hood), must be reinforced.

4.28. As a continuous QA/QC measure, the supervisor should observe the DNA IQ™ protocols at regular intervals for critical assessment and possible re-evaluation of the impact and suitability of changes in the methods.

As an outcome of the recommendations, the authors have raised three OQI's that are listed in Table 3.

Table 3. List of OQI's generated from process audit 8227.

OQI	Description	Recommendations
20367	Automated DNA IQ™ process, including documentation	4.10 – 4.15, 4.19 – 4.22
20368	Enhancement of the MP II extraction platforms, including environment	4.16 – 4.18, 4.23 – 4.27
20369	Training and personnel related to the DNA IQ™ process	4.1 – 4.9, 4.28

5. Acknowledgements

The authors would like to acknowledge DNA Analysis (FSS) for the opportunity to conduct this audit. The authors would also like to thank all staff members in the Analytical Section for making themselves available throughout the duration of the audit process.

6. Documentation and Storage

A hard copy of this report, along with the footage on DVD, is stored with the Quality Management Team in DNA Analysis (FSS). An electronic copy of the report is available in PDF format from the authors and the Senior Scientist (Analytical Section). A summary of the audit findings is available in QIS for Audit 8227.

7. References

QIS 23651 R2 (2008). Forensic and Scientific Services Learning and Development Manual [Guideline]. Scientific Skills Development Unit, FSS: Coopers Plains, Brisbane, Australia.

QIS 23939 R2 (2008). Operation and Maintenance of the MultiPROBE® II Plus HT EX and MultiPROBE® II Plus HT EX with Gripper™ Integration platform [Standard Operating Procedure]. DNA Analysis, FSS: Coopers Plains, Brisbane, Australia.

QIS 24256 R1 (2008). Procedure for the use of the STORstar unit for automated sequence checking [Standard Operating Procedure]. DNA Analysis, FSS: Coopers Plains, Brisbane, Australia.

QIS 24450 R1 (2008). MultiPROBE® II PLUS HT EX Robotic Platform Training Module [Training Module]. DNA Analysis, FSS: Coopers Plains, Brisbane, Australia.

QIS 24896 R0 (2008). Automated DNA Extraction with the DNA IQ Kit Training Module [Training Module]. DNA Analysis, FSS: Coopers Plains, Brisbane, Australia.

QIS 24897 R3 (2008). Automated DNA IQ Method of Extracting DNA from Reference and Casework samples [Standard Operating Procedure]. DNA Analysis, FSS: Coopers Plains, Brisbane, Australia.

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Audit 8227: Process Audit of the Automated DNA IQ™ System (including Off-Deck Lysis)

17 September 2008

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Queensland Government
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Background

- Potential instances of well-to-well cross contamination were identified and investigated (OQI 19477, 19768, 19349).
- The exact cause of the contamination could not be identified.
- Audits of processes were already scheduled for a later date, but these were put forward.
- Audit team was formed consisting of Amy Cheng, Peter Clausen and Iman Muharam.

Objective

To identify any steps in the automated DNA IQ™ protocol where a potential for quality breakdown was present, and also to identify areas of improvement that may benefit the protocol.

Why perform audits?

- Why do internal audits?
  - Verify the integrity of the system to meet internal objectives.
  - Assess if the quality system is effective and sustainable.
- Process audits:
  - Any activity, or set of activities, that uses resources to transform inputs into outputs.
  - Often, the output from one process will directly form the input into the next process.
- Factors affecting a process may include:
  - Environment.
  - Safety.
  - Documents.
  - Records.
  - Equipment.
  - People.
  - Output from the previous process.

Audited batches (15-28 July 2008)

Batch ID	Protocol Type	MP II Platform	Auditor[s]	Date
[redacted]	Off-deck (RS)		IAM, AC	15 July 2008
	STORstar lysate		IAM	15 July 2008
	Off-deck (NRS)		IAM, AC	15 July 2008
	DNA IQ (Ref)	MP II B	IAM	16 July 2008
	DNA IQ (Ref)	MP II A	AC	16 July 2008
	Off-deck (NRS)		PAC	16 July 2008
	Off-deck (NRS)		PAC	16 July 2008
	DNA IQ (CW)	MP II B	IAM	17 July 2008
	DNA IQ (Ref)	MP II B	PAC	28 July 2008

Video footage was captured for some processes.
Training records for 16 staff members were also reviewed.

Audit recommendations

4.1. Add KPC's for off-deck lysis and STORstar components into the DNA IQ training module (QIS 24896 R0).

4.2. As part of DNA IQ™ training delivery and the associated training module, incorporate more aspects into the background and theory of the system, including discussions on the composition and function of each buffer reagent.

4.3. A Training Delivery Plan needs to be developed for training in the automated DNA IQ™ extraction process. Note that a TDP already exists for training on the use of the MultiPROBE® II platforms (used in conjunction with QIS 24450).

4.4. "Statement of Competence to Train" records must be finalised for appropriate Automation Project team members.

4.5. Review the expected timeframes to complete training modules QIS 24450 (Operation and Maintenance of the MultiPROBE® II PLUS HT EX Robotic Platform) and QIS 24896 (Automated DNA Extraction with the DNA IQ Kit).

4.6. Trainers and supervisors need to progress the completion of training modules with staff. Consider adding progress reports as an agenda item in weekly team meetings or an appropriate alternative.

Audit recommendations

4.7. Apart from staff identified as trainers, it is recommended that all DNA Analysis staff attend Trainer the Trainer. This will assist with 1) trainer and trainee responsibilities, 2) adult learning styles, 3) introduces the FSS Learning and Development Manual (QIS 23651).

4.8. A re-evaluation of pipetting skills should be performed in order to benchmark and standardise techniques. The evaluation can incorporate demonstrations on differences in the pipetting behaviour of hot, warm and cool liquids; reagents containing a high proportion of solvents (e.g. ethanol), etc. A SOP and TM detailing and assessing pipetting techniques (e.g. forward versus reverse pipetting) should be created, if not yet available (e.g. see QIS 23899). The re-evaluation should also assess the use of multichannel and multistep pipettors in combination with various tip types.

4.9. The issue of overworked staff in the Analytical Section needs to be investigated further.

4.10. In-tube sample submissions to the Analytical Section must contain the appropriate amount/length of sample in the first instance, in order to eliminate the need for reprocessing and reduce the risk of contamination.

4.11. If proceeding with a checkerboard format for DNA extractions, the method for preparing the water blanks must be reviewed and standardised (see point 2.2.3).

4.12. Standardisation of the method for transferring substrates to spin baskets should be considered (see 2.2.6).

Audit recommendations

4.13. Investigate the isolation of all DNA IQ™ reagents and off-deck lysis protocols in one working area. The authors are aware, however, that the current physical design of the DNA Suite may not allow this.

4.14. Investigate the advantages of separating the DNA IQ™ SOP (QIS 24897) into two separate documents, e.g. off-deck lysis (including STORstar) and automated DNA IQ™, and implement as appropriate. The SOP needs to be updated to reflect changes and correct minor errors (e.g. see points 2.4.7, 2.4.13.2, 2.4.13.13).

4.15. Finalise configuration of the appropriate AUSLAB worksheets for use throughout the DNA IQ™ method, so that operators are using the correct worksheets and are able to record all of the necessary batch details in designated fields.

4.16. The automated DNA IQ™ protocols must be reviewed and further optimised to increase liquid handling performance (e.g. incorporate the use of different syringe sizes and tip types) with the assistance of a qualified PerkinElmer specialist (e.g. see points 2.4.13.7, 2.4.13.9, and resin transfer in points 2.5.9, 2.5.16). The optimised protocol should be tested and verified prior to routine use, as per current practice.

Audit recommendations

4.17. Further to 16, the applicability of a different magnet in order to minimise the need to manually secure the plate to the magnet should be investigated. Alternatively, a 96-deep well plate that is not prone to heat warping should be sourced.

4.18. The option for using pierceable film or septa on plates during the automated DNA IQ™ protocol should be investigated (see point 2.4.13.14).

4.19. A procedural checklist should be considered for each protocol so that individual operators can keep track of each specific step as they are performed. This checklist can be added as an appendix to SOP's in QIS that can be printed out by operators prior to performing the procedure. Alternatively, the checklist can be configured in AUSLAB and printed out together with the batch worksheet.

4.20. Checking of calculation results for reagent volumes by a different operator should be introduced, as should the dispensing of reagents into the correct troughs.

4.21. The use of "working" containers and aliquots should be enforced where appropriate so that the possible contamination of stock solutions is minimised.

4.22. Appropriate calibrated volumetric devices should be sourced to measure the volume of critical reagents such as TNE buffer.

Audit recommendations

4.23. A process to change syringes more frequently at regular intervals should be implemented. Because of this, the process to calibrate or check new syringes will be time consuming and therefore alternative calibration or pipetting verification systems should be sourced (e.g. Artel MVS).

4.24. The BSD Duet 600 instrument can be moved to a different location in order to decrease human traffic and increase the amount of working space available around the MP II extraction platforms. A portable biohazard hood can be introduced into Room 6125 to enable some sample processing outside of the MP II hoods (e.g. manual addition of DNA IQ™ resin).

4.25. Investigate the use of a tip catcher that is made of a material not prone to rusting (e.g. plastic).

4.26. The procedure for washing and drying the MP II tip chutes must be reviewed (see point 2.4.13.18). Designate a rack position or location for drying of the tip chute and tip catcher, separate from the rack used for reagent troughs. Furthermore, a spare tip chute can be made available for each MP II, therefore used tip chutes can be allowed to decontaminate in a Decon bucket to fully decontaminate the tip chute, without compromising throughput of the MP II.

4.27. The cleaning regime of the MP II, including surroundings and enclosure (e.g. top of MP II hood), must be reinforced.

4.28. As a continuous QA/QC measure, the supervisor should observe the DNA IQ™ protocols at regular intervals for critical assessment and possible re-evaluation of the impact and suitability of changes in the methods.

OQI's

- Three OQI's were raised as an outcome of the audit.
  - 20367: Automated DNA IQ process, including documentation.
    - 4.10-4.15, 4.19-4.22
  - 20368: Enhancement of MP II platforms, including environment.
    - 4.16-4.18, 4.23-4.27
  - 20369: Training and personnel related to the DNA IQ process.
    - 4.1-4.9, 4.28

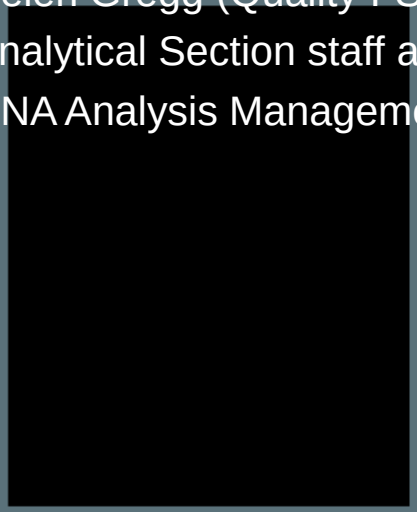
[redacted]

Audit handover

- Audit findings were presented to Allan, Cathie and Tom on 11 August 2008.
- The report (including video footage disc) was handed over to DNA Analysis Quality Management.
- Some recommendations were already being investigated and changes implemented at the time of handover.

Acknowledgements

Peter Clausen (SSDU)
Amy Cheng (Analytical)
Helen Gregg (Quality FSS)
Analytical Section staff and supervisor
DNA Analysis Management Team



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Meeting Attendance – DNA Analysis

Date of Meeting: 17/09/08 **Chair:** Cathie Allen.

NAME	PRESENT/ ABSENT	NAME	PRESENT/ ABSENT	NAME	PRESENT/ ABSENT	NAME	PRESENT/ ABSENT
Abigail HOULDING	✓	Hung CHAN	REC	Mitchell FINGER	✓	Vojtech HLINKA	✓
Adrian PIPPIA	✓	Ian HOBDAY	—	Owanna FRANCISCA	✓	Wendy HARMER	—
Alicia QUARTERMAIN	✓	Iman MUHARAM	✓	Paula TAYLOR	✓		
Allan McNEVIN	✓	Inga SULTANA	✓	Penny TAYLOR	✓		
Allison LLOYD	✓	Ingrid MOELLER	✓	Peter CLAUSEN	—		
Amanda STORER	✓	Jacqui WILSON	✓	Pierre ACEDO	✓		
Amy CHENG	✓	Janine SEYMOUR-MURRAY	✓	Rebecca GREGORY	✓		
Angela ADAMSON	✓	Jayde KEATING	✓	Rebecca THUELL	✓		
Angelina KELLER	✓	Jenny MUNOZ	✓	Rhys PARRY	✓		
Anne FINCH	SICK	Josie ENTWISTLE	✓	Robert MORGAN	✓		
Belinda ANDERSON	✓	Julie CONNELL	✓	Robyn SMITH	✓		
Biljana MICIC	✓	Justin HOWES	✓	Rose HIGGINS	✓		
Catherine ALLEN	✓	Kate LEE	✓	Sandra McKEAN	✓		
Cecilia IANNUZZI	✓	Katrina HEGARTY	LEAVE	Shannon MERRICK	✓	Admin Team not required to attend meeting.	
Chiron WEBER	✓	Kerry-Anne LANCASTER	SICK	Sharon CUMMINGS	LEAVE		
Christopher LOCK	RDO	Kirsten SCOTT	✓	Shirley ELLIS	NON WORK		
Claire PERRIN	✓	Kylie RIKA	✓	Stephanie MIDDAP	SICK		
Courtney RUIZ	—	Lai-Wan CHEN	Working in Lab	Susan BRADY	✓		
Crystal REVERA	✓	Lee TAYLOR	✓	Susan GILLESPIE	✓		
Deborah NICOLETTI	✓	Lisa WESTON	✓	Suzanne SANDERSON	✓		
Emma CAUNT	✓	Lynette DENT	✓	Thomas NURTHEN	✓		
Emma STEWART	—	Margaret BRIAN	✓	Thu NGUYEN	✓		
Evan LECKENBY	✓	Maria AGUILERA	✓	Timothy GARDAM	LEAVE		
Generosa LUNDIE	✓	Megan HARVEY	Working in Lab	Tracey GARTH	—		
Helen WILLIAMS	SICK	Melissa CIPOLLONE	✓	Valerie CALDWELL	✓		

- Please Indicate reason why absent from Staff Meeting

Queensland Government
Queensland Health

TN-24

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Extraction Batch Audit

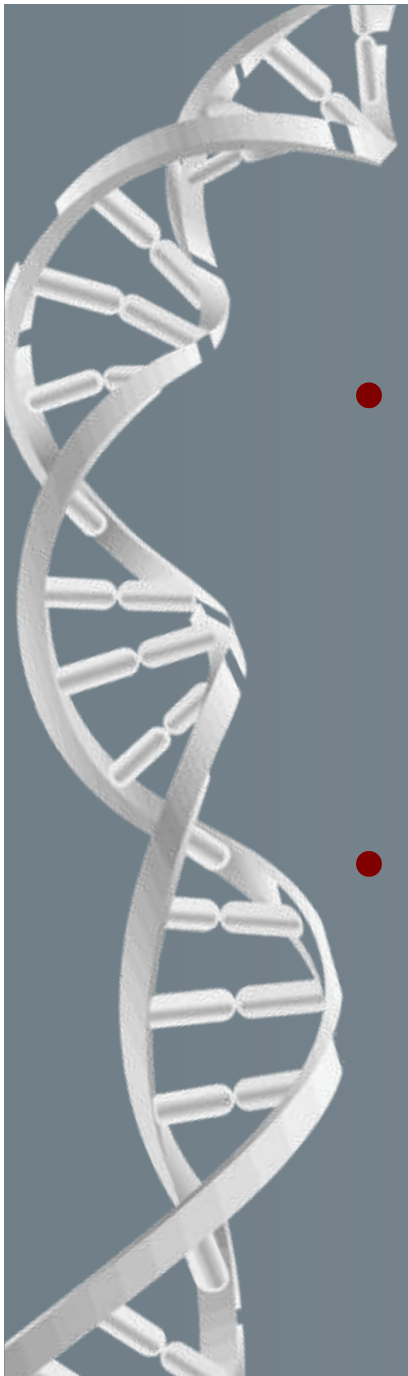
Angelina Keller, Rebecca Gregory and Susan Brady

DNA Analysis, QHFSS

17th September 2008

Queensland Government

Queensland Health



Aim :

- To evaluate casework data obtained from extractions processed with DNA IQ™ on the MultiPROBE® II PLUS HT EX platforms for contamination events.
- This was achieved by comparing initial results for each sample from within an extraction batch.

Background:

- This audit was conducted after the identification of five OQI's [redacted] and 20231).
- All casework samples processed through DNA IQ™ and on the MultiPROBE® II platforms were investigated.
- The extraction audit covered the period from the 23 October 2007 to the 28 July 2008.
- A working party was later established to manage the reporting of all results, with a focus on the release of urgent statements.

Background:

- LISS in association with Thomas Nurthern created an export file from AUSLAB. This function exported the initial results obtained from an extraction batch to a text file.
- Timothy Gardam developed a results comparison macro that identified any matches of >12 alleles between samples from within an extraction batch.

Methods:

The methods involved six stages

- Extraction Audit Progress
- Virtual Platemap of Extraction Batch
- Extraction Batch Comparison
- Checking Matches
- Further Investigations
- Contamination Events

Methods: Extraction Audit Progress

- The status of each extraction batch was recorded in an Excel spreadsheet titled Batch Audit Progress, located in I:/Extraction Audit.

Methods: Extraction Audit Progress

Microsoft Excel - Batch Audit Progress

File Edit View Insert Format Tools Data Window Help

D52 On Hold

	A	B	C	D	E	F	G	H
1	[redacted]	Date Started	Date Complete	Outcome	Batch Allocated To	Specimen Note Added	Batch Audit Entry	Comments
2		15/08/2008	15/08/2008	Plate OK		Yes	Yes	All matches checked, no queries
3		15/08/2008	15/08/2008	Plate OK		Yes	Yes	All matches checked, no queries
4		15/08/2008						
5		15/08/2008						
6		15/08/2008						
7		15/08/2008						
8		15/08/2008						
9								
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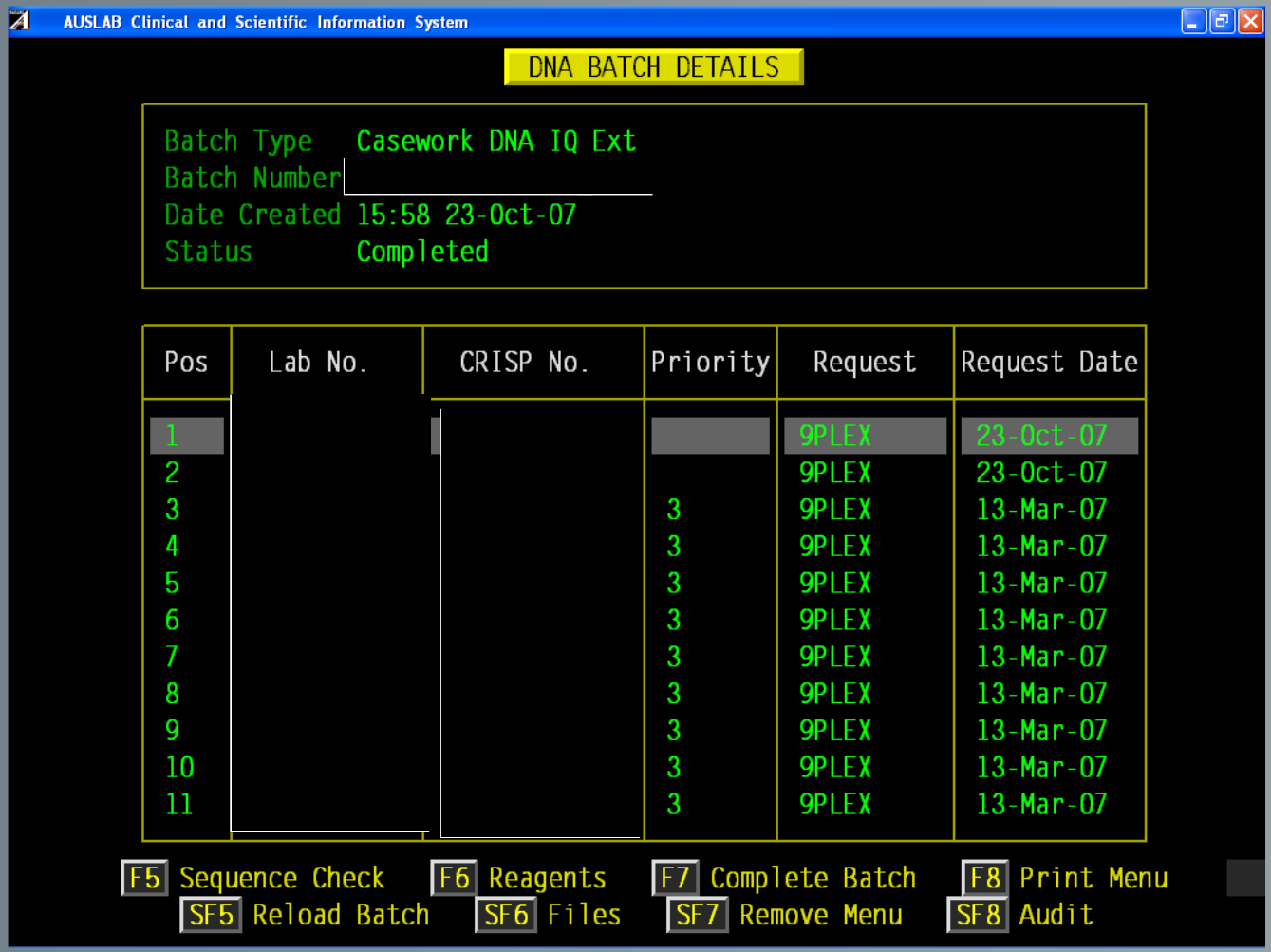
Sheet1 Sheet2 Sheet3

Ready NUM

Methods: Virtual Plate Map

- The Extraction Batch Details were accessed through AUSLAB.

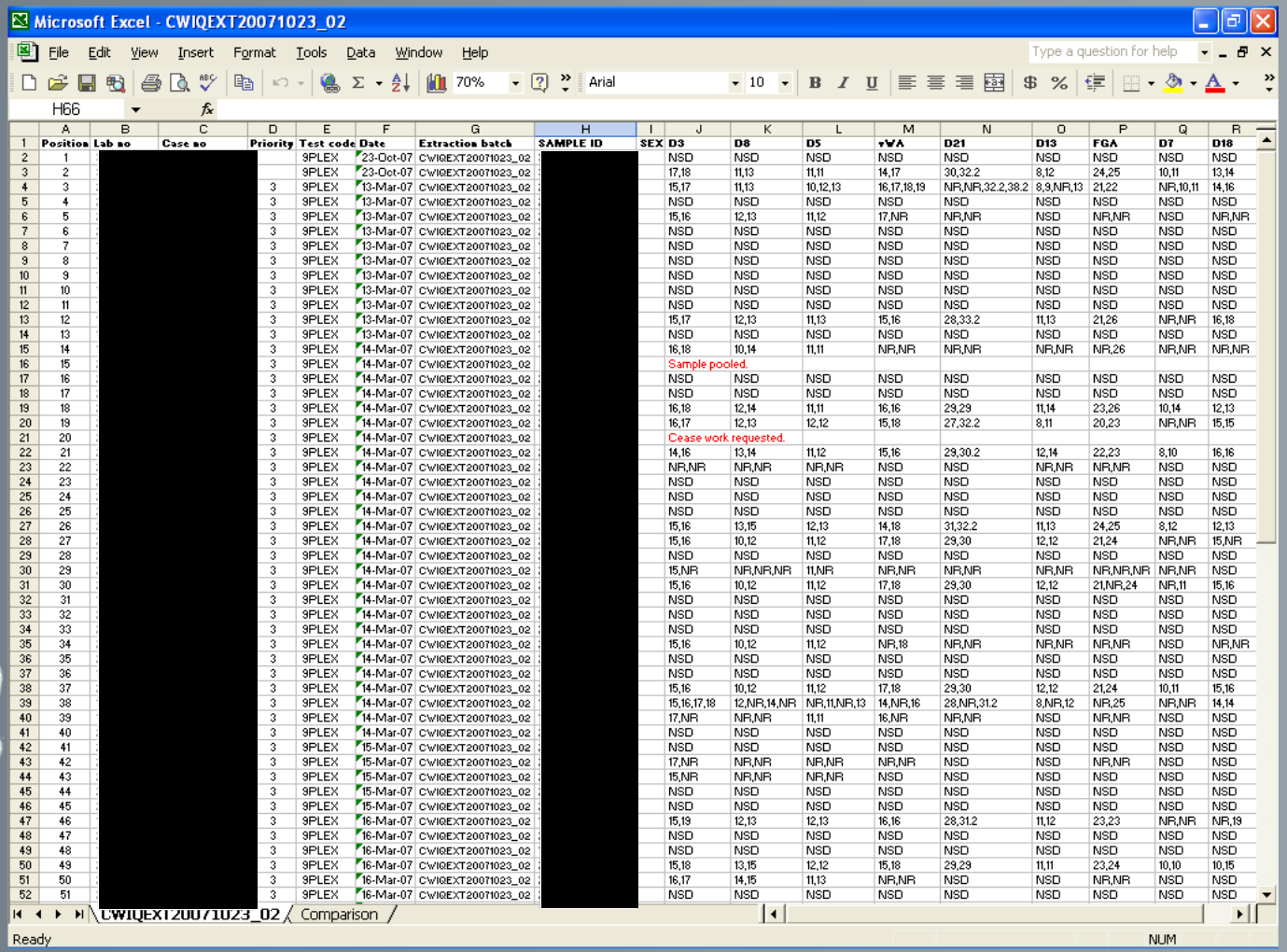
Methods: Virtual Plate Map



Methods: Virtual Plate Map

- Reload Batch (Shift F5) was selected to export the extraction batch results from AUSLAB.
- The save function (Ctrl F11) was then selected to export all of the extraction batch details from AUSLAB.
- The extraction batch number was entered for all samples.
- A new worksheet titled “Comparison” was inserted into the extraction batch details spreadsheet.

Methods: Virtual Plate map



Position	Lab no	Case no	Priority	Test code	Date	Extraction batch	SAMPLE ID	SEX	D3	D8	D5	vWA	D21	D13	FGA	D7	D18
1	[redacted]	[redacted]		9PLEX	23-Oct-07	CWIQEXT20071023_02	[redacted]		NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
2				9PLEX	23-Oct-07	CWIQEXT20071023_02			17,18	11,13	11,11	14,17	30,32.2	8,12	24,25	10,11	13,14
3			3	9PLEX	13-Mar-07	CWIQEXT20071023_02			15,17	11,13	10,12,13	16,17,18,19	NR,NR,32.2,38.2	8,9,NR,13	21,22	NR,10,11	14,16
4			3	9PLEX	13-Mar-07	CWIQEXT20071023_02			NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
5			3	9PLEX	13-Mar-07	CWIQEXT20071023_02			15,16	12,13	11,12	17,NR	NR,NR	NSD	NR,NR	NSD	NR,NR
6			3	9PLEX	13-Mar-07	CWIQEXT20071023_02			NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
7			3	9PLEX	13-Mar-07	CWIQEXT20071023_02			NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
8			3	9PLEX	13-Mar-07	CWIQEXT20071023_02			NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
9			3	9PLEX	13-Mar-07	CWIQEXT20071023_02			NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
10			3	9PLEX	13-Mar-07	CWIQEXT20071023_02			NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
11			3	9PLEX	13-Mar-07	CWIQEXT20071023_02			NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
12			3	9PLEX	13-Mar-07	CWIQEXT20071023_02			NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
13			3	9PLEX	13-Mar-07	CWIQEXT20071023_02			15,17	12,13	11,13	15,16	28,33.2	11,13	21,26	NR,NR	16,18
14			3	9PLEX	13-Mar-07	CWIQEXT20071023_02			NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
15			3	9PLEX	14-Mar-07	CWIQEXT20071023_02			16,18	10,14	11,11	NR,NR	NR,NR	NR,NR	NR,26	NR,NR	NR,NR
16			3	9PLEX	14-Mar-07	CWIQEXT20071023_02			Sample pooled.								
17			3	9PLEX	14-Mar-07	CWIQEXT20071023_02			NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
18			3	9PLEX	14-Mar-07	CWIQEXT20071023_02			NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
19			3	9PLEX	14-Mar-07	CWIQEXT20071023_02			16,18	12,14	11,11	16,16	29,29	11,14	23,26	10,14	12,13
20			3	9PLEX	14-Mar-07	CWIQEXT20071023_02			16,17	12,13	12,12	15,18	27,32.2	8,11	20,23	NR,NR	15,15
21			3	9PLEX	14-Mar-07	CWIQEXT20071023_02			Cease work requested.								
22			3	9PLEX	14-Mar-07	CWIQEXT20071023_02			14,16	13,14	11,12	15,16	29,30.2	12,14	22,23	8,10	16,16
23			3	9PLEX	14-Mar-07	CWIQEXT20071023_02			NR,NR	NR,NR	NR,NR	NSD	NSD	NR,NR	NR,NR	NSD	NSD
24			3	9PLEX	14-Mar-07	CWIQEXT20071023_02			NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
25			3	9PLEX	14-Mar-07	CWIQEXT20071023_02			NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
26			3	9PLEX	14-Mar-07	CWIQEXT20071023_02			NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
27			3	9PLEX	14-Mar-07	CWIQEXT20071023_02			15,16	13,15	12,13	14,18	31,32.2	11,13	24,25	8,12	12,13
28			3	9PLEX	14-Mar-07	CWIQEXT20071023_02			15,16	10,12	11,12	17,18	29,30	12,12	21,24	NR,NR	15,NR
29			3	9PLEX	14-Mar-07	CWIQEXT20071023_02			NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
30			3	9PLEX	14-Mar-07	CWIQEXT20071023_02			15,NR	NR,NR,NR	11,NR	NR,NR	NR,NR	NR,NR	NR,NR,NR	NR,NR	NSD
31			3	9PLEX	14-Mar-07	CWIQEXT20071023_02			15,16	10,12	11,12	17,18	29,30	12,12	21,NR,24	NR,11	15,16
32			3	9PLEX	14-Mar-07	CWIQEXT20071023_02			NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
33			3	9PLEX	14-Mar-07	CWIQEXT20071023_02			NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
34			3	9PLEX	14-Mar-07	CWIQEXT20071023_02			NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
35			3	9PLEX	14-Mar-07	CWIQEXT20071023_02			15,16	10,12	11,12	NR,18	NR,NR	NR,NR	NR,NR	NSD	NR,NR
36			3	9PLEX	14-Mar-07	CWIQEXT20071023_02			NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
37			3	9PLEX	14-Mar-07	CWIQEXT20071023_02			NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
38			3	9PLEX	14-Mar-07	CWIQEXT20071023_02			15,16	10,12	11,12	17,18	29,30	12,12	21,24	10,11	15,16
39			3	9PLEX	14-Mar-07	CWIQEXT20071023_02			15,16,17,18	12,NR,14,NR	NR,11,NR,13	14,NR,16	28,NR,31.2	8,NR,12	NR,25	NR,NR	14,14
40			3	9PLEX	14-Mar-07	CWIQEXT20071023_02			17,NR	NR,NR	11,11	16,NR	NR,NR	NSD	NR,NR	NSD	NSD
41			3	9PLEX	14-Mar-07	CWIQEXT20071023_02			NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
42			3	9PLEX	15-Mar-07	CWIQEXT20071023_02			NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
43			3	9PLEX	15-Mar-07	CWIQEXT20071023_02			17,NR	NR,NR	NR,NR	NR,NR	NSD	NSD	NR,NR	NSD	NSD
44			3	9PLEX	15-Mar-07	CWIQEXT20071023_02			15,NR	NR,NR	NR,NR	NSD	NSD	NSD	NSD	NSD	NSD
45			3	9PLEX	15-Mar-07	CWIQEXT20071023_02			NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
46			3	9PLEX	15-Mar-07	CWIQEXT20071023_02			NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
47			3	9PLEX	16-Mar-07	CWIQEXT20071023_02			15,19	12,13	12,13	16,16	28,31.2	11,12	23,23	NR,NR	NR,19
48			3	9PLEX	16-Mar-07	CWIQEXT20071023_02			NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
49			3	9PLEX	16-Mar-07	CWIQEXT20071023_02			NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
50			3	9PLEX	16-Mar-07	CWIQEXT20071023_02			15,18	13,15	12,12	15,18	29,29	11,11	23,24	10,10	10,15
51			3	9PLEX	16-Mar-07	CWIQEXT20071023_02			16,17	14,15	11,13	NR,NR	NSD	NSD	NR,NR	NSD	NSD
52			3	9PLEX	16-Mar-07	CWIQEXT20071023_02			NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD

Methods:

Extraction Batch Comparison

- The Batch Comparison Macro was opened and Compare Profiles was selected.
- The extraction batch results to be compared were selected. This generated the batch profile comparison results.
- This page was copied and pasted into the comparison worksheet of the virtual plate map, and then printed to include the extraction batch number as a hard copy record.

Methods:

Extraction Batch Comparison

- Matching alleles were highlighted in yellow. Light yellow indicated one matching allele, dark yellow indicated at least two matching alleles.

Microsoft Excel - Batch Profile Comparison

File Edit View Insert Format Tools Data Window Help

C54

	Sample ID	Amel	D3	D8	D5	vWA	D21	D13	FGA	D7	D18	Match Score	Compare Profiles
2	[redacted]		15,16	10,12	11,12	17,18	29,30	12,12	21,24	NR,NR	15,NR	15	
3			15,16	10,12	11,12	17,18	29,30	12,12	21,NR,24	NR,11	15,16	15	
4													
5			15,16	10,12	11,12	17,18	29,30	12,12	21,24	NR,NR	15,NR	15	
6			15,16	10,12	11,12	17,18	29,30	12,12	21,24	10,11	15,16	15	
7													
8			15,16	10,12	11,12	17,18	29,30	12,12	21,NR,24	NR,11	15,16	17	
9			15,16	10,12	11,12	17,18	29,30	12,12	21,24	10,11	15,16	17	

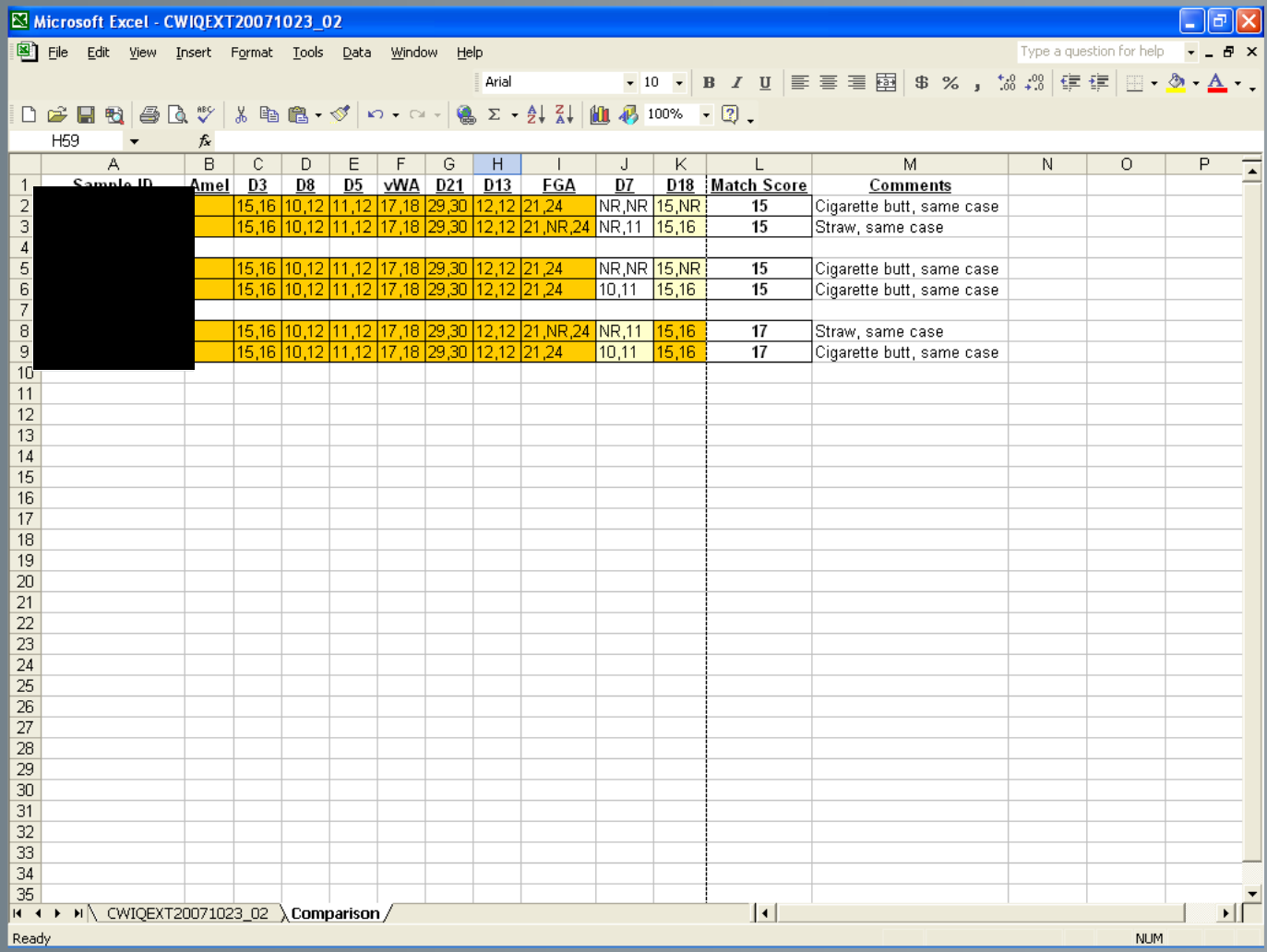
Sheet1

Ready NUM

Methods: Checking Matches

- From the extraction batch the first matching sample highlighted by the Macro was selected.
- Sample details were noted (eg. exhibit type). This was repeated for the second matching sample.
- If both samples were from the same exhibit or the same case this match was passed.
- Comments for each match were entered into the comparison worksheet within the virtual plate map.

Methods: Checking Matches



	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Sample ID	Amel	D3	D8	D5	vWA	D21	D13	FGA	D7	D18	Match Score	Comments
2	[redacted]		15,16	10,12	11,12	17,18	29,30	12,12	21,24	NR,NR	15,NR	15	Cigarette butt, same case
3			15,16	10,12	11,12	17,18	29,30	12,12	21,NR,24	NR,11	15,16	15	Straw, same case
4													
5			15,16	10,12	11,12	17,18	29,30	12,12	21,24	NR,NR	15,NR	15	Cigarette butt, same case
6			15,16	10,12	11,12	17,18	29,30	12,12	21,24	10,11	15,16	15	Cigarette butt, same case
7													
8			15,16	10,12	11,12	17,18	29,30	12,12	21,NR,24	NR,11	15,16	17	Straw, same case
9			15,16	10,12	11,12	17,18	29,30	12,12	21,24	10,11	15,16	17	Cigarette butt, same case

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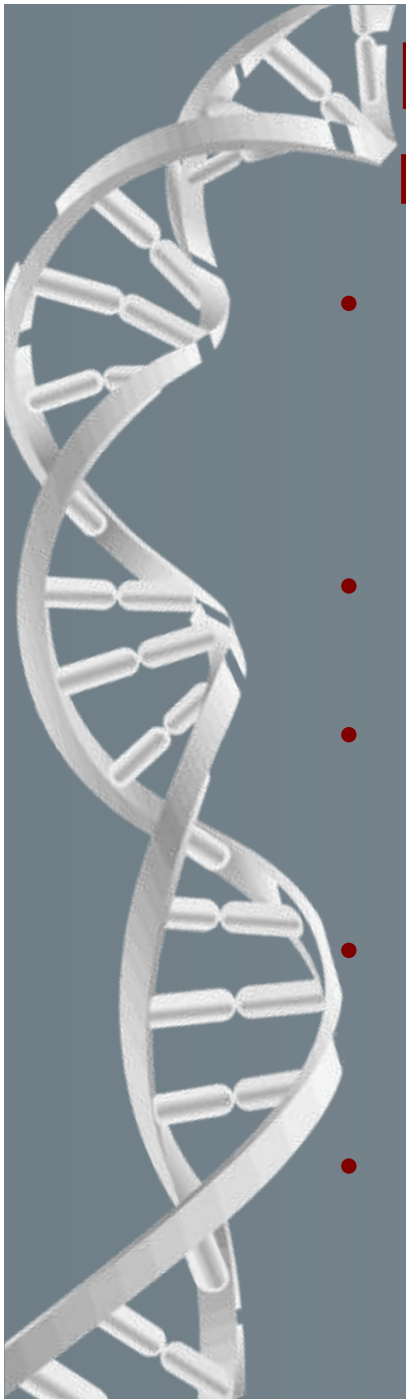
Methods: Checking Matches

- A batch audit entry was inserted “Extraction batch audit complete. Plate released” and a specimen note entered for each sample.
- Any matches that could not be passed were highlighted in red and the entire batch was placed on hold pending further investigation.
- The Batch Audit Progress spreadsheet was updated with all outcomes.
- Both the Batch Audit Progress spreadsheet and all finalised virtual plate maps were password protected to ensure the integrity of these documents.



Methods:

Further Investigations

- For matches requiring further investigation, Genotyper printouts were obtained and case details were noted eg. address, date and type of alleged offence.
- Nominated case scientists performed both mixture and single source interpretations.
- If matches were excluded, the paperwork was returned for filing, and both AUSLAB and the Batch Audit Progress spreadsheet were updated.
- Re-sampling was conducted to confirm matches between single source profiles from different cases.
- If re-sampling was not possible the original spin baskets were re-extracted.

Methods:

Contamination Events

- Mixed or single source matches that were identified as contamination events were brought to the attention of the team leader.
- An attempt was made to establish the source of the contamination through the reconstruction of a plate map.
- An OQI was then raised by a senior scientist.

Results:

- Six extraction folders contained the extraction batches (Volumes 28-33) located in the Analytical Section .
- 278 extraction batches were processed through DNA IQ™ and on the MultiPROBE® II platforms from the 23 October 2007 to the 28 July 2008

Total number of extraction batches	**278**
Extraction batches released	202
Extraction batches on hold	14
Extraction batches removed	62

Discussion:
Extraction batches released (202)

- where no matches were returned after the use of the Batch Comparison Macro (22), or

- where any matches could be explained by samples originating from the same exhibit or from exhibits within the same case (157).

Discussion: Further Interpretations:

- Matches that could not be eliminated included partial and full profiles from both single and mixed source samples.
- Nominated case scientists performed interpretations to determine if the matches could be excluded or whether further investigation was required before the batch could be released.
- 20 batches were released after further investigations.
- 3 batches were released after re-sampling.

Discussion: On Hold Batches (14)

- If part of previously raised OQI's (five)

  - OQI's included 18580, 19349, 19477, 19768 and 20231.
- If included in new OQI's identified (three)

  - OQI's 20351, 20422 and 20437.
- If re-sampling was required to confirm initial results (six). Re-sampling has been conducted and results are pending.

Discussion: On Hold Batches

- Five extraction batches had 53 samples that had not progressed to Quantification. To finalise initial results a new test code entry was required in AUSLAB.
- Extraction batches were removed in AUSLAB for several reasons. These included:
  - controls not being added
  - incorrect sample type (tapelifts or manual extraction)
  - incorrect batch type (off-deck lysis)
  - requiring smaller batch sizes (urgent samples)

Summary:

•Evaluation of the casework data obtained from extractions using DNA IQ™ and the MultiPROBE® II platforms involved 216 extraction batches.

• 202 batches were released, 14 batches were placed on hold (six batches still awaiting results).

•This audit identified three new contamination events and three OQI's were raised to address these issues.

•The new methodology developed and applied in this audit provides scientists with a strengthened quality system that may prevent similar adverse events from occurring in future and simultaneously increase the confidence scientists have in reporting results.

Recommendations:

- The Batch Comparison Macro could be applied to all new extraction batches to assist in the identification of any adverse events prior to the release of results.

- If an adverse event is identified, a streamlined process needs to be in place to address the issue effectively with efficient laboratory communication (eg. e-mail alert system).

- Availability of AUSLAB functions to export all results from any batch type, facilitating other types of audits and quality measures.

- A previously identified system fault in AUSLAB needs to be addressed to ensure that all samples progress from extraction to quantification.

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Extract

Angelina Kelle , Cathie Allen
DNA Analysis

1. Abstra

An internal a ocess was deemed necessary to address adverse quality events identified through the laboratory quality system and Opportunity for Quality Improvemer urpose of this audit was to maintain the continuous high quality stand alysis laboratory.

All initial res samples processed with DNA IQ™ and the MultiPROBE between the 23 October 2007 and the 28 July 2008 were invest f 216 extraction batches. Of these, 202 batches w %), six batches were placed on hold pending the release of results (3%) a the involvement in previous OQI's (2%). An a rough the identification of new contamination .

ew tool to reinforce the e strengthening the confidence scientists have in rep ave applications outside the scope of this audit and b

2. A

- te ca a obta xtractions processed with DNA IQ™ on ROBE® II PLUS HT EX platforms for contamination events by comparing lts for each sample from within an extraction batch.

3. B

aboratory quality system (OQI process) a number of adverse quality events on the MultiPROBE® II platforms. Five OQI's (18580, 19349, 19477, 19768 and 20231) had previously been raised to address a dropped Slicprep™ 96 Device plate and profiles in the negative controls. The management team convened and a decision was made to conduct an internal audit of the entire extraction process.

Initial investigations were unable to determine whether the contamination events originally identified were due to the MultiPROBE® II platforms alone or a combination of the platforms and the DNA IQ™ process. The management team subsequently decided to audit the initial results obtained from every casework sample processed through DNA IQ™ and on the MultiPROBE® II PLUS platforms. This covered the period from the 23 October 2007 to the 28 July 2008, when both processes were temporarily halted. A working party was later established to manage the reporting of all results, with a focus on the release of urgent statements.

Laboratory [redacted] olutions (LISS) in association with Thomas Nurthern (DNA Analy[redacted]vices (FSS)) created an export file from AUSLAB. This functio[redacted] obtained from an extraction batch to a text file that could then [redacted]xcel. Timothy Gardam (DNA Analysis, FSS) developed [redacted] that identified any matches of >12 alleles between samples fr[redacted]n. This matching stringency was determined by the managemer[redacted] the upload st[redacted] National Criminal Investigatio[redacted]0).

4. Equipm[redacted]

i. AU[redacted]initial results from an extraction batch
ii. AU[redacted]details of samples from an extraction batch (eg [redacted]se number, position, priority)

[redacted]

5. Metho[redacted]

5.1. Ex[redacted]s

i. The [redacted]n batch was recorded in an Excel spreadsheet titled Bat[redacted]ed in I:/Extraction Audit (See Figure. 1 – Batch Audit Progress).

[redacted]

Microsoft Excel - Batch Audit Progress

File Edit View Insert Format Tools Data Window Help

D52 On Hold

	A	B	C	D	E	F	G	H
1	[redacted]	ate Started	Date Complete	Outcome	Batch Allocated To	Specimen Note Added	Batch Audit Entry	Comments
2		15/08/2008	15/08/2008	Plate OK		Yes	Yes	All matches checked, no queries
3		15/08/2008	15/08/2008	Plate OK		Yes	Yes	All matches checked, no queries
4		15/08/2008						
5		15/08/2008						
6		15/08/2008						
7		15/08/2008						
8		15/08/2008						
9								
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33								
34								
35								

Sheet1 Sheet2 Sheet3

Ready NUM

[redacted]**en shot of Batch Audit Progress**

[redacted]

[redacted]

5.2. V[redacted]action Batch

i. AU[redacted]
ii. Op[redacted]ment) and 2. (DNA Batch Details) were selected.
iii. The[redacted]r was entered[redacted]XT20071023_02). The DNA[redacted]s were displayed (See Figure 2. – AUSLAB DNA Extr[redacted]

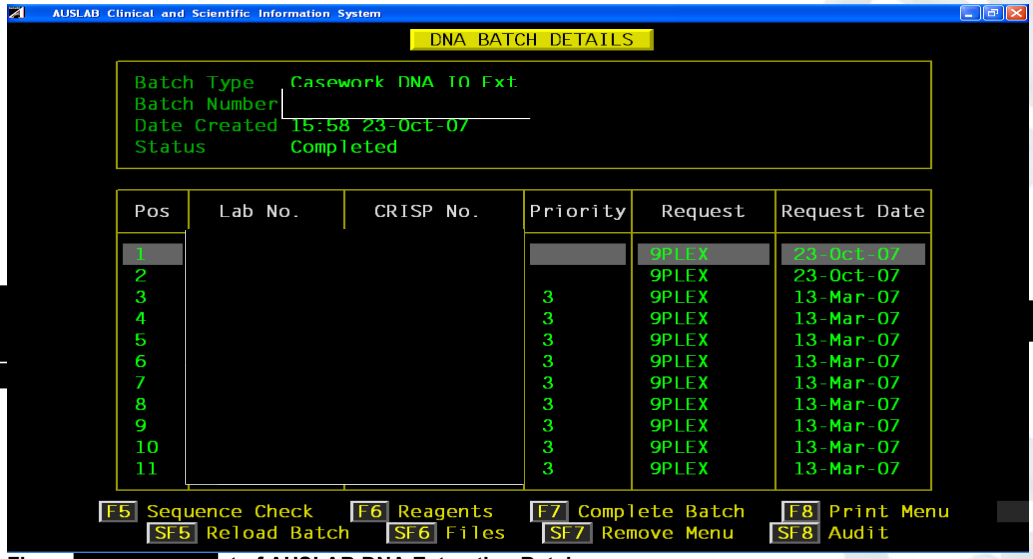


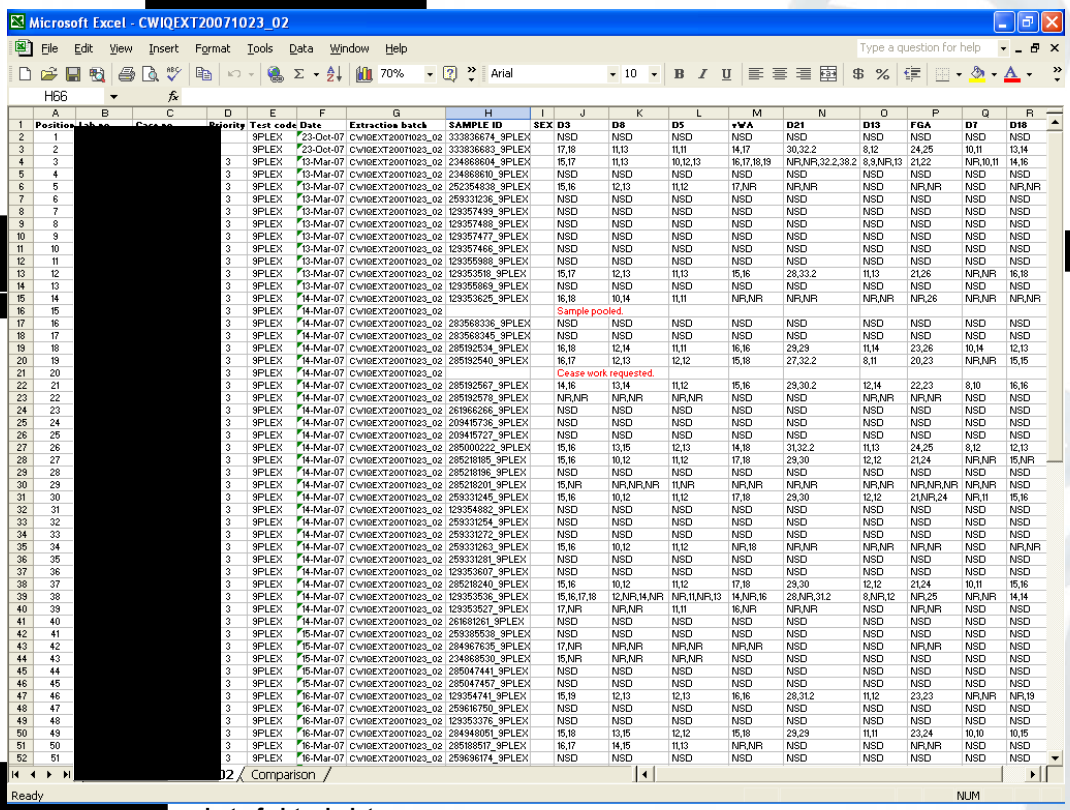
Figure [redacted]ot of AUSLAB DNA Extraction Batch

iv. [redacted]tch [redacted]as se[redacted]ort the extraction batch results from [redacted] These results were saved as a text file to C:/AUSLAB, which was then [redacted]o the I:/Extraction Audit/Work in Progress/AUSLAB Shift F5 folder.
v. [redacted]function (Ctrl F11) was selected to export all of the extraction batch [redacted]m AUSLAB. The prompt "OK to save table to disk (y/n)?" was displayed, [redacted]lected for yes and the file name I:/Extraction Audit/Work in [redacted]CWIQEXT20071023_02.xls was entered.
vi. [redacted]ction batch details spreadsheet was opened, a header row was inserted [redacted]Position, Lab no, Case no, Priority, Test Code, Date, Extracton Batch, Sample [redacted]ex, D3, D8, D5, vWA, D21, D13, FGA, D7 and D18.
vii. [redacted]xtraction batch number was entered for all samples.
viii. A new worksheet titled "Comparison" was inserted into the extraction batch details spreadsheet.

[redacted] [redacted]

ix. The [redacted] xt file (located in the Shift F5 folder) was opened with Mic[redacted]ts were copied and then pasted into the extraction bat[redacted]s entire spreadsheet was the virtual plate map of the ext[redacted] – Virtual plate map).

x. If re[redacted] virtual plate map, it was necessary to check the sta[redacted] Reasons for no results included samples still being proc[redacted]ate reworking, [redacted] samples had not auto[redacted]quantification. Any explanations for blank result fields wer[redacted]e map. For samples that had failed to progress to quantification, a re-quant had to be ordered in AUSLAB. The test code for this was REQ[redacted]

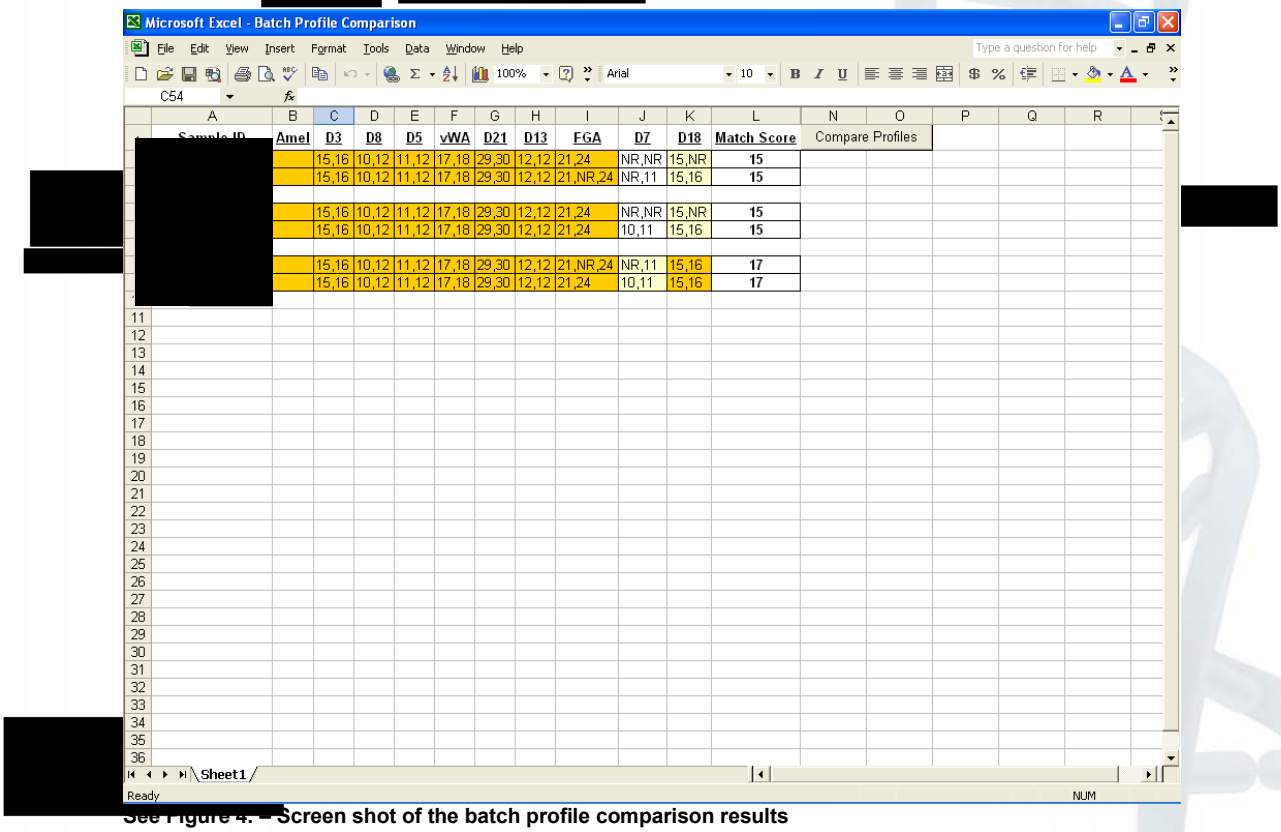


[redacted]en shot of virtual plate map

[redacted]

5.3. [redacted]arison

i. The [redacted]in I:/Macros/testing was opened.
ii. Co[redacted].
iii. A pr[redacted]file for match [redacted] was displayed. The extr[redacted]file to be compared (located in the Shift F5 folder) was sele[redacted]e batch profile comparison results (See Figure 4. – Batch profile comparison results).
iv. This page was copied and pasted into the comparison worksheet of the virtual plat[redacted]to include the extraction batch number as a hard copy reco[redacted]



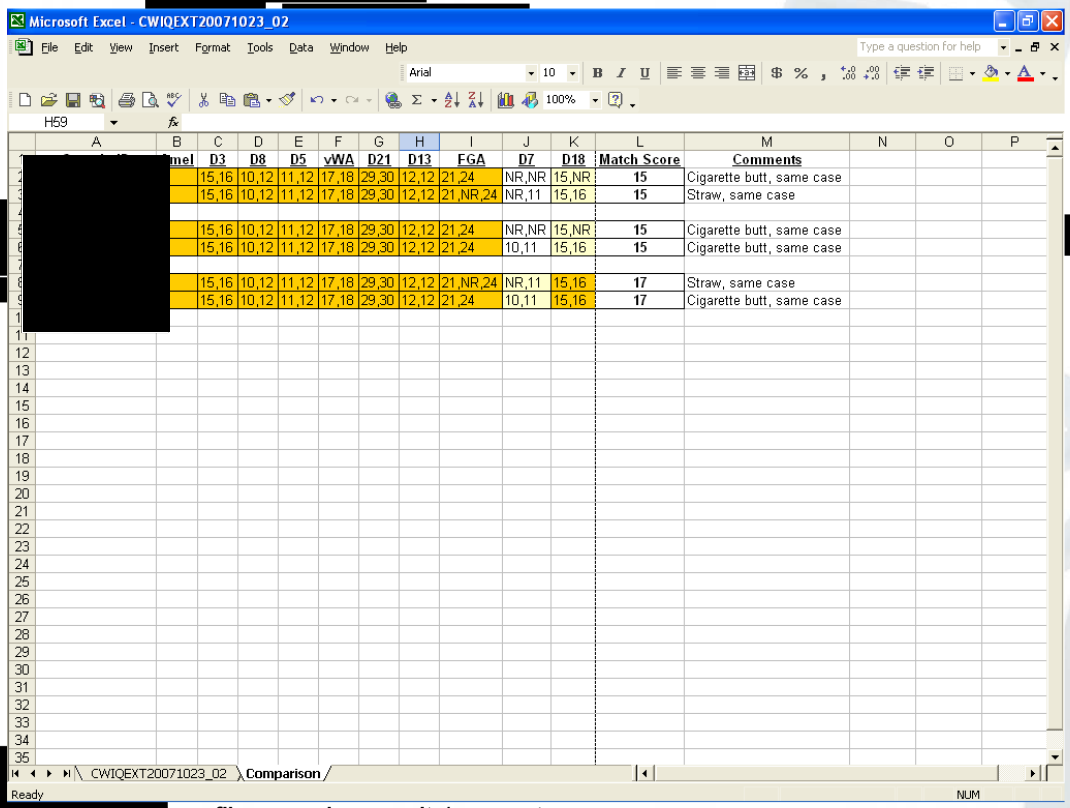
Sample ID	Amel	D3	D8	D5	vWA	D21	D13	FGA	D7	D18	Match Score
[redacted]		15,16	10,12	11,12	17,18	29,30	12,12	21,24	NR,NR	15,NR	15
		15,16	10,12	11,12	17,18	29,30	12,12	21,NR,24	NR,11	15,16	15
		15,16	10,12	11,12	17,18	29,30	12,12	21,24	NR,NR	15,NR	15
		15,16	10,12	11,12	17,18	29,30	12,12	21,24	10,11	15,16	15
		15,16	10,12	11,12	17,18	29,30	12,12	21,NR,24	NR,11	15,16	17
		15,16	10,12	11,12	17,18	29,30	12,12	21,24	10,11	15,16	17

See Figure 4. – Screen shot of the batch profile comparison results

The batch comparison macro identified any matches of >12 alleles between samples from within an extraction batch. Matching alleles were highlighted in yellow. Light yellow indicated one matching allele[redacted]yellow indicated at least two matching al[redacted] number of matching alleles between samples was listed in the column titled Match Score.

5.4. [redacted]

i. AUSLA[redacted]
ii. Options [redacted]) and 2. (DNA Batch Details) were selected.
iii. The Ext[redacted]s entered (eg[redacted]0071023_02).
iv. The first [redacted]ghted by the Macro was selected. Sample details were noted (e[redacted]s repeated for the second matching sample. If both samples were from the same exhibit and/or case this match was passed. Comments for each match were entered into the comparison worksheet within the virtual plate map (See Fig[redacted]mparison results/comments).



	D3	D8	D5	vWA	D21	D13	FGA	D7	D18	Match Score	Comments
	15,16	10,12	11,12	17,18	29,30	12,12	21,24	NR,NR	15,NR	15	Cigarette butt, same case
	15,16	10,12	11,12	17,18	29,30	12,12	21,NR,24	NR,11	15,16	15	Straw, same case
	15,16	10,12	11,12	17,18	29,30	12,12	21,24	NR,NR	15,NR	15	Cigarette butt, same case
	15,16	10,12	11,12	17,18	29,30	12,12	21,24	10,11	15,16	15	Cigarette butt, same case
	15,16	10,12	11,12	17,18	29,30	12,12	21,NR,24	NR,11	15,16	17	Straw, same case
	15,16	10,12	11,12	17,18	29,30	12,12	21,24	10,11	15,16	17	Cigarette butt, same case

[redacted]h profile comparison results/comments

v. If the extraction batch was released at this stage, Audit (Shift F8) and Insert Audit Trail (F5) was selected from the extraction batch and "Extraction batch audit complete. Plate released" was entered. A specimen note was also entered for each sample on the extraction batch. [redacted]
vi. Any matches that could not be passed were highlighted in red and the entire batch was placed on hold pending further investigation.
vii. The Batch Audit Progress spreadsheet was updated with all outcomes.
viii. Both the Batch Audit Progress spreadsheet and all finalised virtual plate maps were password protected to ensure the integrity of these documents.

5.5 [redacted]

i. For ma[redacted]tigation, Genotyper printouts were obtained and case de[redacted]s, date and type of alleged offence.
ii. Nomina[redacted]ed both mixture and single source interpretations.
iii. If matc[redacted]aperwork was [redacted]iling, and both AUSLAB and the Bato[redacted]sheet were updated.
iv. If possib[redacted]ducted to confirm matches between single source profiles from different cases. Where re-sampling was not possible, the original spin baskets were re-extracted.

5.6 [redacted]s

M[redacted]urce matches that were identified as contamination events were brought to[redacted]pt was made to establish the source of the d[redacted]a plate map. An OQI was then raised by a [redacted]

6. Result

It was ident traction batches processed through DNA IQ™ and on the Mult e 23 October 2007 to the 28 July 2008 (See Table lited extraction hese extraction batches were filed in olumes 28-33) located in the Analytical Section.

Table 1. – Current status of audited extraction batches

Total number of extraction batches	**278**
released	202
on hold	14
Extraction batches removed	62

Extraction b

matches were returned after the use of the Batch Comparison Macro, or ed by samples originating from the same me case.

les from both single and ntists performed interpretations to determine if th urther investigation was required before the batch

Extrac s were placed on hold if they were part of previously raised OQI's (five), new O as a result of this audit (three), or if re-sampling was required to confirm initial).

- OQI's included 18580, 19349, 19477, 19768 and 20231. These reports cces QIS.
- new OQI's 20351, 20422 and 20437 identified three previously unknown tion events in three different extraction batches. These batches are on ing further investigation. For further details please refer to 1. - OQI 20351, Appendix 2. - OQI 20422 and Appendix 3. - OQI 20437.
- between different cases were identified on nine extraction batches. Re- or re-extraction of the original spin baskets has been conducted and still pending for six batches.

ve extraction batches where 53 samples had not progressed to Quantification. ial results a new test code entry was required in AUSLAB.

Extraction batches were removed in AUSLAB for several reasons. These included:

- controls not being added
- incorrect sample type r manual extraction)
- incorrect batch type (off-deck lysis)
- requiring smaller batch sizes (urgent samples)

7. Discus[redacted]

From the 21[redacted]ed in this audit, a total of 202 batches were released. C[redacted]

- 22 b[redacted]ches after the [redacted]tch Comparison Macro.
- 157[redacted]es that could be explained without further investigation.
- 20 b[redacted]ntified as requiring further interpretation by a nominated case scientist, but were subsequently passed as there was no evidence of cont[redacted]
- 3 ba[redacted]and passed as the profiles obtained from re-sampling mat[redacted]

A total of 14[redacted]aced on hold. These included:

- Five[redacted]us OQI's
  [redacted]our of these OQI's (19349, 19477, 19768 and 20231) were from [redacted]nation events. The initial results were reported [redacted]
  [redacted]ed from a dropped Slicprep™ plate [redacted]
  [redacted]ed.
  [redacted]ously unknown [redacted]
  [redacted]on batch CWIQEXT20080402_01. This batch [redacted]file present in the positive extraction control - [redacted] (position 1). This profile matched to the corresponding [redacted]mponents present in the profiles obtained from samples of a sexual [redacted]ssault case - 209039621(position 25), 209039610 (position 26), [redacted]09039596 (position 27), 209039585 (position 28), 209039604 (position [redacted]) and 209039579 (position 32). This profile also matched to the profiles [redacted]btain[redacted] samples [redacted]rent volume crime cases - 209066683 [redacted]osition 42), 209066674 (position 43), 209066660 (position 44) and [redacted]59718144 (position 51). The contaminating profiles appeared to be [redacted]stributed in both directions across the plate.
  [redacted]QI 20422 involved extraction batch CWIQEXT20080506_02. This batch [redacted]ad a mixed DNA profile present in sample - 365296308 (position 7). The [redacted]ajor component of this DNA profile matched to the profile obtained from [redacted]e deceased in this case. The minor component of this profile matched to the profile obtained from the complainant in what appears to be an unrelated sexual assault case - 320124514 (position 23) and 320124503 (position 24).
  - OQI 20437 involved extraction batch CWIQEXT20080630_01. This batch had a bent pipette tip at position 6 which appeared to have made contact with the horizontally adjacent well at position 14. No DNA profile was [redacted] obtained for [redacted] 323288136 (position 6), whilst a partial profile [redacted] obtained for sample - 365366424 (position 14). In addition, a full profile obtained for sample - 320126679 (position 5) from a sexual assault case matched to sample - 323288127 (position 7) a case involving the unlawful use of a motor vehicle.

These adverse events did not appear to have a uniform distribution across the plate.

- Six [redacted] that required the re-sampling of exhibits to confirm the [redacted] any potential contamination events. If re-sampling was [redacted] from within the case not previously examined were sam[redacted]skets were re-extracted. Results are pending.

A total of [redacted] ontained 53 samples that had not progressed to quantificatio[redacted]al results were still outstanding. A new test code entry was require[redacted]e samples to progress. This was either due to the processing comments not being checked by the MultiPROBE® II platform operator or a previously identified system fault in AUSLAB.

8. Miscel[redacted]

An outcome[redacted] recommend the use of the filter function in the vi[redacted]n conjunction with normal quality processes to manage the reporting of all re[redacted]

Evalu[redacted]m extractions using DNA IQ™ and the MultiP[redacted]ction batches. 202 batches were released, 14 batch[redacted]ed on hold (six batches still awaiting results) and 62 batches were remov[redacted]

This a[redacted]d three new contamination events and three OQI's were raised to addre[redacted]ues i[redacted]e wit[redacted]tory quality system. The new metho[redacted]loped and applied in this audit provides scientists with a strengthened quality [redacted]t may prevent similar adverse events from occurring in future and simult[redacted]crease the confidence scientists have in reporting results.

[redacted]dations

[redacted]mmendations from this audit are listed below:

- The Batch Comparison Macro could be applied to all new extraction batches to assist in the identification of any adverse events prior to the release of results.
- If an adverse event is [redacted] a streamlined process needs to be in pl[redacted] address the issue effectively with efficient laboratory communication (eg. e-mail alert system).
- Availability of AUSLAB functions to export all results from any batch type, facilitating other types of audits and quality measures.
- A previously identified system fault in AUSLAB needs to be addressed to ensure that all samples progress from extraction to quantification.

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APPEND[redacted]

OQI No.: [redacted]

What Stage? [redacted]TION OF ROOT CAUSE

Created By: [redacted]

On: [redacted]08

Corporate Unit*: Forensic and Scientific Services

Site/Location [redacted]ins

Centre/Group [redacted]ences (FS)

Department*: [redacted]Forensic Biology (FSS)

Work Area: [redacted]gy - Major Crime

Desc [redacted] CWIQEXT20080402_01 was found to have a partial minor DNA [redacted]e extraction positive control (346792908). This [redacted]profile matches alleles present in samples [redacted] 25) [redacted] These samples are from a [redacted]nd the profile from these samples is the same. This [redacted] profiles from four separate volume crime cases [redacted]ction batch. It appears as though the contaminating profiles have not only contaminated from right to left across the plate but also from left to right. Part of the investigation into this event has been researched by the Extraction Audit Team and a word document [redacted]de[redacted] drafted and sent to the receiver of this OQI so that it can be included in the investigation part of the OQI process.

Source [redacted]: Audit

What N[redacted]?: Process

Action [redacted]: MCNEVIN, Allan

In Corp[redacted]: Forensic and Scientific Services

[redacted]**n:** Coopers Plains

[redacted]**up:** DNA Analysis

In Department: Forensic Biology (FSS)

In Work Area: Forensic Biology - Analytical

Root Cause: Other[redacted]

Date Actioned or Due: 18-AUG-2008

How Fixed?: Other

Accepted?: Pending

Approval Type: Pending

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APPEND[redacted]

OQI No.: [redacted]

What Sta[redacted]ON OF ROOT CAUSE

Created B[redacted]n [redacted]

On: [redacted]

Corporate Unit*: Forensic and Scientific Services

Site/Locat[redacted]

Centre/Grou[redacted]: Forensic Sciences (FS)

Departme[redacted](FSS)

W[redacted]: Forensic Biology - Major Crime

D[redacted] DNA profile was obtained for lab no. [redacted]5382) that had a major DNA profile [redacted] in th[redacted] complainant in an [redacted]exual assault matter (QP800088413).

Sour[redacted]PSS)

Wha[redacted]: Process

Fixin[redacted]

Actio[redacted] Who: MCNEVIN, Allan

In C[redacted] Unit: Forensic and Scientific Services

In [redacted] Site/L[redacted]: Coopers Plains

In [redacted]: Forensic Sciences (FS)

[redacted]ent: Forensic Biology (FSS)

Root Cause: Other

Date Actioned or Due: 30-AUG-2008

How Fixed?: Other [redacted]

Accepted?: Pending

Approval Type: Pending

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APPEND[redacted]
OQI No.:
What Sta[redacted]ON OF ROOT CAUSE
Created B[redacted]nda [redacted]
On:
Corporate Unit*: Forensic and Scientific Services
Site/Locat[redacted]
Centre/Grou[redacted] Forensic Sciences (FS)
Departme[redacted]FSS)
W[redacted] Forensic Biology - Major Crime
D[redacted]01 : Whilst this process was running on the [redacted]d that the plate was misaligned on the [redacted]tip a[redacted]ive tip at position 6 was [redacted]ave made contact with the well of position [redacted]

Position 6 did not result in a DNA profile. (QT700518732, UUMV, Greenslopes). Position 14 resulted in an incomplete male DNA profile. (QT800323269, burglary, Noosaville)

[redacted]

During further checks of this extraction batch, it was discovered that the DNA profile in position 5 matched the DNA profile in position 7.

Position 5 contained extract from the oral swab of a SAIK (QT800131925, Townsville), and this DNA profile matches to the DNA profiles obtained from the other intimate samples from the SAIK of that case, so it appears to be a true result. Position 7, however, was supposed to contain extract from a swab from the right throttle of a motorbike involved in the UUMV case mentioned above (QT700518732). The other sample for this case was in p[redacted]which did not result in a profile, and [redacted] reference samples were received in relation to this matter for further comparison.

Summary : position 5 (sample 320126679) from a sexual assault case has matched to position 7 (sample 323288127) from an

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[redacted]ed unlawful use of motor vehicle case in [redacted] State.

Source of [redacted] (QHPSS)

What Nee [redacted]
Fixing?: [redacted]

Action By [redacted]lan
Whom?:

In Corpor [redacted] Forensic and Scientific Services
Unit:

In [redacted]
Site/Locat [redacted]

In [redacted]
C [redacted]
I [redacted])

[redacted]

How [redacted]
Acce [redacted] Pending
Appr [redacted] Pending

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

Queensland Government
Queensland Health

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DNA Analysis (Forensic Biology) | Forensic & Scientific Services | Queensland Health

The FSS DNA Analysis laboratory was experiences programming problems with their extraction robotics. The problems included drops forming on the tips, leading to cross-contamination. I was asked to take a look at the programming to determine whether any of the liquid handling settings could be improved. The test was physically set up and run and observed for problems. Following is a list of the steps which required some modifications to the liquid handling settings.

9. ADD LYSIS BUFFER
    Increased dispense height
    Increased tracking
    Inserted a *post-dispense transport air gap* to remove bubbles
    Decreased dispense height after mix step

17. REMOVE LYSIS
    Decreased scan in speed
    Decreased aspirate speed
    Decreased *Retract From Liquid* speed
    Decreased dispense height
    Inserted a *post-dispense transport air gap* to remove bubbles

20. DISPENSE LYSIS BUFFER
    Was splashing, bubbles after dispense
    Decreased dispense speed

28. REMOVE LYSIS
    Increased dispense height
    Inserted a *post-dispense transport air gap* to remove bubbles
    Decrease tracking on second "remove"

37. ADD WASH
    Use dispense speed from 20

38. REMOVE WASH
    Inserted a *post-dispense transport air gap* to remove bubbles

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62. ADD ELUTION BUFFER
Remove Flush

72. TRANSFER ELUANT
Decreased apirate and dispense speeds
Decreased *Retract From Liquid* speed

74. ADD ELUTION 2
Remove Flush

Where possible, dispense heights were used to allow the liquid to just touch the tip as dispensing ended. For example, if adding 600ul to a well, dispense at 550-600ul from the bottom. This enables any drops to be drawn off of the tip by the liquid in the well.

Using a *post-dispense transport air gap* ensures that any liquid remaining in the tip is drawn back up before the pipetting arm moves in an X or Y direction, thus negating any contamination of neighbouring wells.

Slowing down the *tip retraction speed* also helps to remove droplets from the test. After dispensing, if the tips come out of the liquid at the "usual" speeds (100mm/sec), you can often see drops being pulled out with the tip. This is just due to the surface tension in the well. By slowing down the tip retraction speed, the tip comes out of the liquid slowly, allowing any excess liquid on the outside of the tip to drain off the tip and remain in the well.

In many steps in this extraction test, the volume of liquid in the wells is quite high. As a result, it was useful to slow down the dispense speeds to avoid splashing.

While observing the test, the problems were noted and then the modifications done and the test carried out again. Actual extraction protocol liquids were used to completely mimic a "real" extraction run. The test was run several times with continuous modifications to improve the liquid handling settings. The final runs showed no dripping and a much neater and cleaner test.

Fwd: Re:

Justin Howes [redacted]

Thu 23/10/2008 3:14 PM

To: McNevin, Allan [redacted] Allen, Cathie [redacted] Taylor, Paula [redacted] Nurthen, Thomas <[redacted]>;Harmer, Wendy [redacted]

1 attachments (10 KB)

jbsikwjaeyym.image.jpeg;

no probs
JAH

>>> Cathie Allen Thursday, 23 October 2008 3:13 pm >>>
Hi,

Theo and David would like to start at about 9.30am on the 12th.

Allan/Tom - can you please arrange to have an appropriate person to show Theo & David through the process (thought we would meet him in 611 [Al that means you will have to move your appointment for that morning - sorry] and have a chat about our processes etc in general then into the audit)

Paula & Justin - thought you could be involved in the initial chat in the morning

Wendy - could you please arrange for lunch to be provided for Theo & David

Thanks,
Cathie.

>>> Greg Shaw 21/10/2008 10:06 am >>>
Hi Theo, sounds fine, I shall ask Cathie Allen DNA Managing Scientist to forward the relevant SOPs etc. Look forward to meeting you then Greg

>>> "Theo SLOOTS" [redacted] 21/10/2008 9:39 am >>>

Hi Greg

Yes 12 November would suit me. Do you have any time in mind. Probably start at 9.300 and plan to spend as long as it takes, if that's OK with you.

With your permission I will also bring my molecular scientist David Whiley, who has a really good insight into operational issues.

If possible I would like to have a copy of the SOP for the specimen handling and extraction process before I come.

Thanks

Theo

****

Theo Sloots PhD, GCM, MASM
Associate Professor
Unit Director (Research)
Queensland Paediatric Infectious Diseases Laboratory

[redacted]

[redacted]

****

Sir Albert Sakzewski Virus Research Centre
celebrating 20 years of research
1987–2007

****

Save a tree. Please don't print this email unless it's really necessary!

From: Greg Shaw [redacted]
Sent: Tuesday, 21 October 2008 9:24 AM
To: Theo SLOOTS
Subject: Good morning Prof Sloots, we have not met however, I am the Director at Qld Health Forensic & Scientific Services at Coopers Plains. I understand that Greg Smith has contacted you regarding assisting us with a scientific review of our response to a contam

Good morning Prof Sloots, we have not met however, I am the Director at Qld Health Forensic & Scientific Services at Coopers Plains. I understand that Greg Smith has contacted you regarding assisting us with a scientific review of our response to a contamination problem we have been addressing with liquid handlers in our DNA analysis laboratory. I believe that a tentative date of 12 November was set.

I should now like to confirm that is remains convenient for you and make any arrangements that may be necessary. Would you kindly confirm by return mail and note any action or information that you might require prior to your visit? Alternatively I would be happy to discuss the matter with you on [redacted] at your convenience.

Thank you in advance for your help, Greg Shaw.

****

Thomas Nurthen

From: Emma Caunt [redacted]
Sent: Tuesday, 28 October 2008 9:16 AM
To: Pippia, Adrian; McNevin, Allan; Storer, Amanda; Allen, Cathie; Moeller, Ingrid; Howes, Justin; Rika, Kylie; Taylor, Paula; Nurthen, Thomas
Subject: Process for reporting IQ results
Attachments: Guidelines for reporting results from DNAIQ batches.doc

Hi all

The Investigation Team is currently working through the IQ cases and assessing which samples can be reported and which cannot. We have put together a process for assessing these samples and have been working from this process for a couple of weeks. I now wish to have the process included in the case management SOP and just wanted to give you the opportunity to have a look at it for any glaring issues before I do this.

Could I please have any feedback by next Tuesday.

Thanks

Emma

Guidelines for Reporting Results from DNAIQ Batches

Step 1

This step uses the information that you already have available from the extraction process.

1. Find the extraction batch relating to your sample and enter into it in AUSLAB or Extraction Audit files. Check each sample on the batch to determine whether any of these profiles match your profile. This includes checking any profiles that contain NR peaks and may require the use of Genotyper. Report if there are no matches.

   If your profile matches something else on the batch from a different case you need to investigate the circumstances of each case and document as possible contamination event. In this case it is safer to report as 'failed to pass QC'.

2. If samples on the extraction batch have given NSD profiles, check the quant, IPCCT (should be <28) and sample type and determine whether this is the true result for this sample or if the sample could be inhibited.

3. If the NSD profile indicates inhibition then you need to consider whether a small amount of this sample could have contaminated your sample by dilution of the inhibitors. If you are happy that this has not occurred, for example using quant values, sample types and profiles from other samples in the same case from a different extraction batch, then this result is OK to report.

Step 1 covers cases with one sample. The following checklist needs to be applied to cases with multiple samples on the one extraction batch in addition to step 1.

Checklist 1

1. Full profile and partial profiles that do not match any other profiles on extraction batch including samples within the case can be reported. A partial profile with several alleles which do not match anything else on the batch can be reported. A partial profile (e.g., 3 alleles) with matches on the batch can be reported as 'insufficient for reliable interpretation'. NR peaks in the profile need to be looked at for exclusion of contamination.

2. More than one single source profile from the same item on the same batch that match each other can be reported (applies to clothing only, not weapons or areas on body e.g., knife blade vs knife handle, fingernails vs other body part).

3. Same profiles from different items from the same person can be reported (e.g., John's blood on Jack's top and trousers - which item of clothing his blood is on doesn't matter).

4. Same profiles from different items from different people (same batch) can not be reported until confirmed or quant values check out (e.g., John's blood on Adam, Jack and Jim – Adam and Jack's samples could have cross-contaminated Jim who is innocent (if the quant values of Adam and Jack's samples are high enough to render a profile in another position)).

5. Profiles can be reported if they match the ref sample of the owner/donor of the exhibit (not unexpected/incriminating).

6. If mixed DNA profile, and can either condition on known reference sample or call both major and minor, and individual contributions meet above criteria result can be reported.

7. Mixed DNA profile where the major or conditioned can be called confidently, but the minor/remaining is partial and not unique (not an obvious contamination event but insufficient information to rule out contamination) – report major, and report the remaining/minor as 'insufficient for meaningful interpretation'. If any of the individual components of the mixed profiles appear to be a result of a contamination event then the entire profile cannot be called. These contaminating profiles would be more than likely single source and of a quant value high enough to render a profile in another position.

8. If a mixed DNA profile (2 person) is obtained that can't be separated then the extraction batch needs to be checked for other profiles that could have contributed to the mixture. If all profiles can be excluded then the mixture is ok to be reported.

9. Complex mixed DNA profile, even if you would normally report as 'can't exclude..' should be reported as 'too complex for meaningful interpretation'. There is a possibility that a random profile from the batch might not be able to be excluded, therefore the profile itself is further indicating that it is too complex for a reliable interpretation.

10. Partial insufficient and NSD results can be reported,

Any uncertainty regarding reporting samples needs to be discussed with someone else.

After checking all samples comment in UR notes 'all samples quality checked and OK'.

Quants

Single source profiles which are the same as each other and have similar quant values are unlikely to have contaminated each other. If they had, the contaminating profile would not account for the whole profile. The majority of the profile would be from the original sample.

If you have a mixture, quant values can be applied in some instances.

Reworks:

If a profile is still questionable once the above processes have been followed, consider the following actions:

- re-extraction of spin baskets
If a profile is obtained which confirms the original profile (even if it has fewer alleles including NRs), then the best profile can be reported. If no profile is obtained from the spin basket then the original profile cannot be accepted. Consider lowering thresholds for low level interpretation.

- re-sampling of items
If the item is re-sampled and this profile confirms all other profiles from the item then all profiles can be accepted. If the re-sampled result is different or NSD then the original results cannot be confirmed.

Examples:

Extraction batch has samples with profile matching complt. On same batch, samples from deft have been extracted, and a mix of the two is amongst the results off that batch. The mixture cannot be reported as it is not possible to tell if the mixed result is true or the result of the complt sample contaminating the deft sample. (e.g., John kicked Adam - a swab of John's toe gave a mixed profile of John and Adam. This sample however has been cross-contaminated by a swab from Adam's hand which was extracted on the same batch.)

Extraction batch contains samples from 2 defts clothing. All samples show match to complt within case. These results cannot be reported (without resampling) as it is not possible to tell if the results are a true reflection of the samples, or if the samples from one deft (true results) have contaminated the samples from the other deft.

DNA Analysis (Forensic Biology) | Forensic & Scientific Services | Queensland Health

The FSS DNA Analysis laboratory made some changes to their existing DNA IQ extraction protocol and asked PerkinElmer to observe and ensure that the liquid handling was optimum. The test was physically set up and run and observed for problems. Following is a list of the steps which required some modifications to the liquid handling settings.

7. TRANSFER LYSIS
   Increase aspirate height and decrease tracking

9. MIX RESIN
   Change aspirate and dispense heights to be equal

13. ADD LYSIS BUFFER
   Decrease dispense height and tracking

17. REMOVE 905UL…
   May require slower aspirate speed, if resin is transferred – customer to check.

20. DISPENSE LYSIS
   Drops and bubbles after dispense, on tip and top of well
   Decrease blowout volume to 5ul, customer to check
   If problem persists, switch to waste mode, with a waste volume of 3-5ul

28. REMOVE LYSIS
   Decrease dispense height and tracking

31. ADD WASH BUFFER
   See response for 20

39. REMOVE WASH BUFFER
   Syringes are not homing during procedure, since it is a custom
   Insert a "Wash Tip" after each remove

41. ADD WASH
    See response for 20

49. REMOVE WASH
    See response for 39

51. ADD WASH
    See response for 20

59. REMOVE WASH
    See response for 39

73. TRANSFER ELUTION
    Tiny amount left in tips.
    Change System Gap to zero and Transport gap to 5ul.

Where possible, dispense heights were used to allow the liquid to just touch the tip as dispensing ended. For example, if adding 600ul to a well, dispense at 550-600ul from the bottom. This enables any drops to be drawn off of the tip by the liquid in the well.

Using a *post-dispense transport air gap* ensures that any liquid remaining in the tip is drawn back up before the pipetting arm moves in an X or Y direction, thus negating any contamination of neighbouring wells.

Slowing down the *tip retraction speed* also helps to remove droplets from the test. After dispensing, if the tips come out of the liquid at the "usual" speeds (100mm/sec), you can often see drops being pulled out with the tip. This is just due to the surface tension in the well. By slowing down the tip retraction speed, the tip comes out of the liquid slowly, allowing any excess liquid on the outside of the tip to drain off the tip and remain in the well.

While observing the test, the problems were noted and then the modifications done and the DNA Analysis Team was advised to run the test again with the modifications. Actual extraction protocol liquids were used to completely mimic a "real" extraction run. With these modifications, the DNA IQ extraction protocol is a sound, neat protocol.

Background

Following a request from the Director, Mr Greg Shaw, a review of procedures was conducted by Drs Sloots and Whiley (the reviewers) at the Forensic and Scientific Services laboratory, Clinical and State-wide Services, Coopers Plains, pertaining to the extraction of nucleic acids from samples submitted for analysis.

The reason for this review related to a previous episode in the laboratory which resulted in anomalous results and which appeared to be linked to the operation of robotic instrumentation utilised in the nucleic acid extraction process.

During their visit, the reviewers were made aware of the operations applied in the general laboratory from receipt of specimens to issuing of results, and then examined in detail the bench process relating to the pre-digestion of specimens and the extraction of nucleic acids using the Perkin Elmer MultiPROBE II PLUS HT EX with Gripper Integration Platform.

All aspects of these operations were scrutinised including staff input and instrument operation.

Findings

It was obvious to the reviewers that extensive measures were applied by all staff to prevent the misidentification or cross contamination of samples. There was appropriate use of personal protection equipment and other protective measures to prevent contamination of the work environment with extraneous nucleic acid.

The procedures currently in place for the Off-Deck Lysis and MPII extraction appeared to be adequate and specifically designed to prevent cross contamination of test samples.

We agree with the Forensic Services Management team that the previous issue of possible cross-contamination of samples most likely related to the use of adhesive film in sealing the deep-well plates used in the Off-Deck lysis procedure. The type of plate used, and the period of storage at reduced temperatures have in our experience caused similar problems in molecular diagnostics. The subsequent decision to change this procedure to the use of capped tubes has clearly solved this problem.

The use of robotic equipment for the extraction of nucleic acids has some considerable benefits for a busy laboratory, and prevents human error introduced as a result of repetitive actions. However, the efficient use of such instruments requires the proper maintenance and calibration be performed at the requisite time intervals. These appeared to be adequately performed at the time of review.

It may appear that the original issue concerning the cross-contamination of samples in the deep-well plates could have been prevented if this change in procedure had been fully validated against existing protocol when the new method was introduced. Although most

laboratories would have considered this change to be minor and therefore accepted without validation, it clearly demonstrates that all changes in procedure, no matter how minor, need to be validated according to a standardised protocol before their introduction as standard operating procedure.

Items for Further Consideration

During the review process some items were identified which may require further consideration by the management staff of the Forensic and Scientific Services laboratory.

These are:

1. Develop a standard validation protocol for each procedure based on the guidelines described by J Butler (www.promega.com; September 2006). Incorporate these into the Standard Operating Procedures for the laboratory.
2. We advise that the number of negative controls included in each batch of extractions be increased to comprise at least 10% of the total number of specimens tested. These controls should ideally be distributed randomly over the plate. Currently one negative control is included with 47 samples.
3. Quality assessment might be increased by testing a control plate once every 3-4 weeks on each of the MultiPROBE II PLUS platforms. We would suggest alternating between the soccer ball, zebra and checkerboard formats.
4. It was noted that the magnetic particles used for the nucleic acid extraction had a tendency to settle quickly, thereby blocking the filter tip and potentially producing a false-negative result. At the time of review this was not a problem as the attending operator was diligent in observing all stages of this process. We would like to reiterate however, that constant observation by the operator of all processes leading up to and including the addition of magnetic particle is necessary to ensure that failure of the robotic system does not occur.
5. Finally, it was noted that the laboratory design allowed traffic from the amplification/post-amplification area into the lysis/extraction areas. Presently this carries moderate contamination risk, as the amplification protocol is limited to 28-32 cycles. However, if this protocol is changed in the future to detect low copy nucleic acid (greater than 32 cycles) the risk of carrying post-PCR product into the extraction area would be high, and work-flow dynamics must then be carefully examined to minimise that risk. Likewise, sample cross-contamination during specimen handling and extraction processes will assume greater relevance when contemplating detection of low copy nucleic acid, and would necessitate stringent validation of all steps.

[redacted]

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Report [redacted] gation of contamination in the Automa [redacted] action protocol

Thomas Nurth[redacted]
, DNA Analysi[redacted]

1. Abstra[redacted]

2. [redacted]

E[redacted] IQ protocol began on the x October 2007. [redacted] number of batches were extracted on both [redacted] per Ir[redacted]

[redacted] nts (OQIs) had been raised but no obvious link w[redacted]

15 OQ[redacted] ve been raised.

3. A[redacted]

To do[redacted] well [redacted] mated DNA IQ extraction batches.

4. E[redacted] and Materials

- [redacted] E® II PLUS HT EX with Gripper™ Integration Platform (PerkinElmer, Downers [redacted] USA)
- [redacted] Performance Evaluation Option with Mettler SAG285/L balance (Mettler-Toledo, [redacted], Switzerland)
- [redacted] Q™ System (Promega Corp., Madison, WI, USA)
- [redacted] tion Buffer (10mM Tris, 1mM EDTA, 100mM NaCl, 20% SDS)
- [redacted] Rep™ 96 Device (Promega Corp., Madison, WI, USA)
- Nunc™ Bank-It tubes (Nunc)
- 175µL non-conductive sterile filter RoboRack tips (PerkinElmer, Downers Grove, IL, USA)
- 1000µL Conductive sterile filter Robotix tips (MBP)
- ABI Prism® 7000 SDS [redacted] ystems, Foster City, CA, USA) [redacted]
- 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)
- For mock samples:
  - FTA® Classic Card (Whatman Inc., Florham Park, NJ, USA)
  - Sterile cotton swabs (Medical Wire & Equipment, Corsham, Wiltshire, England)

- [redacted]

5. Metho[redacted]

5.1 Automa[redacted]

As per QIS [redacted]Q Method of Extracting DNA from Reference and Casework samples

5.2 Re-extr[redacted]es

As per QIS [redacted]Q Method of Extracting DNA from Reference and Casework s[redacted]

5.3 Manual[redacted]

A[redacted] Automated DNA IQ Method of Extracting DNA from Reference and C[redacted]

[redacted]t from an extraction

5.5 L[redacted]

Samp[redacted]lysed at 30 ru and 20 ru in GeneMapper ID*x* by a competent scientist.

5.6 P[redacted]d program alteration

The P[redacted]Liqui[redacted]pecia[redacted]ed to review the automated DNA IQ protoc[redacted]

5.7 A[redacted]

An Au[redacted]ole DNA extraction procedure was performed

5.8 R[redacted]tions

[redacted]check batches

A Microsoft Excel macro was created to check the DNA profiles within a batch for matching DNA profiles

6. OQIs

18 OQIs have been raised in relation to these events. See Table 1 for the list of OQIs raised and the batches that were affected.

Table 1-List of OQIs and batches

OQI#	Ext Batch ID
19330	FTAEXT20080205_01
19349	CWIQEXT20080225_02
19477	CWIQEXT20080430_01
19767	FTAEXT20080515_01

19768	[redacted]
20231	
20351	
20422	
20437	
20615	[redacted]
20617	
20690	
20925	CWIQEXT20080403_01
21222	[redacted]
21309	
22880	[redacted]
22882	[redacted]
19703	[redacted]

6 [redacted]

[redacted]XT20080205_01) appears to have a single [redacted] The [redacted]

[redacted]nt area of the FTA yielded a single source DNA [redacted]

- [redacted] (12) (Store plate) yielded a single source DNA [redacted] matched the expected reference profile.
- [redacted]tion of the original FTA card D2 (12) yielded single source DNA profile [redacted]ed the expected reference profile.

- Dire[redacted]amination - right to left.

Conta[redacted]he sample has occurred at a point after the removal of the lysate in the extrac[redacted], as the lysate profile is a single source profile matching the expected refere[redacted] The right to left direction of contamination is consistent with robotic move[redacted]mination may have occurred by robotic dripping during elution, during cappi[redacted]g, during storage or during preparation of amplifications.

9

[redacted] casework samples (CWIQEXT20080225_02) appears to have a single [redacted] event.

- The negative extraction control (B1/ 2) was found to contain a DNA profile. The source of this DNA profile was found to be the extraction positive control in position A1 (Pos 1).
- Re-extraction of the s[redacted]e (B1/2) (Store plate) did not yield a DN[redacted] the negative extraction control.
- No substrate exists for a negative extraction – therefore no substrate extraction profile for comparison

- Direction of contamination - top to bottom of plate.

Contamination of the sample has occurred at a point after the removal of the lysate in the extraction process, as the lysate profile for the negative extraction control is NSD. The top to bottom direction of contamination is consistent with robotic movement. Contamination

may have c[redacted]during elution, during capping/decapping, during storage or [redacted]cations.

6.3 20351

This batch [redacted]QEXT20080402_01) appears to have multiple contaminati[redacted]

- The [redacted]ol (A1/1) was found to contain a mixed DNA profile. The mixed profile was found to contain a profile consistent with the DNA in 6 pos[redacted]27, D4/28, G4/31, H4/32).
- Re-[redacted]e (A1/1) (Store plate) yielded a mixture consistent with the [redacted]
- Re-[redacted]strate (A1/1) yielded a single source DNA profile
- Pos[redacted]ontain a mixed DNA profile. The mixed profile was [redacted]ontain a profile consistent with the DNA in 6 positions (A4/25, B4/26, [redacted]
- [redacted]) (Store plate) yielded a mixture consistent [redacted]
- [redacted]artial single source DNA [redacted]
- [redacted]n a mixed DNA profile. The mixed profile was [redacted]with the DNA in 6 positions (A4/25, B4/Pos26, [redacted]/28, G4/31, H4/32).
- [redacted]tion of stored lysate (B6/42) (Store plate) yielded a mixture consistent [redacted]itial mixture obtained.
- [redacted]tion of the original substrate (B6/42) yielded a partial single source DNA [redacted]
- [redacted]C6/43) was found to contain a mixed DNA profile. The mixed profile was [redacted]ontain a profile consistent with the DNA in 6 positions (A4/25, B4/26, [redacted]/28, G4/31, H4/32).
- [redacted]tion of stored lysate (C6/43) (Store plate) yielded a DNA profile consistent [redacted]iginal contamination
- [redacted]tion of the original substrate (C6/43) yielded an NSD profile with [redacted]ermined Quant.
- Position (D6/44) was found to contain a DNA profile consistent with the DNA in 6 positions (A4/25, B4/26, C4/27, D4/28, G4/31, H4/32).
- Re-extraction of stored lysate (D6/44) (Store plate) yielded a DNA profile consistent with the original contamination
- Re-extraction of the [redacted]strate (D6/44) yielded a partial profile ([redacted]) which was not consistent with the original 9PLEX or lysate DNA profiles.

- Position (C7/51) was found to contain a DNA profile consistent with the DNA in 6 positions (A4/25, B4/26, C4/27, D4/28, G4/31, H4/32).
- Re-extraction of stored lysate (C7/51) (Store plate) yielded a DNA profile consistent with the original contamination
- Re-extraction of the original substrate (C7/51) yielded an NSD profile with undetermined Quant.

- Position (C11/83) contains a mixture.
- Original profile, lysate and substrate have same alleles – referred for mixture interpretation to confirm uncontaminated profile.
- Sampled at different times, places and by different people.

- Direction [redacted]ft and left to [redacted]

A contamina[redacted]occurred at a point during the extraction process before the r[redacted]after removal of the substrate, as the lysate profiles are contaminated (consistent with original 9PLEX contamination). The right to left direction of contaminati[redacted]obotic movement; however the left to right contaminati[redacted]ion may have occurred during storage, during plate agitation on [redacted]o[redacted]ate seals.

6.4 [redacted]20[redacted]

T[redacted]work samples (CWIQEXT20080417_01) appears to have multiple c[redacted]

- [redacted]) was found to contain a DNA profile. The [redacted] to P[redacted]
- [redacted]a DNA profile consistent
- [redacted]traction – therefore no substrate extraction

- [redacted]A2/9) was found to contain a profile consistent with the DNA profile [redacted]n Pos (A4/25)
- [redacted]tion of stored lysate of (A2/9) (Store plate) did not yield any DNA profile
- [redacted]tion of the original (A2/9) substrate (spin basket) did not yield any DNA [redacted]

- [redacted]G2/15) was found to contain a profile consistent with the DNA profile [redacted]n Pos (A4/25)
- [redacted]tion of stored lysate of (G2/15) yielded a partial DNA profile consistent [redacted]5).
- [redacted]tion of the original substrates for (G2/15) did not yield any DNA profile.

- [redacted]traction of the original substrate for A4 (25) yielded a DNA profile consistent [redacted]he initial profile, re-extraction of the lysate yielded a consistent partial profile.

- Direction of contamination - right to left.

A contamination of the samples has occurred at a point during the extraction process before the removal of the lysa[redacted]r removal of the substrate, as some of th[redacted] profiles are contaminated (consistent with original 9PLEX contamination). The right to left direction of contaminations are consistent with robotic movement. Contamination may have occurred during storage, during plate agitation on the robot, by robotic dripping, or by the removal of plate seals.

6.5 19477

This batch of casework samples (CWIQEXT20080225_02) appears to have multiple contamination events.

- The [redacted] (A1/1) was found to contain a DNA profile. The sou[redacted]traced to 6 Positions (H2/16, A3/17, B3/18, E3/21, H3/[redacted]
- Re[redacted]A1/ 1) (Store plate) did not yield any DNA profile.
- No[redacted]ive extraction – therefore no substrate extraction pro[redacted]
- Pos[redacted] contain a mixed DNA profile. The mixed profile was found to contain a profile consistent with the DNA profile obtained in positions (H2/16, A3/17, B3/18, E3/21, H3/24, A4/25).
- Re-[redacted]e (E1/5) (Store plate) yielded a mixture consistent with the [redacted]
- Re-[redacted]e (E1/5) (spin basket) yielded a mixture, but the [redacted] had fewer alleles than the original contamination.
- [redacted] was found to contain a profile consistent with the DNA profile [redacted] Pos (H2/16, A3/17, B3/18, E3/21, H3/24, A4/25).
- [redacted] (Store plate) yielded a profile consistent with [redacted]
- [redacted]e (H1[redacted]

- [redacted]

A con[redacted]d at a point during the extraction process before [redacted]l of the lysate but after removal of the substrate, as some of the lysate profile[redacted]minated (consistent with original 9PLEX contamination). The right to left direct[redacted]minations are consistent with robotic movement. Contamination may have [redacted]ing storage, during plate agitation on the robot, by robotic dripping, or by the re[redacted]te se[redacted]

6.6

This b[redacted]work samples appears to have a single contamination event.

- [redacted]ction negative control (A1/Pos 1) was found to contain a DNA profile. The [redacted]the DNA profile was traced to 2 Positions (G4/31 & H4/32).
- [redacted]ion of stored lysate (A1/Pos 1) (Store plate) yielded a partial DNA profile [redacted]o only)
- [redacted]bstrate exists for a negative extraction – therefore no substrate extraction profile for comparison

- Direction of contamination - right to left.

Contamination of the extracti[redacted]e control has occurred at a point during t[redacted] extraction process before the removal of the lysate, as the lysate is also contaminated (consistent with original 9PLEX contamination). The right to left direction of contamination is consistent with robotic movement. Contamination may have occurred during storage, during plate agitation on the robot, by robotic dripping, or by the removal of plate seals.

6.7 20422

This batch of casework samples appears to a single contamination event.

- Pos[redacted]ure. The mixture was consistent with the DNA profile fou[redacted]& H3/24).
- Re[redacted]te (G1/7) of the contaminated sample yielded a mix[redacted]ith the initial mixture obtained.
- Re[redacted]bstrate (G1/7) yielded a single source DNA profile.

- Direction o[redacted] left.

Contaminati[redacted]curred at a point during the extraction process before the removal of the lysate but after removal of the substrate, as the lysate profile is contaminate[redacted]al 9PLEX contamination). The right to left direction of contaminati[redacted]otic movement. Contamination may have occurred during stora[redacted]p[redacted]obot, by robotic dripping, or by the removal of plate seals.

6[redacted]

T[redacted]have a single contamination event.

- [redacted]rce of this mixture was
- [redacted]9/65) of the contaminated sample (Store plate) [redacted]nt with the initial mixture obtained.
- [redacted]nt area of the FTA yielded a single source DNA
- [redacted]tion of the original FTA card substrate (A9/65) yielded single source DNA [redacted]sistence with the single source profile obtained from the re-punch of a [redacted]rea of the FTA card.

- Dire[redacted]amination - left to right.

Conta[redacted]he sample has occurred at a point during the extraction process before the re[redacted] lysate but after removal of the substrate, as the lysate profile is conta[redacted]sistent with original 9PLEX contamination). The left to right direction of conta[redacted]ot consistent with robotic movement. Contamination may have occurred durin[redacted]ring plate agitation on the robot, or by the removal of plate seals.

6.9 20617

This batch of casework samples appears to have multiple contamination events.

- Position F6 (46) cont[redacted]ure. The source of this mixture was foun[redacted] position G6 (47).
- Re-extraction of the stored lysate (F6/46) of the contaminated sample (Store plate) yielded a mixed DNA profile consistent with the initial mixture obtained
- Re-extraction of the original substrate(F6/46) yielded a single source DNA profile

- Position G7 (55) contains a mixture. The source of this mixture was found to be position G6 (47).
- Re-extraction of the stored lysate (G7/55) of the contaminated sample (Store plate) yielded a mixed DNA profile consistent with the initial mixture obtained

- Re- [redacted] bstrate (G7/55) yielded a single source partial DNA pro[redacted]

- Direction [redacted]t and bottom to top of plate.

A contamin[redacted] curred at a point during the extraction process before the re[redacted] after removal of the substrate, as some of the lysate profiles are [redacted]t with original 9PLEX contamination). The bottom to top direction[redacted] onsistent with robotic movement, however the left to right contaminations are not. Contamination may have occurred during storage, during plate agitati[redacted]ic dripping, or by the removal of plate seals.

6.10 20[redacted]

This batch [redacted]s to have multiple contamination events

- [redacted]7 (53) is a pooled sample which contains a mixture. The mixture was [redacted]d in 4 positions (A7/49, B7/50, C7/51, and [redacted]
- [redacted]7/53) [redacted]ooled profile.
- [redacted]e (E7/55), was found to contain a mixture with [redacted] profile but matches the lysate mixture profile.

- [redacted]n a partial profile (low RFU peaks). The partial [redacted]onsistent with the pooled DNA profile in (E7/53).
- [redacted]tion of the stored lysate (F7/54) was found to contain a partial profile (low [redacted]s), consistent with the DNA profile found in 3 positions (E7/53, G7/55 and [redacted]hich are later pooled).
- [redacted]tion [redacted]al sub[redacted]4) yielded an NSD profile with [redacted]ned Quant.

- [redacted]7 (55) is a pooled sample with (E7/53): Refer above for details.
- [redacted]tion of the stored lysate (G7/55) which had not been pooled, was found to [redacted]mixture with fewer alleles than the original pooled profile.
- [redacted]tion of the original substrate (G7/55), was found to contain a mixture with [redacted]es than the original pooled profile. The substrate profile was consistent [redacted]e lysate mixture profile, but did have 3 additional alleles due to higher RFU [redacted]s in the substrate mixture profile in comparison with the lysate mixture profile.

- Position H7 (56) is a pooled sample with (E7/53): Refer above for details.
- Re-extraction of the stored lysate (H7/56) which had not been pooled, was found to contain a mixture with fewer alleles than the original pooled profile.
- Re-extraction of the [redacted]strate (H7/56), was found to contain a [redacted] fewer alleles than the original pooled profile but matched the lysate mixture profile.

- Position A8 (57) is a pooled sample with (E7/53): Refer above for details.
- Re-extraction of the stored lysate (A8/57) which had not been pooled, was found to contain a mixture consistent with the initial pooled mixture which was contaminated.
- Re-extraction of the original substrate (A8/57) yielded a mixture, but the mixture from the substrate had fewer alleles than the lysate re-extraction.

- Pos[redacted]xture. The mixture was consistent with the DNA pro[redacted]/49, B7/50, C7/51, and D7/52).
- Re-[redacted]te (A9/65) (Store plate) was found to contain a mix[redacted]al mixture profile.
- Re-[redacted]bstrate (A9/65) yielded a single source DNA profile

- Direction o[redacted]ight and top to bottom of plate.

Contaminati[redacted]s extraction plate may have occurred at more that one point during the extraction process, as some samples appear to have become contaminate[redacted]the lysate from the magnetic beads, with other samples indicating co[redacted] eluate has occurred after the removal of the lysate from the pla[redacted]o [redacted] contaminations are consistent with robotic movement, however the left to right contaminations are not. Contaminations may have occurred du[redacted]gitation on the robot, by robotic dripping, or by the removal of r[redacted]

6[redacted]

[redacted]have [redacted]

- [redacted]e with undetermined Quant
- [redacted]1/6) (Store plate) was found to contain a profile [redacted]ned in Pos (F2/14).
- [redacted]e (F1/6) yielded an NSD profile with [redacted]ned Quant
- [redacted]perator of robot noted that 8tip arm contacted the lysate plate at location [redacted]osition 6 and 14 on this plate.

- [redacted]1 (7[redacted] to co[redacted]le consistent with the DNA profile [redacted]n Pos (E1/5)
- [redacted]tion of the stored lysate (G1/7) (Store plate) was found to contain a profile [redacted] with the DNA profile obtained in Pos (E1/5).
- [redacted]tion of the original substrate (G1/7) yielded an NSD profile with a Quant [redacted]000995.

- [redacted]2 (10) contains a mixture.
- [redacted]traction of the stored lysate (B2/10) (Store plate) was found to contain a [redacted]e consistent with the initial mixture profile.
- Re-extraction of the original substrate (B2/10) was found to contain a mixture consistent with the initial mixture profile.
- This sample has produced consistent profiles from substrate, lysate and initial amplification. This sample may not be contaminated.

- Position E2 (13) was found to contain a full DNA profile
- Re-extraction of the stored lysate (E2/13) (Store plate) was found to contain a profile consistent with the initial DNA profile.
- Re-extraction of the original substrate (E2/13) yielded an NSD profile with an undetermined Quant value.

- Position F2 (14) was found to contain a partial DNA profile.
- Re-extraction of the stored lysate (F2/14) (Store plate) was found to contain a profile consistent with the initial DNA profile.

- Re- [redacted] bstrate (F2/14) yielded an NSD profile with an unc
- NO [redacted] that 8tip arm contacted the lysate plate at location bet [redacted] his plate.

- Direction [redacted] ttom of plate [redacted]

Contaminati [redacted] s extraction plate has likely occurred at more that one point during [redacted] Some samples appear to have become contaminated before the removal of the lysate from the magnetic beads (lysate profile consistent with original 9PL [redacted] vever the sample in position F1 (6) on this extraction plate is NSD [redacted] nd in the substrate, with the lysate profile being the only contam [redacted] b. [redacted] f the lysate in this position can be explained by the operator or robot noting that 8tip arm contacted the lysate plate at location between position 6 a [redacted]

T [redacted] direction of contaminations are consistent with robotic movement. C [redacted] torage, during plate agitation on the robot, by r [redacted] val of plate seals.

[redacted]

T [redacted] have multiple contamination events.

- [redacted] The mixture was consistent with the DNA [redacted] nd in 4 positions (D4/28, E4/29, F4/30, and G4/31).
- [redacted] tion of the stored lysate of the contaminated sample (F3/22) yielded a [redacted] e consistent with the original contamination.
- [redacted] tion of the original substrate (F3/22) yielded a mixed DNA profile.
- [redacted]
- Position D4 (28) was found to contain a partial DNA profile. (referred to Tom for allele confirmation)
- Re-extraction of the stored lysate (D4/28) (Store plate) was found to contain a mixture which had alleles not present in either the initial product or substrate re-extraction. SOURCE?
- Re-extraction of the original substrate (D4/28) yielded a full profile consistent with the initial partial profile.

[redacted] contamination – right to left.

Contamination of the sample has occurred at a point during the extraction process before the removal of the lysate but after removal of the substrate, as the lysate profile is contaminated (consistent with original 9PLEX contamination). The right to left direction of contamination is consistent w [redacted] movement. Contamination may have oc [redacted] during storage, during plate agitation on the robot, by robotic dripping, or by the removal of plate seals.

6.13 20925

This batch of casework samples appears to have multiple contamination events.

- Position H2 (16) contains a mixture. The mixture was consistent with the DNA profile found in 2 positions (G3/23 & H3/24).

- Re- [redacted] ate of the contaminated sample (H2/16) yielded a DN [redacted] original contamination.
- Re- [redacted] bstrate (H2/16) yielded a partial profile (low RFU) whi [redacted] he lysate DNA profile.

- Pos [redacted] ixture. The mi[redacted]nsistent with the DNA prof [redacted] F8/62, F9/70, G9/71 & H9/72)
- Re- [redacted] ysate of the contaminated sample (F6/46) yielded a DNA profile consistent with the original contamination.
- Re-extraction of the original substrate (F6/46) yielded an NSD profile with und [redacted]

- Direction o [redacted] a [redacted]

A contamina [redacted] curred at a point during the extraction process before the r [redacted] of the lysate but after removal of the substrate, as some of the lysate pr [redacted] inated (consistent with original 9PLEX contamination). The right to left d [redacted] th robotic movement. Contamination may h [redacted] gitation on the robot, by robotic dripping, or by [redacted]

6.14
This b [redacted] work samples appears to have a single contamination event.

- [redacted] 11 (86) contains a mixture. The mixture was consistent with the DNA [redacted] nd in 5 positions (B10/74, C10/75, F10/78, G10/79, and H10/80).
- [redacted] tion of the stored lysate of the contaminated sample (F11/86) yielded a [redacted] e consistent with the original contamination.
- [redacted] tion of the original substrate (F11/86) yielded an NSD profile with an [redacted] ned Quant value.

[redacted] contamination – left to right.

A contamination of the samples has occurred at a point during the extraction process before the removal of the lysate but after removal of the substrate, as some of the lysate profiles are contaminated (consistent with original 9PLEX contamination). The right to left direction of contaminations are consistent with robotic movement. Contamination may have occurred during storage [redacted] te agitation on the robot, by robotic drip [redacted] the removal of plate seals.

6.15 21309
This batch of casework samples (CWIQEXT20080531_01) appears to have multiple contamination events

- Position G11 (87) contains a mixture. The mixture was consistent with the DNA profile found in 1 position (H11/88).

- Re- [redacted] ate of the contaminated sample (G11/87) yielded a DN[redacted] original contamination.
- Re- [redacted] bstrate (G11/87) yielded a partial DNA profile (low RF[redacted]

- Pos[redacted] ixture. The [redacted] consistent with the DNA prof[redacted] 11/88).
- Re-[redacted] ysate of the contaminated sample (H12/96) yielded a DNA profile consistent with the original contamination.
- Re-extraction of the original substrate (H12/96) yielded an full DNA profile

- Direction o[redacted] right and bottom to top of plate.

Contaminat[redacted] red at a point during the extraction process before the removal[redacted] val of the substrate, as the lysate profile is contaminat[redacted] sistent with original 9PLEX contamination). The left to right direction and bo[redacted] tion of contamination is not consistent with robotic movement. C[redacted] orage, during plate agitation on the robot, or by [redacted]

[redacted] have a single contamination event.

- [redacted] The mixture was consistent with the DNA [redacted] d in 3 positions (B10/74, C10/75, F10/78, G10/79, and H10/80).
- [redacted] tion of the stored lysate of the contaminated sample (F11/86) yielded a [redacted] e consistent with the original contamination.
- [redacted] tion of the original substrate (F11/86) yielded an NSD profile with an [redacted] ned Quant value.

- Dire[redacted] amination – left to right.

Conta[redacted] he sample has occurred at a point during the extraction process before the re[redacted] lysate but after removal of the substrate, as the lysate profile is conta[redacted] sistent with original 9PLEX contamination). The left to right direction of conta[redacted] ot consistent with robotic movement. Contamination may have occurred [redacted], during plate agitation on the robot, or by the removal of plate seals.

[redacted] 03?
This batch of casework samples appears to have a single contamination event.

- Position F5 (45) contains a mixture. The mixture was consistent with the DNA profile found in 3 pos[redacted] 7, E6/38, and E7, 39).
- Re-extraction of the stored lysate of the contaminated sample (F5/45) yielded a DNA profile consistent with the original contamination.
- Re-extraction of the original substrate (F5/45) yielded a full profile consistent with other samples from the same case (F2/42, F3/43 and F4/44).

Contamination of the sample has occurred at a point during the extraction process before the removal of the lysate but after removal of the substrate, as the lysate profile is contaminated (consistent with original 9PLEX contamination). The left to right direction and

bottom to to[redacted]n is not consistent with robotic movement. Contaminat[redacted]ng storage, during plate agitation on the robot, or by the remova[redacted]

6.18 228[redacted]

This batch o[redacted]IQEXT20080507_01 has not been found to contain any adverse events.

- Pos[redacted]acted to
- Re-[redacted]of[redacted]he contaminated sample (C5/21) yielded a mixed DNA profile inconsistent with the original DNA profile.
- Re-[redacted]ostrate (C5/21) yielded a mixed DNA profile con[redacted]om the same case (F2/42, F3/43 and F4/44).

T[redacted]occurred:

[redacted]41161178) and the spin [redacted]on. This statement is b[redacted]

- [redacted]ual DNA IQ extraction batch with only samples [redacted]e DNA profile obtained does not match any of [redacted]A profiles
- [redacted]profile obtained from this sample was checked against all other samples [redacted]nique DNA profile.
- [redacted]ce of the DNA profile was the reagents; then the other samples in this [redacted]batc[redacted]the c[redacted]ld display the same profile or mixtures [redacted]y did not.
- [redacted]o database of plastics manufacturers to confirm if the DNA profile's [redacted]s the plastic.

The n[redacted]nt DNA profiles between the original sample (C5/21) and the lysate (4035[redacted]e attributed to user error. The profile obtained is consistent with the user rotatin[redacted]plate 180'. The DNA profile matches sample (76/D10).

[redacted]NA profile obtained for the lysate (342254626) is consistent with the original [redacted]16178) and contamination from the adjacent well (Pos 29/E4) 301864386 and possibly other wells (minor contributors).Contamination of the sample has occurred at a point during the extraction process before the removal of the lysate but after removal of the substrate, as the lysate profile is contaminated (consistent with original 9PLEX contamination). The left to ri[redacted]n and bottom to top direction of contamin[redacted] not consistent with robotic movement. Contamination may have occurred during storage, during plate agitation on the robot, or by the removal of plate seals.

7. Discus

The resul 6 taken indicate that there was not a single type of ev all of the contamination events. Broadly the events fit i

- Can't exclude the instrument
- Exc
- Sor n not identified

An extens ken to evaluate the performance of the DNA IQ m e PerkinElmer MultiPROBE II extraction p

Wiley & Sloots

8. S

9. R

r fr Law from Solicitor General.pdf
9330 Report
19349 Report
19477 Report
19767 Report
19768 Report
20231 Report
QI 20351 Report
QI 20422 Report

- OQI 20437 Report
- OQI 20615 Report
- OQI 20617 Re
- OQI 20690 Report
- OQI 20925 Report
- OQI 21222 Report
- OQI 21309 Report
- OQI 22880 Report
- OQI 22882 Report
- OQI 19703 Report
- Audit OQI 21718

- [redacted] #62
- [redacted]

10. Action [redacted]

Cases [redacted] re subject to rigorous investigation to ensure that qu [redacted] included the addition of Quality paragraphs to all cas [redacted] the affected time period.

[redacted]

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- **Appendices**

OQI 19930 FTAEXT20080205_01 Original

1	9	17	333941730	33	41	49	57	65	73	81	89
2	10	18	26	34	42	50	58	66	74	82	90
3	11	19	27	35	43	51	59	67	75	83	91
4	333993604	20	28	36	44	52	60	68	76	84	92
5	13	21	29	37	45	53	61	69	77	85	93
6	14	22	30	38	46	54	62	70	78	86	94
7	15	23	31	39	47	55	63	71	79	87	95
8	16	24	32	40	48	56	64	72	80	88	96

OQI 19349 CWIQEXT20080225_02

346790253	9	17	25	33	41	49	57	65	73	81	89
346790262	10	18	26	34	42	50	58	66	74	82	90
3	11	19	27	35	43	51	59	67	75	83	91
4	12	20	28	36	44	52	60	68	76	84	92
5	13	21	29	37	45	53	61	69	77	85	93
6	14	22	30	38	46	54	62	70	78	86	94
7	15	23	31	39	47	55	63	71	79	87	95
8	16	24	32	40	48	56	64	72	80	88	96

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OQI 19477	CWIQEXT20080430_01										
346795477	9	17	320124335	334116189	41	49	57	65	73	81	89
2	10	18	320124326	34	42	50	58	66	74	82	90
3	11	19	27	35	43	51	59	67	75	83	91
4	12	20	28	36	44	52	60	68	76	84	92
333810182	13	21	320124371	37	45	53	61	69	77	85	93
6	14	22	30	38	46	54	62	70	78	86	94
7	15	23	31	39	47	55	63	71	79	87	95
288908564	320124349	24	320124362	40	48	56	64	72	80	88	96

OQI 19767	FTAEXT20080515_01										
1	9	17	308802586	33	41	49	57	184858899	73	81	89
2	10	18	26	34	42	50	58	66	74	82	90
3	11	19	27	35	43	51	59	67	75	83	91
4	12	20	28	36	44	52	60	68	76	84	92
5	13	21	29	37	45	53	61	69	77	85	93
6	14	22	30	38	46	54	62	70	78	86	94
7	15	23	31	39	47	55	63	71	79	87	95
8	16	24	32	40	48	56	64	72	80	88	96

OQI 19768	CWIQEXT20080506_01										
346796064	9	17	25	33	41	49	57	65	73	81	89
2	10	18	26	34	42	50	58	66	74	82	90
3	11	19	27	35	43	51	59	67	75	83	91
4	12	20	28	36	44	52	60	68	76	84	92
5	13	21	29	37	45	53	61	69	77	85	93
6	14	22	30	38	46	54	62	70	78	86	94
7	15	23	342270241	39	47	55	63	71	79	87	95
8	16	24	342270230	40	48	56	64	72	80	88	96

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OQI 20231	CWIQEXT20080417_01											
346794568	346802405	17	346802502	33	41	49	57	65	73	81	89
2	10	18	26	34	42	50	58	66	74	82	90
3	11	19	27	35	43	51	59	289009815	334742062	83	91
4	12	20	28	36	44	52	60	68	76	84	92
5	13	21	29	37	45	53	61	69	77	85	93
6	14	22	30	38	46	54	62	70	78	86	94
7	346802482	23	31	39	47	55	63	71	79	87	95
8	16	24	32	40	48	56	64	72	80	88	96

OQI 20351	CWIQEXT20080402_01										
346792908	9	17	209039621	33	41	49	57	65	73	81	89
2	10	18	209039610	320110714	209066683	50	58	66	74	82	90
3	11	19	209039596	35	209066674	259718144	59	67	75	209439271	91
4	12	20	209039585	36	209066660	52	60	68	76	84	92
5	13	21	29	37	45	53	61	69	77	85	93
6	14	22	30	38	46	54	62	70	78	86	94
7	15	23	209039604	39	47	55	63	71	79	87	95
8	16	24	209039579	40	48	56	64	72	80	88	96

OQI 20422	CWIQEXT20080506_02										
1	9	17	25	33	41	49	57	65	73	81	89
2	10	18	26	34	42	50	58	66	74	82	90
3	11	19	27	35	43	51	59	67	75	83	91
4	12	20	28	36	44	52	60	68	76	84	92
5	13	21	29	37	45	53	61	69	77	85	93
6	14	22	30	38	46	54	62	70	78	86	94
365296308	15	320124503	31	39	47	55	63	71	79	87	95
8	16	320124514	32	40	48	56	64	72	80	88	96

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OQI 20437 CWIQEXT20080630_01

1	9	17	25	33	41	49	57	65	73	81	89
2	365366399	18	26	34	42	50	58	66	74	82	90
3	11	19	27	35	43	51	59	67	75	83	91
4	12	20	28	36	44	52	60	68	76	84	92
320126679	365366413	21	29	37	45	53	61	69	77	85	93
323288136	365366424	22	30	38	46	54	62	70	78	86	94
323288127	15	23	31	39	47	55	63	71	79	87	95
8	16	24	32	40	48	56	64	72	80	88	96

Appendix 2. Quality Paragraphs used

Category A – samples tested during the period of Oct 07 to July 08 but no adverse event associated with the results of this case.

Testing for this case has been conducted in a period where some results were the subject of adverse events. This period was between October 2007 and July 2008. Testing for this case was not the subject of any adverse results. This conclusion has been reached by assessing the individual results by comparison to all other results from samples processed along side each other.

Include the following if Retesting has been conducted: Retesting has been conducted on identified samples which have confirmed the alleles in the original result.

These samples have been reported as they have been assessed as no adverse event having been detected and the results have passed all quality assurance checks.

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Category B – samples tested during the period and an adverse event occurred on those samples – results cannot be reported (QC failure).

Testing for this case has been conducted in a period where some results were the subject of adverse events. This period was between October 2007 and July 2008. Within this case, the adverse event is demonstrated to have affected a result or results and the integrity cannot be verified, then this result has therefore been reported as follows: "These samples did not pass our Quality System requirements at the DNA Analysis stage and therefore the DNA profiling results relating to these samples cannot be reported".

Category C – samples tested during the period, an adverse event has occurred on that sample/s, retesting has been conducted and the retesting results can be reported.

Testing for this case has been conducted in a period where some results were the subject of adverse events. This period was between October 2007 and July 2008. Testing for some samples within this case has been the subject of an adverse event. The cause of the adverse event was identified to have occurred within the extraction process performed on an automated platform. Where sample is remaining (which could include, but is not limited to, additional stain remaining or previously extracted portion), retesting has been conducted, using an alternative manual extraction method. The retesting has confirmed the alleles in the original result.

These samples are reported as they have been assessed as no adverse event has been detected and the results have passed all quality assurance checks.

Report for QIS Audit as of 7/10/2022 2:04:42 PM

Report for QIS Audit -

9642 DNA IQ follow up audit

Audit Contact Details

Contact	Thomas NURTHEN
Organisational Unit/s	Quality and Projects
Site/Locations	Coopers Plains

Audit (Lead/Internal) Contact Details

Auditor (Internal/Lead)	Inga SULTANA
Organisational Unit/s	Quality and Projects
Site/Locations	Coopers Plains

Other Auditors

Auditors (Other)	Susan BRADY

Audit Details

Date Audit Performed	24/08/2009
Audit Type	OQI Follow up
Audit Status	Closed
Audit Subject	Review the changes made to the DNA IQ process for improvements in quality of results
Audit Objective	Check that the changes made to the DNA IQ program sufficiently improve the quality and reliability of results.
Audit Scope	The new DNA IQ program v6.3 run on Extraction MulitPROBEs A and B, using reference samples in checkerboard patterns
Audit Criteria	Nil contamination, SOP compliance

Audit Outcome

Audit Findings

1. Abstract

A follow-up internal audit was performed on the DNA IQ method for extracting DNA from Casework and Reference Samples. The purpose of this audit was to maintain the continuous high quality standards within the DNA Analysis laboratory.

2. Aim

To determine if the DNA IQ extraction process is fit for purpose as well as to ensure that the recommendations from Audit 8227 have been implemented.

3. Background

Through the laboratory quality system (OQI process) a number of adverse quality events were identified on the MultiPROBE® II platforms. Three OQI's (19349, 19477, and 19768) had previously been raised to address contamination events, which were investigated

in Audit 8227. This is a follow-up audit to ensure quality measures that were suggested have been implemented successfully. Audit findings and recommendations are outlined in this report.

4. Findings and Observations

Observations from the Off-Deck lysis process are outlined below:

• Table 2 – Table of Reagent Volumes, on Page 5 of the SOP (24897 Automated DNA IQTM Method of Extracting DNA) is unclear. The table lists reagent volumes all in mL, most of the reagent volumes required are less than 1mL. The addition of a formula to determine reagent volumes would assist in the event of non-standard batch sizes.

• The Auslab OFF-Deck Lysis worksheet has 20% Sarcosyl, this should be 40%. As a result the operator is required to amend the volume on the worksheet.

• The process of transferring the substrate to the spin basket is not defined. Operators can do this in 2 different ways.

• The preparation of the plate map using the BSD program for the validation configuration of batches i.e. Soccerball, is not mentioned in the SOP at all.

• Staff were using a draft copy of the SOP

Observations from the MultiPROBE II element of the automated extraction process are outlined below:

• In the new deck arrangement, the reagents are placed at the back of the deck. This was identified as a possible Workplace Health and Safety concern as the operators are required to lean over the entire platform in order to place the reagents in the reservoirs.

• It was observed that whilst filling the reservoir, some lysis buffer was spilt onto the shaking platform.

• There were bubbles present on the meniscus of the resin after addition to the reservoir; the operator was required to pipette mix to reduce the bubbles.

• The Schott bottle containing Lysis Buffer + DTT was not labelled.

• Section 4.4 DNA IQ Resin, Point 3 was not performed at this stage, as the tube was inverted just prior to transferring reagent into container on platform.

• The second operator performed a platform check before commencing run to ensure everything is in the appropriate position.

• The cabinet doors are not ergonomically designed. This is a Workplace Health and Safety concern as they are difficult to open, and they must be removed monthly to enable environmental cleaning. It is also difficult to manoeuvre the uncapped nunc tubes to a new position on the deck after uncapping due to the cabinet doors.

• The manual de-capping process is inefficient, time consuming and has the potential to be a possible source of contamination. The operator is required to use their finger to remove the cap off from the end of the de-capper. Some operators are uncomfortable with this manual de-capping process. De-capping manually can also be difficult due to the position of the cabinet. The de-capper can only be placed in the corner of the cabinet, making it difficult for operators to utilise.

• In the SOP, a specific de-capping order is mentioned; however it was

observed that not all operators perform the task in this order.

• Some operators are concerned with the shaking of the slicprep plate on the magnet. It has been observed by some operators to dislodge occasionally.

• The sequence checking of the first and last nunc tube samples of both sets of tubes to the batch paperwork is not included in the SOP.

• The reservoir 'F/W G13', where the fixed tips are rinsed with nanopure water is only cleaned monthly during environmental cleaning. There is a potential for a contamination event to occur.

• Bubbles/droplets were observed on the outer surface of the disposable tips. This was observed on both 1mL and 125uL tips, after dispensing both the resin and the lysis buffer.

• It was sometimes difficult to follow the SOP as some steps are only outlined in the prompts on the computer program running the MultiPROBE II. These prompts are only mentioned in the SOP as 'Follow the directions as outlined in the user prompt', and the specific step details are not mentioned at all.

• During the transfer of lysates to the slicprep, some of the tubes are temporarily lifted from their position. No tubes were completely removed from their position.

• The seal on the slicprep plate was not completely sealed on one corner after sealing with the heatsealer.

• It was observed that during the wash stage, a 1mL tip flicked out of the tip chute after being discarded by the fixed tip. One operator has witnessed this occurring 2 times out of 4 runs.

• A 125uL tip could not be removed automatically from the fixed tip on 2 occasions. The operator was required to remove them manually.

• There is currently a check at the end of the extraction process to ensure there is no lysate left in the original nunc tubes. This step may be more useful if performed before the extraction process.

• A new magnet is now being used. The plate now sits on the magnet with no operator intervention.

• It was observed that the de-capper on Platform A has begun to rust.

• The shaker component needs to be removed from the deck every Friday in order to perform weekly maintenance on the Monday. This could be a Workplace Health and Safety concern as the shaker is heavy and is difficult to manoeuvre with the current cabinet doors.

5. Summary

Overall, the recommendations of Audit 8227, Process Audit of the Automated DNA IQTM System (including Off-Deck Lysis) have been addressed. Some sections are still to be addressed and have been included in this audit's recommendations. Further improvements were identified during this audit and have been outlined below.

6. Recommendations

There have been several places identified where improvements can be made. These are outlined below:

1. The addition of a formula to determine reagents amounts in Section 4 of the SOP.

2. In Table 2 in the SOP, change the reagent volumes to appropriate measurements. Currently all volumes are listed in mL, but for most of the reagents uL would be more appropriate.

3. Update the Auslab Off-Deck Lysis worksheet to state 40% Sarcosyl instead of 20%.

4. The addition of the specific methods for transferring the substrate to the spin basket to the SOP.

5. Addition of the method for the preparation of the BSD plate map for the validation configuration of batches i.e. Soccerball to the SOP.

6. Consider acquiring an automatic de-capping machine. The manual de-capping method is inefficient, difficult in the current cabinet and has the potential to cause contamination events.

7. Investigate ergonomically designed cabinet doors for the MultiPROBE II platforms. The current cabinet doors are a Workplace Health and Safety concern.

8. The SOP requires the addition of the tube to batch paperwork sequence checking step.

9. Implement weekly cleaning of the F/W G13 reservoir.

10. Investigate the bubbles/droplets on the outer surface of the disposable tips.

11. Elaborate on the prompts in the SOP. This will make the SOP easier to follow for operators.

12. Investigate an alternative tip chute to prevent tips flicking out after being discarded from the fixed tips.

13. Investigate a more appropriate way to perform the weekly maintenance rather then to remove the shaker component every week.

7. OQI's that will be raised from this audit:

1. OQI 23911: The cabinet doors are non ergonomical and are not fit for purpose. They are heavy, difficult to move and inefficient, and are a Workplace Health and Safety concern.

2. OQI 23912: The manual de-capping tool is non ergonomical and is a potential source for contamination.

3. OQI 23913: The used disposable tips flick out of the tip chute on Platform A when being discarded.

8. Acknowledgements

The auditors would like to thank all DNA Analysis staff involved in the audit for their time, assistance and cooperation.

Contact Comments

1. The addition of a formula to determine reagents amounts in Section 4 of the SOP.
This point will be addressed in the next revision of the SOP 24897, it has been added as a comment and communicated to the appropriate staff and will be included as an appendix in the future.

2. In Table 2 in the SOP, change the reagent volumes to appropriate measurements. Currently all volumes are listed in mL, but for most of the reagents ?L would be more appropriate.
This point will be addressed in the next revision of the SOP 24897; it has been added as a comment and communicated to the appropriate staff.

3. Update the AUSLAB Off-Deck Lysis worksheet to state 40% Sarcosyl instead of 20%.
A change request will be submitted to LISS for amendment of this mistake.

4. The addition of the specific methods for transferring the substrate to the spin basket to the SOP.
This was discussed at length previously amongst the Analytical team and a decision was made to include both methods; as each was valid and an operator was free to choose a method that they would most prefer using.

5. Addition of the method for the preparation of the BSD plate map for the validation configuration of batches i.e. Soccerball to the SOP.
This point will be addressed in the next revision of the SOP 24897; it has been added as a comment and communicated to the appropriate staff.

6. Consider acquiring an automatic de-capping machine. The manual de-capping method is inefficient, difficult in the current cabinet and has the potential to cause contamination events.
Whilst the current manual de-capping of Nunc tubes is inefficient the procedure has been redesigned to minimise the potential for cross contamination. Given the success and increase in efficiency of de-capping with the Pre-PCR automated de-capper; additional de-cappers are a priority but current budget constraints prohibit DNA Analysis purchasing additional units at this time. As noted in the following response, purchase and implementation of improved cabinets has been delayed due to budgeting issues as well

7. Investigate ergonomically designed cabinet doors for the MultiPROBE II platforms. The current cabinet doors are a Workplace Health and Safety concern.
New cabinets have already been applied for and are expected to be delivered during the move to block 3. The Perspex doors slide along a metal channel, to make sliding of the doors easier for the time being – soap will be used on the metal channels. Improved cabinets have not been sourced prior to this date due to budget restrictions.

8. The SOP requires the addition of the tube to batch paperwork sequence checking step.
This point will be addressed in the next revision of the SOP 24897; it has been added as a comment and communicated to the appropriate staff.

9. Implement weekly cleaning of the Flush Wash G13 reservoir.
This will be discussed at an Analytical meeting to determine if cleaning or renewing of the reservoir is necessary.

10. Investigate the bubbles/droplets on the outer surface of the disposable tips.
This has been investigated as part of the DNA IQ re-implementation project. The surface of the conductive tips attracts small droplets/bubbles of the lysis buffer through static attraction. There is a low risk that these small droplets/bubbles will separate from the end of the tip. The step where the droplets/bubbles remain adhering to the conductive tips is a post dispense step in the store plate. The store

plate is discarded after the extraction and there are no consumables between the plate and the tip chute where the tips are discarded. The instrument does not pass over any labware that may be contaminated by the adhering bubbles/droplets.

The droplets observed adhering to the fixed tips after the addition of resin to the lysate plate is of minimal risk. The tips dispense well above sample and do not contact sample. The droplets are clean reagent adhering to the tip. There is minimal risk of contamination occurring as a result of adhering reagent to the tips.

11. Elaborate on the prompts in the SOP. This will make the SOP easier to follow for operators.
This will be discussed at an Analytical meeting to determine which prompts require elaboration. It has been added as a comment in QIS for revision in the next version of the document.

12. Investigate an alternative tip chute to prevent tips flicking out after being discarded from the fixed tips.
Alternative tip chutes are currently being investigated with a view to purchase of a plastic based chute that could be easily decontaminated without corrosion. The tips observed flicking out of the tip chute on extraction platform A were as a result of incorrect tip chute dispensing position calibration. This has been addressed and no further tips have flicked out of the tip chute. This issue was previously addressed on extraction platform B and no tips have been observed flicking out of the tip chute.

13. Investigate a more appropriate way to perform the weekly maintenance rather then to remove the shaker component every week
The shaker must be removed from the deck during the weekly maintenance as the eight tip arm and gripper would crash into it during the random arm movement test. Quotes have been requested from the manufacturer (PerkinElmer) for an expansion deck and new shakers that would be smaller in size and easier to move. Once the quotes have been assessed against the current budget a decision to replace the DPC shaker will be made. Appointments/intervention by appropriate staff for the removal of the DPC shaker will be sent to ensure that staff who cannot remove the DPC shaker easily will not have to.

Associations

Module	Document		
QIS Record	Automated DNA IQ Method of Extracting DNA from Reference and Casework samples		
QIS Record Number	24897	**Associated Version**	5.0DNA IQ Method of Extracting DNA from Casework and Reference Samples
Status	Superseded	**Current Version**	
Association Description			

Module	OQI		
QIS Record	From Audit 9642: Tip chute on Platform A		
QIS Record Number	23913	**Associated Version**	The used disposable tips flick out of the tip chute on Platform A when being discarded.
Status	Closed Approved	**Current Version**	
Association Description			

Module	OQI		
QIS Record	From Audit 9642: The manual de-capping tool		
QIS Record Number	23912	**Associated Version**	The manual de-capping tool is non ergonomical and is a
Status	Closed Approved	**Current Version**	
Association Description			

potential source for contamination.

Module	OQI		
QIS Record	From Audit 9642: The cabinet doors are not fit for purpose		
QIS Record Number	23911	**Associated Version**	The cabinet doors are non ergonomical and are not fit for purpose. They are heavy, difficult to move and inefficient, and are a Workplace Health and Safety concern.
Status	Closed Approved	**Current Version**	
Association Description			

Module	Audit		
QIS Record	DNA Extraction Process (DNA IQ)		
QIS Record Number	8227	**Associated Version**	Process Audit of the Automated DNA IQ System (including Off-Deck Lysis)
Status	Closed	**Current Version**	
Association Description			

Records

No Records found

9642 DNA IQ follow up audit

Report for QIS Audit as of 14/10/2022 3:42:06 PM

Report for QIS Audit -

9175 DNA IQ external audit

Audit Contact Details

Contact	Iman MUHARAM
Organisational Unit/s	DNA Analysis
Site/Locations	Coopers Plains

Audit (Lead/Internal) Contact Details

Auditor (Internal/Lead)	Iman MUHARAM
Organisational Unit/s	DNA Analysis
Site/Locations	Coopers Plains

Other Auditors

Auditors (Other)	

Audit Details

Date Audit Performed	12/11/2008
Audit Type	Process
Audit Status	Closed
Audit Subject	DNA IQ external audit
Audit Objective	No Objective
Audit Scope	No Scope
Audit Criteria	No Criteria

Audit Outcome

Audit Findings	External auditors from the Sir Albert Sakzewski Virus Research Centre were requested by Mr Greg Shaw (Senior Director, Forensic and Scientific Services) to review the automated DNA IQ procedure, specifically version 6.1, including associated procedures such as off-deck lysis and arranging tubes using STORstar.
Contact Comments	Refer to linked OQI's.

Associations

Module	Document		
QIS Record	Automated DNA IQ Method of Extracting DNA from Reference and Casework samples		
QIS Record Number	24897	**Associated Version**	4.0Migrated Data from QIS version 1
Status	Superseded	**Current Version**	
Association Description		**Module**	OQI
QIS Record	No Title Provided		
QIS Record Number	21718	**Associated Version**	

Status	Closed Approved	**Current Version**	Migrated Data from QIS version 1
Association Description			
Module	OQI		
QIS Record	No Title Provided		
QIS Record Number	21715	**Associated Version**	Migrated Data from QIS version 1
Status	Closed Approved	**Current Version**	
Association Description			

Records

No Records found

9175 DNA IQ external audit

Report for QIS OQI as of 14/10/2022 3:43:33 PM

Report for QIS OQI -

21718 No Title Provided

OQI Details

Status	Closed Approved
Subject	Refer to Audit 9175 (DNA IQ external audit) and report by Sloots & Whiley (14 November 2008). A summary of items for further consideration is listed below: 1. Develop a standard validation protocol based on Butler (Profiles in DNA, September 2006) and incorporate into a SOP. 2. Increase the number of negative controls to comprise at least 10% of the total number of samples in the batch. 3. Test a control plate once every 3-4 weeks on each of the MPII platforms (alternate between soccerball, checkerboard and zebra stripe). 4. Constant observation by the operator of all processes leading up to and including the addition of magnetic particle is necessary to ensure that resin is added correctly. 5. Workflow dynamics for future detection of LCN DNA must be carefully examined to minimise the risk or post-PCR carryover from amplification or post-amplification areas into pre-amplification areas.
Source of OQI	Audit
Date Identified	15/12/2008

OQI Creator Contact Details

Creator	Thomas NURTHEN
Organisational Unit/s	DNA Analysis
Service/s	Forensic and Scientific Service
Site Location/s	Coopers Plains

Investigator/Actioner Contact Details

Actioner	Kirsten SCOTT, Thomas NURTHEN, Chiron WEBER
Organisational Unit/s	Quality and Projects
Service/s	Forensic and Scientific Service
Site Location/s	Coopers Plains

Investigation Details

Investigation Completed	02/11/2009 **Root Cause Type** Procedure/Method/Process
Investigation Details	Refer to Audit 9175 (DNA IQ external audit) and report by Sloots & Whiley (14 November 2008). A summary of items for further consideration is listed below: 1. Develop a standard validation protocol based on Butler (Profiles in DNA, September 2006) and incorporate into a SOP. 2. Increase the number of negative controls to comprise at least 10% of the total number of samples in the batch.

3. Test a control plate once every 3-4 weeks on each of the MPII platforms (alternate between soccerball, checkerboard and zebra stripe).

4. Constant observation by the operator of all processes leading up to and including the addition of magnetic particle is necessary to ensure that resin is added correctly.

5. Workflow dynamics for future detection of LCN DNA must be carefully examined to minimise the risk or post-PCR carryover from amplification or post-amplification areas into pre-amplification areas.

Preformed By Chiron WEBER

Investigation Completed 13/01/2009 **Root Cause Type** Otherreassign to Chiron Weber.
Investigation Details
Preformed By Quality Information System

Action Details

Action Complete 14/02/2014 **Action Fix Type** Changed ProcessActions from Audit 9175 (DNA IQ external audit)
Title

Action Description

1. Develop a standard validation protocol based on Butler (Profiles in DNA, September 2006) and incorporate into a SOP.

2. Increase the number of negative controls to comprise at least 10% of the total number of samples in the batch.

3. Test a control plate once every 3-4 weeks on each of the MPII platforms (alternate between soccerball, checkerboard and zebra stripe).

4. Constant observation by the operator of all processes leading up to and including the addition of magnetic particle is necessary to ensure that resin is added correctly.

5. Workflow dynamics for future detection of LCN DNA must be carefully examined to minimise the risk or post-PCR carryover from amplification or post-amplification areas into pre-amplification areas.

Action Complete 13/01/2009 **Action Fix Type** Changed Processreassign to
Title **Action Description** Chiron Weber.

Task Details

Title Standardise validation
Task Status Completed **Task Completed** 14/02/20141. Develop a
Task Fix Type Documentation **Task Assignee** Thomas NURTHENstandard validation
Task Undertaken
protocol based on Butler (Profiles in DNA, September 2006) and incorporate into a SOP.

Title Increased negative controls in automated extraction batches
Task Status Completed **Task Completed** 02/02/2010Increase the
Task Fix Type Changed Process **Task Assignee** Thomas NURTHENnumber of negative
Task Undertaken
controls to comprise at least 10% of the total number of samples in the batch.

As of the 02/02/2010 the number of controls on the extration plate is as follows:

2x Pos, 2x neg, 10 x blank

Title	Automated control plates
Task Status	Completed
Task Completed	14/02/2014
Task Fix Type	Changed Process
Task Assignee	Thomas NURTHEN
Task Undertaken	Test a control plate once every 3-4 weeks on each of the MPII platforms (alternate between soccerball, checkerboard and zebra stripe).

Title	Observation of automated extraction procedure
Task Status	Completed
Task Completed	14/02/2014
Task Fix Type	Changed Process
Task Assignee	Thomas NURTHEN
Task Undertaken	Constant observation by the operator of all processes leading up to and including the addition of magnetic particle is necessary to ensure that resin is added correctly.

Title	LCN lab processes
Task Status	Completed
Task Completed	14/02/2014
Task Fix Type	Changed Process
Task Assignee	Thomas NURTHEN
Task Undertaken	Workflow dynamics for future detection of LCN DNA must be carefully examined to minimise the risk or post-PCR carryover from amplification or post-amplification areas into pre-amplification areas.

Follow-up And Approval

Follow-up Status	Accepted
Follow-up Status Comment	14/02/2014 10:20:00 AM Thomas NURTHEN: Accepted 13/03/2009 12:00:00 AM Iman MUHARAM: reassign to Chiron Weber.
Approver	Thomas NURTHEN
Approval/Rejection Date	14/02/2014
Approval/Rejection Comment	14/02/2014 10:20:41 AM Thomas NURTHEN: Accepted as per managment team as at 13/02/2014

Associations

Module	Audit		
QIS Record	DNA IQ external audit		
QIS Record Number	9175	**Associated Version**	Migrated Data from QIS version 1
Status	Closed	**Current Version**	
Association Description			

Records

No Records found

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Biology Management Team Minutes

PRESENT AND APOLOGIES			
Chairperson:	CJA	**Date and Time:**	**2008 – November 14**
Venue:	Conference Room 102	**Secretariat :**	WH
Attendees:	CJA, JAH, AAP, AMc, AJR, IM, KDR, PT, TEN, EJC, WH		
Apologies:	Iman, PAC		
Guests:	Alice (Training Update)		

1.0 PREVIOUS MINUTES ENDORSED
Minutes of previous meeting held on 30 October 2008 are endorsed by CJA & WH without amendment or amended as follows:

Item	Topic	Discussion	Action required (inc: Officer, Due date)	Communications to go out
		2.0 STANDING AGENDA ITEMS		
2.1	**Training Update** *(PAC)* a. Court Training Program progress b. Biology	SSDU update 131108.doc PAC to supply to PT / JAH a spreadsheet which includes names of staff members with workplace skills yet to be completed / marked / uploaded into QIS. QIS 2 – looks good. Release in March / April 2009.	PAC	

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Biology Management Team Minutes

	Training Calendar progress c. Training modules – review, changes, and additions. d. Staff Training sign off and feedback progress including outstand-ing sign offs from gap analyses of existing staff. e. Review the Issues Register.	JAH to email PAC the feedback from AMc Court training. SSDU to review pending feedback (Train the Trainer) which will be added to the Training Register Issues Log. Agreed there will be one SOP for GMIDX Training (includes Genotyper) a separate Training Module will be required for the GMIDX part. JAH will forward some alternative wording for inclusion in the Paternity SOP. A QIS comment will be added by JAH. The new training room is available for booking if delivering training and participants needs to use computers. (8 spaces). If on-line surveys are to be conducted, staff can us the training room, so that they can access the internet to complete surveys. <u>PREVIOUS</u> TEN / PAC working on audit of training portfolios. Needs Attn: Issues Register – ? OQI re Court Training . KDR to follow-up with Claire P. If there was a problem with the Case file an OQI is definitely required. SSDU will advise DPP of trainee numbers attending 2009 sessions. DNA Analysis to advise of approximately how many staff members they would like to attend 2009 Court Training Program? Mtg set to discuss 17/11/08. On going - Hairs SOP – KDR and PAC to work on model answers prior to KDR's leave. Meeting booked for 20/11/08. Needs Attn - Stats course – PAC to prepare a report on this course / outcomes. PAC to forward this report to JAH this week 13-17 Oct 2008. Report not received as yet 13/11/08. Analytical staff interested in general stats course. Allan will review feedback from PAC as to whether this course suitable. Presidential Website – not what Analytical staff are looking for. AJR advised that the text book from Canberra Stats Course is on her desk, should anyone wish to view it.	JAH PAC JAH TEN / PAC PAC/KDR/ CP JAH / PT / CJA KDR / PAC PAC	

Biology Management Team Minutes

		CJA – chat with JF about SSDU role in future and what DNA Analysis will need to do to assist. Form a working party to work out what we want – AM, TEN, PT, EJC. PT – Chair. Amanda and Kylie to email Paula any comments / feedback for this working group prior to departure this year.	CJA	
2.2	**Automation Update** (Iman)	Visit by External Auditors (12/11/08) – The Auditors looked at off deck lysis, storStar, programming end to end, platforms, OQI – what we did / processes, reporting, analysis, timelines. They identified no areas of risk, and complimented our staff. The auditors agreed with our actions taken and basic principles. A report will be issued from the external Auditors. Some recommendations are – ▪ Locked batches – CJA and Iman to explore this ▪ Reagents to be tested at 35 ▪ Strip Cap seals for PCR Plates – Iman will source these. ▪ # of QC plates per month (i.e. checkerboard) <u>PREVIOUS</u> Seals for Amp Plates – process to be followed, staff are to lift the seals one way only. Adding less lysis, 900 down from 1500, efficiency goes up. AAP, AMc, Iman to think about this prior to the next meeting. On Hold until New Year: Tom working on program for benchmarking extraction methods for all labs using Robots. Mock samples are being prepared (using blood samples first). TEN has spoken with WA, VIC, SA. Yet to speak with NSW & AFP. Week commencing 13 October – Automation looking at auto bead transfer using robots, affecting plates, anti contamination plates checked in soccer / zebra pattern. Run some ref samples to show no contamination. 30/10/08 - DO NOT use Run Ref Samples for this process. Awaiting test results - then the whole team will be asked what they would like to see, to be confident to use the robots again.	Iman IMAN / AAP / AMc IMAN IMAN IMAN / AMc	

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		13/11/08 – Nothing further to update at present A Business Case is currently with Executive for the purchase of Artel system. 28/8/08 – no money available, CJA investigating another avenue. TEN has been working with Kate Holzer on single source supplier information – The alternative is not good for us, as it only does 10ul or above. 25/09/08 – Business Case regarding Artel has been sent again. Needs Attn - With regard to sticky film vs heat film. What is the level of testing required? Agreed to amp some controls using both sticky & heat seals. ie. Amp it twice (using previous REF samples). 28/8/08 – waiting for pre PCR to be up and running. 11/09 – used the heat seal but it appears to come away from the plate during amp – company will come in to see if we are doing it correctly, if we are and it still doesn't work – then we'll send it back. 13/10/08 - Seals – IMAN will follow up with companies that supply seals. Amps will continue as is for now with sticky seals. 30/10/08 – The company rep attended yesterday. Plates are very flexible. Normal processes to be used until this is sorted. IMAN to find out what other labs in the country are using for their seals. 28/8/08 – on hold. Project 24 sperm – IQ vs Chelex - quants. Need to establish if ProtK is the problem. IMAN to carry out differex experiments. 23/5/08 – on hold. - IMAN will investigate nuc /clean ups, 30% of these maybe unnecessary. 14/8/08 – not started as yet, big job.	IMAN IMAN / AMc IMAN	
2.3	**GM IDX Update (Chiron)**	I:\GMIDX validation\PRESENTATIONS\GMIDX Verification Results Management Team Meeting AUG 28 2008.ppt Proposed Implementation / Go Live date – 15 December if not that date then 2nd week of January 2009.		

Biology Management Team Minutes

		PREVIOUS GeneMapper mask now live in AUSLAB. Training has commenced. So once training is complete, will be able to read and upload to AUSLAB. Technical Note for ANZFSS poster – Chiron to follow this up. Chiron to forward QIS document to PAC. PAC to obtain QIS number Sequencing – REFER to item 4.15 Rhys to source a place to have extract sequenced at D3. PT to contact Tracey Dale to get ok to forward it to place for sequencing. (Place needs to be NATA registered) 13/10/08 - Change Proposal #33 and #38 approval. Chiron will forward via email prior to next meeting. Please review, ready for sign off at next meeting.	Chiron Chiron / PAC KDR / PT Chiron / All	
2.4	**Change Proposals / Project Management** *(Tom)*	G:\ForBiol\Quality Assurance and NATA\Management Reports - Biology Management Team Nil PREVIOUS LISS – EXH page – fixed in AUSLAB 29 October. All barcodes of EXH will now go across to QPS in batches so as to not break the interface. Adverse Events SOP - Paula will after locating such, put this SOP into draft. Ongoing - Agreed to purchase a Canon camera to be used only as a card reader. Needs Attn - OQI 20319 - 11/9/08 –Susan & Rebecca working on this. Should be completed by end of November. {2 exhibits repackaged incorrectly} 20/12/07 - ON HOLD – (to be programmed) Suggestion to have photos on screen at Shift F9 and colour to indicate specimen notes. IMAN agreed to look into this.	PT AAP AAP	

Biology Management Team Minutes

Item	Topic	3.0 NEW BUSINESS Discussion	Action	
3.1	**OQIs (Allan)**	Nothing to add further. There are 27 open OQIs at present.		
3.2	**Trial Leave Calendar**	G:\ForBiol\AAA Administration\Timesheets and HR forms\Leave_Calendar_Trial_complete.xls For the period July – 12 November. 24% of staff i.e. 1 in 4 staff members, had not completed and submitted leave forms for leave taken. 13 occurrences of incorrect completion of RAF forms by staff 3 occurrences of discrepancies between timesheet and leave forms. Mtg scheduled 17 November to review timesheets with regard to introduction of 38 hr week. Complete - Wendy to meet with Tom separately. Agreed to use new form of leave calendar. Managers to check this calendar when signing timesheets. Complete - Wendy to email file path to all managers.	All	
3.3	**Update from FSS Workforce Support Mtg held 12 Nov.**	All arrangements for interviews, moderation, printing of applications, obtaining referee reports, etc etc is now the responsibility of the Chair of the panel or their delegated officer.		
3.4	**Microcon to full and microcon to 30ul review (re 320126006) (JAH)**	Reviewed discussion of full to 30uL – targeting 1.2uL and the microcon hasn't been of benefit so case managers have been reverting to microcon to full and no quant. JAH to provide examples of where samples haven't been halted after Quant so feedback can be given to staff. Staff ensure N'Spin before M'con if any sign of inhibition. Change to be made on 17.11.08 – M'con to full means M'con to full (and not to 30uL as previously agreed to).	JAH	

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3.5	**Accuracy of Quants – round up vs round down (re 334727291) (JAH)**	Carried over		
3.6	**Spin baskets and association to cases (JAH)**	Carried over		
3.7	**D3 (20 and 20.1) and data on mismatches (JAH)**	Using PowerPlex to try to confirm as primers are different. In reference to Ingrid's case – Ingrid to arrange for re genescan all samples on one plate.	Ingrid	
3.8	**diluted samples - see FRIT issues log (JAH)**	JAH to see AM with examples so this can be rectified	JAH	
3.9	**Statistics**	Look at getting a Stats expert in to give updated training to all applicable staff CJA to email Jenny Rees re available funds.	CJA	
3.10	**On call arrangements**	Bernice to get back to JAH on pay. Complete - Wendy to obtain for JAH details from 2007. Cathie updated all on Christmas finish. At this stage, Friday 2 January is a working day. Staff will be advised if this day becomes included in closure (if this occurs staff will need to apply for leave of some sort for this day).	JAH All	

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	Topic	4.0 BUSINESS ARISING FROM PREVIOUS MINUTES Discussion	Action required (inc: Officer, Due date)	Communications to go out
	PROJECT	**STATUS, RISKS, ISSUES, COMMUNICATIONS**		
4.1	NucleoSpin cleanup - should we progress to automatically cleaning up all Sperm Lysate samples directly after Diff?? **(Allan)**	Presented to FRIT mtg last week. If samples were not a sperm lysate and there was no DNA detected or inhibition detected then there was no value in doing a nuc clean up. However 80% of sperm lysates that had nuc clean up got better results. Only 30% of sperm lysates give good results straight up. After Analytical have run a couple of experiments – looking into nuc clean up efficiency, decision to be made whether to automatically clean up all sperm lysates or keep as preferred reworking option – AMc to report back to meeting.	AMc	
4.2	**DNA IQ Issues Register**	Found at G Drive, Quality Assurance & Nata folder. DNA IQ issues Dec 2007 – early / late in process. Batches prior to and after. Links 600 IQ investigations. If link has gone out – failure. If REPORTED - chat with QPS, retract. LINKS retract – replace with message. Speci notes phone calls etc. Scan in correspondence / message quality control failure. CJA to brief up.	All CJA	

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4.3	**QPS Matches and whether they warrant an OQI (RS)**	Template EXR – yes quality team to raise and action.	IMAN	
4.4	**External Proficiency Test for Paternity (AJS)**	Agreed this is a good idea. TEN to source such.	TEN	
4.5	**Storage Issues (Quality) (Inga)**	Now at critical point. Suggested storage off site. IMAN to investigate from what we can and can't get DNA profiles from (i.e. spin baskets, beads, etc, testing approx 50 sources) Not a good idea to bring samples back from frozen to room temperature. Agreed that discarding – not a good idea. Working party – what we keep / why / best way to keep it. Major Crime is kept indefinitely, suggested charging QPS for storage / NATA requirement?	IMAN CJA	
4.6	**NCIDD (DN)**	Allele at D3 either 20 or 20.1 difficult to ascertain which is correct. Report allele as NR in NCIDD – but can report in a Link to QPS that it's a 9 loci match. Seek permission from QPS (Tracey Dale) to send (REF / Casework) to another state (NATA accredited) or another Qld Lab for sequencing.	PT	

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4.7	**Missing Audit Trail Restoration (VKI)**	11/9/08 CJA and IMAN to meet to discuss and forward details to Greg Smith. i.e. specific instances 1/8/08 - email_audit trail.pdf AUSLAB is currently a verification of hard copy (case file). Currently no paper trail of exhibits is held in casefile. a. Missing audit trail – court. b. AUSLAB sample tracking (Analytical) Questions 1. How often does this happen? 2. What are the procedures in place if it happens?	CJA / IMAN CJA	
4.8	**How to deal with urgent samples – identify a process**	Presentation of Meeting with QPS. 16/7/08 Question raised by QPS for on-call/weekend assistance for urgent lab based issues. Parameters to be set for urgent assistance, possibly a roster system. CJA to formalise Urgent Case Communication Protocol.	CJA	
4.9	**Methods Page (AUSLAB)**	11/9/08 – Screen mask not updated quickly. A word template similar to statement would be useful. Perhaps a checklist which can be scanned in. PT to follow up with IMAN. 6/12/07 - Needs Attn: Could a rep from V/ C, M/C and Analytical please forward to IMAN a list of ALL the methods used so that these can be added to AUSLAB. QIS document 17092 refers. The Methods Used in Casework page in AUSLAB is not up to date. Some methods are no longer used; some new ones are not listed eg. DNA IQ. 1. Review if required – PT 2. Use manual process – paper rather than AUSLAB – IMAN	PT / IMAN	
4.10	**Future Planning**	**Action** – Project Team to be established. Project – move towards electronic files – how do we get there?	CJA	

NEXT MEETING

The next meeting is scheduled for Thursday 27 November 2008, Conf Room 102, **9am – 11am**

Fwd: Attached Image

Justin Howes [redacted]

Fri 21/11/2008 10:27 AM

1 attachments (132 KB)

0765_001.pdf;

FYI -the external auditor's report. Cathie, for your distribution to the team - Greg commented that it was complementary.
JAH

>[redacted]Friday, 21 November 2008 10:21 am >>>

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

1. PURPOSE AND SCOPE

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

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

2. DEFINITIONS

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

3. PRINCIPLE

Sample Pre-lysis

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

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

DNA IQ™ Kit

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

The in-house protocol includes:

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

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

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

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

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

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

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

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

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

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

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

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

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

4. REAGENTS AND EQUIPMENT

4.1. Reagents

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

- 40% Sarcosyl
- Nanopure Water

These reagents are stored in locations as per Table 1.

Table 1. Reagent storage locations.

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

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

Table 2. Table of reagent volumes.

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

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

4.2. Extraction Buffer

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

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

4.3. Lysis Buffer with DTT Solution

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

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

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

4.4. DNA IQ™ Resin

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

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

4.5. 1x Wash Buffer

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

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

4.6. Elution Buffer

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

5. EQUIPMENT

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

Table 3. Equipment used and location.

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

Table 4. Consumables used for extraction.

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

6. SAFETY

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

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

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

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

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

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

7. SAMPLING AND SAMPLE PREPARATION

7.1. Sample Locations

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

Table 5. Sample storage locations.

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

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

7.2. QC Samples

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

Table 6. Extraction Quality Controls

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

7.2.1. Registration of QC Samples

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

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

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

7.4. Locating Samples

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

8. OFF-DECK LYSIS PROCEDURE

8.1. Off-Deck Lysis (No Retained Supernatant)

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

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

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

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

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

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

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

5. Prepare Extraction Buffer as per Section 4.1.1.

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

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

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

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

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

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

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

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

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

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

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

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

8.2. Off-Deck Lysis (Retained Supernatant)

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

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

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

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

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

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

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

5. Add 450µL of TNE buffer and vortex.

6. Incubate at room temperature for 30 minutes.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

9. AUTOMATED EXTRACTION OF LYSED SAMPLES

9.1. Create the DNA IQ Extraction batch

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

9.2. Locating samples

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

9.3. Sequence checking the Nunc Bank-It™ tubes

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

9.4. MPII Extraction Procedure

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

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

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

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

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

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

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

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

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

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

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

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

9.6. Preparation of reagents for the automated extraction process

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

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

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

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

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

2. Restart or turn on the instrument PC.

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

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

Figure 1. The WinPrep® icon.

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

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

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

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

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

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

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

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

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

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

15. Refer to section 4.1 for the preparation of reagents. Record all lot numbers onto the worksheet and in AUSLAB. Note, for batches of <48 samples, use volumes for 48 samples.

16. Check the syringes and tubing and perform a Flush/Wash if required.

17. Add Wash Buffer to the far right hand side trough of the Amphyl wash station in position **A10**. Add Lysis Buffer to the labelled 150mL reagent trough on the left hand side of the 2 trough holder in position **A13**.

18. Place the 12 channel plate into position **A16**. Add Elution Buffer to the plate by dividing the Elution Buffer between channels 11 and 12. Add Resin to channel 1. It is important to add the resin in a uniform fashion to ensure equal distribution of resin along the channel.

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

    a. Add a B1-Lite generated **'LYSATE'** barcode on the **right hand side** of the Nunc™ Bank-It™ tube rack.
    b. Place the rack onto the MixMate to shake for 1 minute at 1000rpm.
    c. Centrifuge the rack at 3000rpm for 2 minutes in the Eppendorf 5804 centrifuge and then place into position **C13**.

    Note: Do not uncap lids until prompted by program.

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

21. **2mL 96-deep well storage plate:** Label the **left hand side** of the plate with both the correct **AUSLAB batch ID** and **batch ID barcode**. Label the **right hand side** of the plate with a B1-Lite generated "**STORE**" barcode. With a marker, print the word "FRONT-store" on the front of the plate. Place the plate in its correct orientation in position **E16**.

22. **Nunc Bank-It™ extract tubes:** Ensure that the rack is labelled with the correct **AUSLAB batch ID** on the **front** of the Nunc™ Bank-It™ tube rack. Label the **right hand side** of the plate with a B1-Lite generated "**EXTRACT**" barcode. Additionally, randomly check some visible barcodes on the side of lysate tubes against the positions on the worksheet to ensure the correct batch of samples is to be processed. Place the rack in position **G16**.
    Note 1: Do not uncap lids during this step.
    Note 2: If B1-Lite generated barcodes are not available hand-write the labels.

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

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

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

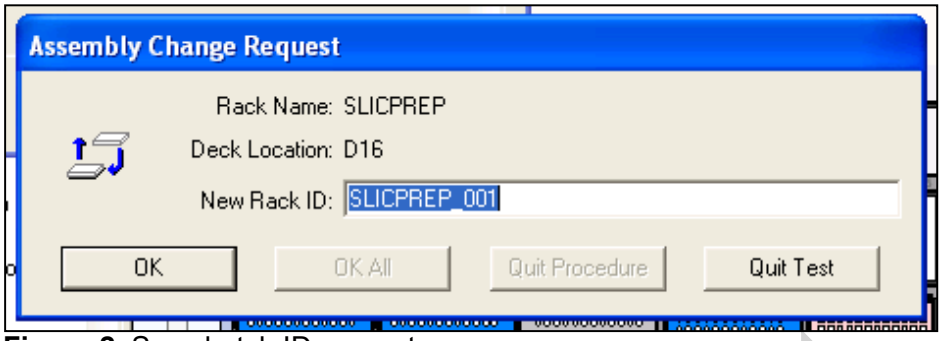


Figure 2. Scan batch ID request

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

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

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

28. For a full batch of 96 samples, ensure that all nodes are checked. For a batch of 48 samples or less, uncheck the node: "Add resin to second half of plate". Click "**Next**" to check all other nodes.

29. Click "**Start**" to continue.

30. The MPII instrument will proceed to scan the required plates on the platform deck in the below order. If barcode reading fails or if B1-Lite barcodes are not available (and hand-written labels have been used), the user is prompted to enter a plate ID. A plate ID can be entered manually into the "Read failed" prompt window for:
    a. Nunc extract tubes, type in **EXTRACT** and press **"Enter".**
    b. 96-deep well storage plate, type in **STORE** and press **"Enter".**
    c. Nunc lysate tubes, type in **LYSATE** and press **"Enter".**

31. After the plates have been identified, two user prompts will appear as a reminder to confirm the deck setup. **Always decap tubes from positions H1 to A1, H2 to A2 etc.**
    a. Ensure all steps on the first prompt have been complete, Click **OK** to continue**.**
    b. Ensure all steps on the second prompt have been complete, Click **OK** to continue**.**

32. The program will progress to transfer the lysates followed by automated addition of the Lysis-DTT buffer and the DNA IQ™ Resin solution. The next user prompt will appear. Follow the steps as outlined in the user prompt. Then click **OK** when ready.
    Note: Ensure that plate is sealed properly with the Pierce Seal. Once the Pierce Seal film is pierced, the PCR Microplate is then discarded (new plate used each time).

33. Once lysis steps have been completed, a user prompt will appear. Follow the directions as outlined in the user prompt. Then click **OK** when ready.
    Note: Nunc lysate tubes (including the rack) should be placed into a clipseal bag, sealed and discarded into a biohazard waste bin.

34. Once the wash steps have been completed, a user prompt will appear. Follow the directions as outlined in the user prompt. Then click **OK** when ready.
    Note: The Nunc extract tubes can be decapped on the bench in the MPII cabinet while the samples are drying and the heating tile is stabilising. At this point, the operator has approximately **12 minutes** to decap the tubes. If the operator has been interrupted and requires more time to decap the tubes, the program may be paused at this step.

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

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

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

38. Once all plates are removed from the deck and sealed, place into a clipseal plastic bag. Click "**OK**" to proceed to the Amphyl wash step.
    Note: Before placing the supernatant storage plate into a clipseal bag, check the plate for the transfer of beads. To do this, place the plate onto the ABI magnet (if beads are present they will settle to the bottom of the plate) and then inspect the bottom of the plate. If beads are present refer to the Section 15, Troubleshooting.

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

9.8. Finalising the MP II Run

1. Transfer left over Resin solution from the 12 channel plate and the Lysis Buffer-DTT (wearing safety glasses) into the glass Lysis-DTT bottle previously used. Discard the 12 channel plate in the biohazard waste bin. Take the bottle to room 6122 and transfer left over reagents into the brown Winchester bottle located in the fume hood.
2. Discard the contents of the Amphyl wash station into the sink and rinse the troughs with Nanopure water.
3. Remove all labware from the deck and clean with 5% TriGene™ followed by 70% Ethanol, and setup for the next run if necessary. In addition, clean the work area around the MPII instrument with 10% (v/v) Bleach 7x and 70% Ethanol.
4. Remove the tip chute and funnel, rinse with warm tap water to remove any residue inside the chute before cleaning with 5% TriGene and 70% Ethanol.
5. Move the platemap to **C:\PACKARD\EXT PLATE MAPS**\Completed Extractions.

9.9. Importing MP II Log File into AUSLAB

1. Click on the Microsoft Access icon in the WinPrep® main menu to open the MultiPROBE log database.
2. Click on the relevant run Test ID in the Test Selection box. In the Report/Query/Action Selection dropdown menu, select "Report: Test Summary (Sorted by Destination Rack ID)"
3. In the Output Selection dropdown menu, select "File". Save the output file as *.csv format to **C:\PACKARD\EXT PLATE MAPS\EXT LOGS** with the same name as the AUSLAB batch ID and click "**Apply**".
4. Open the log file and check for any errors that may have arisen during the extraction process. Compare the listed errors to any that were encountered during the run. Report any critical errors to the line manager.
5. Copy the log file to **I:\EXTRACTION\EXT A MPII\LOGS or I:\ EXTRACTION\EXT B MPII\LOGS** for uploading to AUSLAB.

6. Import the log file, entering the path, filename and extension (e.g. I:\EXTRACTION\EXTA MPII\Logs\CWIQEXT20071115_01.csv) and press **[Enter]**. For more details on importing files into AUSLAB refer to Batch functionality in AUSLAB SOP (QIS 24469).

9.10. Importing Extraction "results" into AUSLAB

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

9.11. Sample Storage

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

10. TROUBLESHOOTING

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

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

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

11. VALIDATION

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

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

12. QUALITY ASSURANCE/ACCEPTANCE CRITERIA

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

13. REFERENCES

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 37ºC. 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 37ºC. 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).

CaSS | Forensic and Scientific Services
A CLINICAL AND STATEWIDE SERVICE

Procedure for the Release of Results

1 PURPOSE

1 To describe the correct format for statements or reports issued from DNA Analysis.

2 To document the procedures for issuing reports within DNA Analysis.

3 To document workflows leading to the releasing of information via Exhibit Reports to the Queensland Police Service.

2 SCOPE

1 This standard operating procedure relates to all statements or reports issued by case analysts to clients.

3 REFERENCES

1 NATA Accreditation Requirements, Supplementary Requirements for Accreditation in the Field of Forensic Science.
2 Evidence Act 1977

4 ASSOCIATED DOCUMENTS

10623	FSS – Laboratory Report Format, Content and Handling
16004	AUSLAB Users Manual – Forensic Biology
22619	Uploading Profiles to NCIDD, Creating and Reviewing Links
23008	Explanations of EXR/EXH Results
23968	Result Communications Procedure
24015	Interstate/International Requests
17117	Procedure for Case Management
26874	Procedure for Paperless Case Management and Review

5 DEFINITIONS

EXR/EXH – Exhibit Report.

6 PRESUMPTIVE EXHIBIT REPORTS

1 The formats of the accepted EXR/EXH comments are located in 23008 Explanations of EXR/EXH results.
2 A Presumptive EXR/EXH should include the following information:

a. **Overall Status**: This should reflect the result. This does not apply to EXHs.

i. Negative (Forensic Value) – Used for items that are examined but not submitted for testing.
ii. Negative (Not examined) – Used for items that are received but not examined.
iii. Not Received at FSS – Used for items that are not received at FSS

iv. Positive (Forensic Value) – Any sample submitted for DNA testing will have this status result.

b. **Lab No**: The results are reported under the individual sub-sample. Refer to Appendix 11 for specific guidelines.

c. **Result Status** – All result options are available using the F1 lookup function. The results status should reflect any presumptive & confirmatory tests that were conducted and include whether the sample was submitted for DNA testing.

Example 1: If a TMB test was performed that was negative and the swab was submitted as cells but also had a hair attached which was observed under microscopy as not suitable for DNA testing the following lines would be entered:

234967280	Presumptive blood test neg. Submitted as cells.
234967280	Hair located. Not suitable for analysis

Example 2: If different testing was performed on 2 sub-samples with a positive TMB test recorded for the first which was submitted and both an AP pos and the presence of spermatozoa detected by microscopic examination on the second the following lines would be entered:

234967280	Presumptive blood test pos. Submitted – results pending
234967281	Presump sem fluid test pos. Submitted results pending.
234967281	Micro positive for sperm. Submitted-Results pending.

d. Linked No and Warm Link name are not required for presumptive EXR/EXHs

7 FINAL EXHIBIT REPORTS

1. The formats of the accepted EXR/EXH comments are located in 23008 Explanations of EXR/EXH results.

A Final EXR/EXH should include the following information. Refer to Appendix 6:

a. **Overall Status**: This does not need to be changed from the Overall Status of the Presumptive Exhibit Report. Note, this does not apply to EXHs.

b. **Lab No**.: The sub-sample no. of the results being reported. This should include the results with most evidential value however any previously reported sub-samples (reported by a presumptive EXR/EXH) should also have a result entered.

c. **Result/Status**: A description of the result (9 loci, partial, no DNA profile). All result options are available using the F1 lookup function. There may be more than one EXR/EXH line which is suitable however the EXR/EXH must fully describe the result. For example if there is a major and minor profile an EXR/EXH line must be entered for both the major and minor profiles.

E.g.

234967280	Mixed DNA Profile. Major component uploaded to NCIDD	UKM1
234967280	Mixed profile, minor component insuff for NCIDD matching	UKM2

d. **Linked No**:
   i. Evidence Sample: The barcode no. of the evidence sample that matches the profile.
   ii. No Evidence Sample: If there is no evidence sample then the profile will be unknown. UK should be used to designate an unknown with F (female) or M (male) used to provide further information. UKP should be used if the sex of the DNA profile is unable to be determined. 1 should be used to denote the first male, female or person profile obtained.

      1. Example: 3 different male profiles would each be reported on a different line with UKM1, UKM2 & UKM3 used to distinguish between the contributors.

      2. Example: A single (1) unknown male would be reported as UKM1.

e. **Warm Link Name**:

   i The name of the evidence sample the profile matches to.
   ii If there is no evidence sample this field is not used.

3. **Suspect Checks** – These are only reported in a final EXR/EXH if they do not match. Any matches are reported via LKRs (22619 Uploading Profiles to NCIDD, Creating and Reviewing Links).
   a. Suspect checks are nominated by the FSS Scientific Services Liaison Unit (SSLU) or from QPS DNA Unit. This information may be found in the UR notes (this must always contain the barcode) and the CS screen (Sus Chk).
   b. A physical comparison of the nominated suspect profile and the crime scene profile. If the profiles match the crime scene sample must be uploaded to NCIDD and the match is recorded via a cold link. No information is released to QPS.
   c. If the profile does not match the result must be recorded in an EXR/EXH
      i. Result/Status: "Suspect Check Actioned – No Match"
      ii. Linked No.: Barcode of the nominated suspect.
      iii. No names are entered.
   d. If there is a suspect check match and the DNA profile is less than the stringency for searching on NCIDD, an Intelligence Report should be issued to QPS Forensic Intelligence Results Management Unit.

4. **Interstate/International Requests** – Refer to 24015

8 STATEMENT OF THE WITNESS

1 AUSLAB TEMPLATE

(i) In the format of a Statement of Witness (Refer to Appendix 1 Example of 1st page)
(ii) With footer on each page that lists the page number, total number of pages and the case reference number, date, name and signature of reporting scientist authorising the statement.
(iii) With appendix that lists test methodology (Refer to Appendix 2).
(iv) With signed Justice's Declaration Act (Refer to Appendix 3).

2 STATEMENT REQUIREMENTS (AUSLAB Test Code: FBSTAT):

i Declaration & Details of the Reporting Scientist (Name, State)
ii Place of Employment (FSS, Forensic Sciences, DNA Analysis)
iii Qualifications held by the Reporting Scientist (B.Sc.)

iv ANZFSS Code of Ethics (if applicable)
v Offence details including Defendant and Complainant details
vi Details relating to the receipt of items & reference samples including the date of receipt, the delivery officer and the method of item receipt (e.g. courier, hand delivered, and mail). A list of the items received with sufficient description to identify the item source.
vii Summaries/ Preambles may include the following (see Appendices 12-14):
- The Role of a Forensic Biologist
- Examinations (if performed by another analyst)
- DNA Profiling
- Mixed DNA Profiles
- Blood Stains
- Seminal Stains
- Saliva
- Semen Staining on Items
- Persistence of Semen in the Vagina
- Statistics

viii A summary of test results of the Reference Samples
ix Description and Results of each of the Items including:
- Description of the Item including barcode information e.g. 123456789. Receipt sub-numbering e.g. 987654321-002 is optional.
- Condition of the Item
- Area of staining
- Areas submitted for testing
- Results obtained (Did it match the reference sample, probability of a match) Where relevant, opinions, explanations for opinions and interpretations or summary. A statement of uncertainty where relevant. Reference to other information which may be relevant to the validity or application of the results, e.g. in support of an opinion, explanation or statement of uncertainty.

Note: If a summary of results is required, it should be included at the beginning of the result section of the statement.

x All items received but not tested are listed
xi Signature of the reporting scientist (at the end of the results)
xii Appendix including information about:
- Statement
- Case Prioritisation
- Procedures
- Methodology
- NATA

xiii Justices Act 1886 – Signature of Reporting Scientist required. The Justices Act must not be on a page by itself. The number of pages to be written within the Justice's Act should be the same as the number of pages for the whole statement.

3 SUBSEQUENT / ALTERNATIVE STATEMENTS:

i **Addendum Statement (AUSLAB Test Code: FBADD)** - If a subsequent statement is issued (This may be due to additional exhibits being delivered or an additional request for further interpretation), it must be clearly marked as an addendum to the original statement.

ii **Amended Statement (AUSLAB Test Code: FBAMD)** - If, after the issue of a statement, an error is detected, the original statement shall be withdrawn and, where

necessary replaced by one which is clearly indicated as being a replacement statement.

iii **Linked Cases / Other (AUSLAB Test Code: FBSHRT)** – If a statement is required for multiple CRISP/ Occurrence no's this statement may be used. This statement does not automatically generate the receipt information contained at the start of FBSTAT. This test code is also used for pre-Auslab cases and other cases that feature manual receipts.

iv **Intelligence Reports** – If there is information that cannot be included in a statement for evidentiary reasons an intelligence report may be produced. This report type shall be approved by the Managing Scientist, Team Leader or Senior Scientist prior to drafting the report. (This would be a rare event). It should follow the format detailed in Appendix 4. Matches on NCIDD that are below our standard match reporting stringency can be reported to FIRMU via intelligence reports. Refer to Appendix 5. These reports must go through the same peer review process as required for all results released from the laboratory. This report shall be scanned into AUSLAB, or uploaded as part of FBIOLW testcode.

v **DNA Evidentiary Certificates** – (Refer to Section 95A Evidence Act 1977)

This is a certificate (in an approved form) that must be signed by a DNA Analyst.

Current staff that hold appointments (Section 133A) as DNA Analysts are held with the Managing Scientist.

It states that any of the following matters is evidence of the matter:

- Receipt and testing of the item/s
- Stated DNA Profile
- That the DNA Analyst examined the records relating to the receipt, storage and testing of the item/s in relation to the matter including any test process that was carried out by someone other than the analyst
- Confirms that the records indicate that all quality assurance procedures for receipt, storage and testing for the item/s that were in place in the laboratory at the time of the test were complied with.

If an Evidentiary Certificate is requested, a workflow has been devised to assist the checking involved in order to sign the certificate (see Appendix 15).

vi **Evidential Reports** – These are used for CMC (Crime and Misconduct Commission Cases). Due to the confidential nature of these cases results cannot be entered into AUSLAB in either EXR/EXH or STATEMENT format (as this information is accessible by QPS and other FSS staff). This report type shall be approved by the Managing Scientist or Team Leader prior to drafting the report. This would be a rare event. Clarification from the requesting party will need to be sought as to the appropriate method of result release.

This report shall be addressed directly to the Investigating Officer and begin with (or equivalent):

" RE : SSFXXXXX (Complainant Jane Smith)"

"I am writing to summarise the results of examination conducted in the DNA Analysis laboratory at Forensic and Scientific Services in relation to the above alleged XXXXXXX incident/s."

This report shall include the following statement elements:

1. Receipt details of reference samples and exhibits
2. Blurbs (Role of a Forensic Scientist, DNA Profiling and appropriate blood or semen blurbs)
3. List of Reference Samples (and results)
4. Results of testing for exhibits submitted
5. Items not examined

And should end with "This information has been peer-reviewed in accordance with standard laboratory Quality Assurance protocols".

This report must go through the same peer review process as required for all results released from the laboratory. This report shall **NOT** be scanned into AUSLAB. **All results are to be included in the case file only.**

4 EXTERNAL TESTING (Example Low Copy No. or Mitochondrial DNA)

i If the results of tests not performed in the laboratory are included in reports, the source of these results shall be clearly and unambiguously identified in the report/statement. (This would be a rare event).

ii If external testing is discussed with the QPS Investigating Officers, these discussions need to be disclosed to the Inspector (or delegate) of QPS Forensic Intelligence and Results Management Unit. Authorisation for external testing must be given and arranged by QPS.

5 RECORDS

i All statements issued must bear a stamp on the front page that lists the date of review, the case analyst's signature and the signature of the analyst who performed the technical review if required.

ii A copy of the statement issued for any test/examination must be retained in the case file. After the statement has been reviewed, F6 to validate will change the statement to PDF format. A time and date stamp will appear in the footer.

iii Further versions can be created of statements and can be viewed in Auslab prior to printing - Press Shift –Insert on the validated statement page (to view PDF Report Table) and F8 to view HTML Report. The original (validated) statement can also be viewed by pressing F5 on this page.

9 STATEMENT/REPORT AUTHORISATION

i Qualified DNA Analysis Reporting Scientists are authorised to sign statements and reports given that all policy and procedure requirements have been satisfactorily fulfilled.
ii All Staff are authorised to sign and initial worksheets, reports etc according to their level of competence.
Iii A staff list with signatures and initials of all staff (17088) is kept for reference. This is located in the Quality area of the Administration compactus.
Iv DNA Analysts can sign Evidentiary Certificates.
v Another scientist with the same or greater level of competence can sign as Peer Reviewer.

10 AMENDMENT HISTORY

Revision	Date	Author/s	Amendments
	24 Feb 1999	V Ientile	
QIS Edition			
1	8 Oct 2001	V Ientile	
2	23 Jan 2004	L Freney	Changes to references, update appendices
3	11 Mar 2004	V Ientile	No interim unchecked results to be issued
4	10 Aug 2006	M Gardam	Combined with 17158, amended the title and updated statement requirements, included intelligence reports, statement blurbs & Evidential Reports. Added Reference to Communication SOP, Added EXR reporting guidelines.
5	31 May 2007	M Gardam	Sub-numbering is optional when giving a description of the item.
6	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
7	August 2009	J Howes	Updated Forensic Biology to DNA Analysis, added EXH, added complete preambles, added Evidentiary Certificate workflow, Quality flag checking workflow, updated Statement of Witness and Appendices examples, DNA Analyst list removed, relative frequency paragraph removed from Intel letter example and updated with match probability, EXH table improvements and current lines added to examples.
8	June 2010	J Howes	Added EB checking workflow, added to Quality Flag workflow, moved Quality paragraphs to own Appendix, deleted Pathology and Scientific services logo

11 DOCUMENTATION

1 **Appendix 1** An example of the format of the front page of a Statement of Witness

2 **Appendix 2** Procedural overview and test methodology (Appendices).

3 **Appendix 3** Example of the Justice's Declaration Act.

4 **Appendix 4** Intelligence Report – Paternity

5 **Appendix 5** Intelligence Report – Below Stringency Links

6 **Appendix 6** Completing Exhibit Reports in AUSLAB

7 **Appendix 7** Review of Exhibit Reports in AUSLAB

8 **Appendix 8** Creating an Addendum Statement in AUSLAB

9 **Appendix 9** Creating a Statement with Receipt Details in AUSLAB

10 **Appendix 10** Creating a Statement without Receipt Details in AUSLAB

11 **Appendix 11** EXR/EXH Reporting (Sub-Sample No. Rules)

12 **Appendix 12** Complete Casework Preamble – Examinations by QHFSS

13 **Appendix 13** Complete Casework Preamble – Examinations by QPS and QHFSS

14 **Appendix 14** Complete Paternity Preamble

15 **Appendix 15** Quality Paragraphs (relating to statements including results of DNAIQ Extractions in period October 2007 – July 2008)

15 **Appendix 16** Evidentiary Certificate Workflow

16 **Appendix 17** Quality Flag Checking Workflow

17 **Appendix 18** Extraction Batch Checking Workflow

APPENDIX 1

Queensland Government
Queensland **Health**

Queensland Health Forensic and Scientific Services
Forensic Sciences

NATA
WORLD RECOGNISED ACCREDITATION

STATEMENT OF WITNESS

39 Kessels Road
COOPERS PLAINS QLD 4108
Phone : (07) 3274 9000
Fax : (07) 3274 9174

QUEENSLAND)
TO WIT)

Our Reference : QPS070148906

I, Clint Justin EASTWOOD, of Brisbane in the State of Queensland, do solemnly and sincerely declare that:-

1. I am employed by Queensland Health Forensic and Scientific Services within Forensic Sciences at Coopers Plains, Brisbane.

2. I am a Scientist in the DNA Analysis Unit.

3. I hold Bachelor of Science and Bachelor of Arts degrees from the University of Queensland.

4. I hold a Masters of Science in Forensic Science from Griffith University.

5. I am a member of the Australian and New Zealand Forensic Science Society and I am bound by the Society's Code of Ethics.

6. This is my statement in relation to the alleged offence that CRISP Number 12/3456789 refers. The defendant in this matter is Alleged Shady CHARACTER. The complainant in this matter is Alleged Unfortunate PERSON.

APPENDIX I

Procedural overview for the Forensic Biology laboratory of Queensland Health Forensic and Scientific Services (QHFSS)

Item Prioritisation

All items submitted in relation to a particular case are prioritized by both the Queensland Police Service and the case scientist, based on their understanding of the case circumstances and the evidentiary value of the item. For example, an intimate medical swab or a pair of underpants will invariably hold more evidential value to a sexual assault investigation than a pair of shoes.

Once biological fluid staining has been detected on an item, the number of samples submitted for DNA analysis will depend on the following factors:

- Whether or not it is thought that more than one person was involved in the incident
- The location of the sample / stain
- The likelihood of obtaining a DNA profile from this type of sample.

Laboratory examinations and subsequent analyses and interpretation have been conducted with the assistance of other qualified scientists as per the standard operating procedures held in the Quality Information System.

Forensic biology case files and any samples remaining are available for independent examination at the laboratory upon request and in accordance with the normal legal rules of disclosure.

Chain of Custody

All forensic biology case files and exhibits are electronically tracked and monitored to ensure that the appropriate chain of custody and continuity measures are maintained. In addition, the packaging of any items that are examined, are signed and dated by the person currently in possession of them and performing the examination. It is the possessor's responsibility to ensure case files and items are secure at all times.

Entry into the laboratory is restricted to authorized persons only via electronically encoded access keys.

Accreditation

The forensic biology laboratory is accredited by the National Association of Testing Authorities (NATA) to conduct forensic DNA analyses. Accreditation provides an internationally recognised means of evaluating the competence of a laboratory to perform specific tests and allows these facilities to determine whether they are performing their work correctly and to appropriate standards. The parameters assessed during accreditation are:

- Organisation and management
- Quality management system
- Personnel
- Evidence management
- Methods and procedures
- Quality assurance equipment
- Reports
- Procurement of services and supplies
- Accommodation and safety

NATA accredits laboratories against criteria based on the internationally recognised standard ISO / IEC 17025:1999. Laboratories that demonstrate compliance with ISO / IEC 17025 at assessment have therefore demonstrated they operate using sound management practices and are technically competent to perform the tests for which they hold accreditation. This Laboratory obtained NATA accreditation in 1998 and is reassessed by NATA at least every two years. This laboratory's NATA Corporate Accreditation number is 41. The NATA laboratory site number is 14171.

APPENDIX II

Technical information relating to DNA profiling at the Forensic Biology laboratory of Queensland Health Forensic and Scientific Services (QHFSS)

Deoxyribonucleic acid (DNA) is the blueprint for life. It carries the genetic information that governs a person's physical characteristics. Except for identical twins, each person's DNA is unique, although the technology currently utilized does not allow the examination of every single difference between people. The technology does however allow the analysis of regions in the DNA which are highly variable between people. These variable portions of the DNA are what enable forensic laboratories to use DNA as a forensic tool.

All samples submitted to this Forensic Biology laboratory are analysed by a method of DNA analysis known as Short Tandem Repeat (STR) profiling. This technique involves the analysis of areas of short, repeated regions which vary in length between individuals. A method known as the Polymerase Chain Reaction (PCR) is used to amplify these specific regions of the DNA and produce numerous copies. In this way, minimal amounts of DNA isolated from small or degraded samples can be increased to a level where they are able to be detected, profiled and compared with other samples.

The STR profiling technique used at QHSS amplifies 10 regions of the DNA (loci), 9 of which contain an STR. For a list of the loci which have been used for samples for this case, refer to Table I, Appendix III. This process is combined with a gender test, which indicates whether the DNA in a particular sample originated from a male or female. The individual components of a DNA profile are represented as a series of peaks which can be measured and given a designation using standard sizing ladders. A person will have two peaks for each STR, one inherited from their mother and the other inherited from their father, unless the same STR is inherited from both parents, in which case only one peak will be seen.

If the DNA profiles obtained from a crime stain and a person's reference sample (for example from a suspect) are found to be indistinguishable, they are said to match. The word match, matches or matching describes the observation that two DNA profiles (one from a reference sample and one from a crime sample for example) are the same when compared. The term match does not impart increased significance to the result it describes. Evaluating the evidential significance of such a match relies on assessing the probability of obtaining the match if in fact the crime stain DNA had originated from someone other than the suspect. In other words, a DNA profile obtained from a crime scene sample is compared with the DNA profile from an individual. If these two profiles do not match, then that individual cannot be the source of the DNA in the crime scene sample. If the two profiles do match, then one of two possibilities could be true; EITHER the individual in question is the source of the DNA in the crime scene sample OR the DNA in the crime scene sample could have come from another individual with the same DNA profile as the individual in question.

When a DNA profile is obtained from a crime stain, three databases consisting of DNA profiles previously obtained from individuals from the Caucasian, Aboriginal and South East Asian populations are used to calculate the probability of another individual having the same DNA profile as the crime stain. The proportion of each DNA component in the crime stain is estimated and

combined to provide a statistical probability that another person would have the same DNA profile by chance.

If more than one person has contributed DNA to a crime stain, the resulting DNA profile is referred to as a mixed DNA profile. In order to assess the evidential significance of the mixed DNA profile a set of hypothesis are considered. These are referred to as Likelihood Ratios. The Likelihood Ratio is based on a calculation of the relative weights of two hypotheses for example:

Hypothesis 1 : the DNA has originated from the victim and another unknown person
Hypothesis 2 : the DNA has originated from two unknown people

The Likelihood Ratio can provide some expectation as to the number of individuals that may have the profile in the population but this is far from being a certainty. The Likelihood Ratio should be assessed in conjunction with the other evidence in the case.

The statistical figure applied to DNA profiles will depend on how closely related people are. The more closely related they are, like brothers for example, the greater the chance that they will have similar DNA profiles. However, due to the random fashion in which DNA from parents combines, the probability that two siblings would share the same 9 STR regions would be very small. As this relationship gets more distant, the probability of two relatives having the same DNA profile becomes smaller still. If it is thought that a relative may have been involved a more meaningful approach would be to submit the relative sample for analysis and comparison to the crime stain DNA profile.

APPENDIX III

Table 1: Loci used in STR Profiling in Forensic Biology, QHFSS

Profiler Plus system

Abbreviated Name	Scientific Name	Chromosomal Name
D3	D3S1358	3
vWA	HUMVWFA31/A	12
FGA	HUMFIBRA	4
Amel	AMELOGENIN	Sex X and Y
D8	D8S1179	8
D21	D21S11	21
D18	D18S51	18
D5	D5S818	5
D13	D13S317	13
D7	D7S820	7

Table 2: Loci used in STR Profiling in Forensic Biology, QHFSS
Cofiler System

Abbreviated Name	Scientific Name	Chromosomal Name
D3	D3S1358	3
D16	D16S539	16
Tho1	Tho1	11
TPOX	TPOX	2
CSF	CSF	5
D7	D7S820	7
Amel	AMELOGENIN	Sex X and Y

APPENDIX 3

JUSTICES ACT 1886

I acknowledge by virtue of S.110A(5)(c)(ii) of the Justices Act 1886 that:-

(i) This written statement by me dated XXXX and contained in the foregoing pages numbered 1 to XXXX is true to the best of my knowledge and belief; and

(ii) I make this statement knowing that, if it were admitted as evidence, I may be liable to prosecution for stating in it anything that I know is false.

.....

Signature

Signed at Brisbane on XXXX, 2009.

APPENDIX 4

Enquiries to:
Telephone:
Facsimile:
File Ref:

<Insert date>

<Insert DRMU Officer's name>
QPS DNA Results Management Unit
[redacted]

THE INFORMATION CONTAINED WITHIN THIS LETTER IS PROVIDED FOR INTELLIGENCE PURPOSES ONLY AND CANNOT BE INCLUDED IN AN EVIDENTIARY STATEMENT.

Dear <Insert officer's name>

RE: CRISP/ OCCURRENCE <insert #> Alleged <insert alleged offence> of <insert name>.

I am writing to advise that in accordance with direction received from <insert officer who requested the action> (QPS), I have compared the DNA profile obtained from the intelligence sample of <insert name>, <barcode #> against the DNA profiles obtained from <insert sample type> of <insert name> and the <insert sample type> registered under Exhibit # <insert lab #> in order to determine paternity.

I advise that at this stage I cannot <insert either include or exclude> <insert name> as a potential parent of the sample. This is based on DNA analysis across the standard 9 DNA systems, in which <insert name> <insert possesses or does not possess> all of the obligate paternal alleles. Further testing across an additional 4 DNA systems can also be performed in this laboratory if required.

An evidentiary reference sample from <insert name> should be obtained and submitted to the laboratory if this information is required in a statement for court purposes.

This information has been peer-reviewed in accordance with standard laboratory Quality Assurance protocols.

Yours sincerely

<Insert scientist name>
<Insert position>
DNA Analysis
Forensic and Scientific Services

[redacted]

APPENDIX 5

DNA Analysis
Forensic and Scientific Services
<Insert Date>

THE INFORMATION CONTAINED WITHIN THIS LETTER IS PROVIDED FOR INTELLIGENCE PURPOSES ONLY AND CANNOT BE INCLUDED IN AN EVIDENTIARY STATEMENT.

ATTN: <Insert DRMU Officer's Name>
Forensic Intelligence Results Management Unit
Queensland Police Service

Re: Comparison of DNA profile from <Insert Name> <Insert barcode #> to the crime scene profile from <Insert CRISP/ OCCURRENCE #>.

In response to the above request, I have compared the DNA profile obtained from the intelligence sample for <Insert Name> <Insert barcode #> against the partial DNA profile (at 5 STR loci) obtained from the scene sample of <Insert CRISP/ OCCURRENCE #>. This partial DNA profile is below our standard DNA match reporting stringency used for the National Criminal Investigation DNA Database.

The DNA profile from <Insert barcode #> matched the DNA profile from the crime scene sample at the 5 available loci. Therefore, the DNA on the scene sample could have come from <Insert barcode #> or from another person who has the same DNA profile at the 5 available loci.

It is estimated that the probability of this DNA profile occurring, had the DNA come from someone other than and unrelated to <Insert barcode #> is approximately one in yyyyyyyyyy using Queensland Caucasian data.

An evidentiary reference sample from <Insert Name> should be obtained and submitted to the laboratory if this information is required in a statement for court purposes.

This information has been peer-reviewed in accordance with standard laboratory Quality Assurance protocols.

Regards

[redacted]

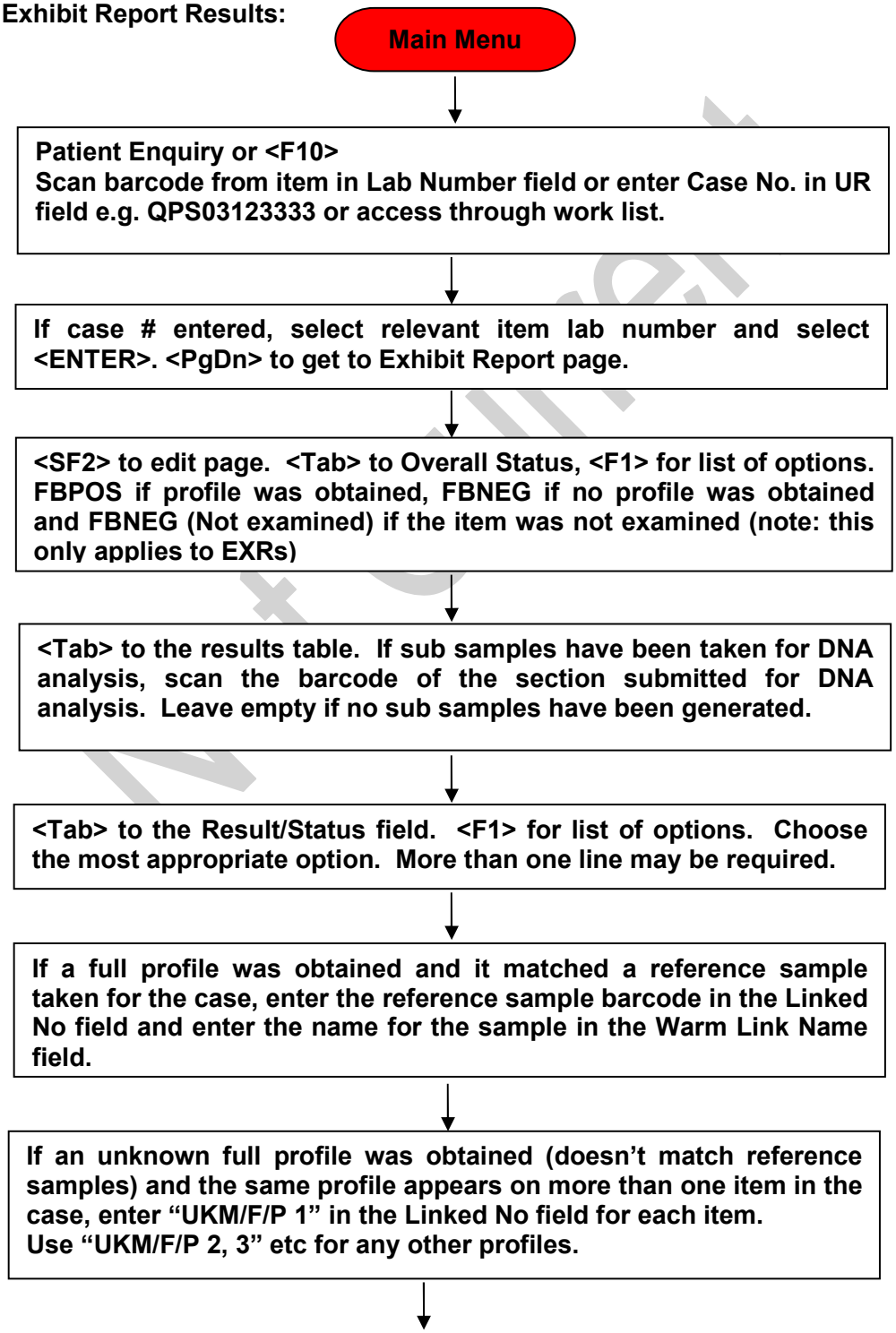
APPENDIX 6

Completing Exhibit Reports

AUSLAB Test Code: EXR/EXH

Purpose: Exhibit Reports are a summary of results for each item received. The information stored in the exhibit reports is transferred to the QPS Forensic Register once the results have been checked and validated. Exhibit Reports can contain information about examinations performed, screening test results and DNA profile results. Interim results can be entered and sent to the QPS Forensic Register once they have been validated.

To enter Exhibit Report Results:



If more than one profile was obtained from a single item or multiple items labelled with one exhibit barcode, enter a line for each profile in the exhibit report table. Enter reference sample barcodes or unknown 1, 2 etc in the Linked No field for each line (or each profile obtained).

↓

<F4> to save entry. Do not validate, Results must be checked and validated by the reviewer. Once results are validated, they are transferred to the QPS Forensic Register.

↓

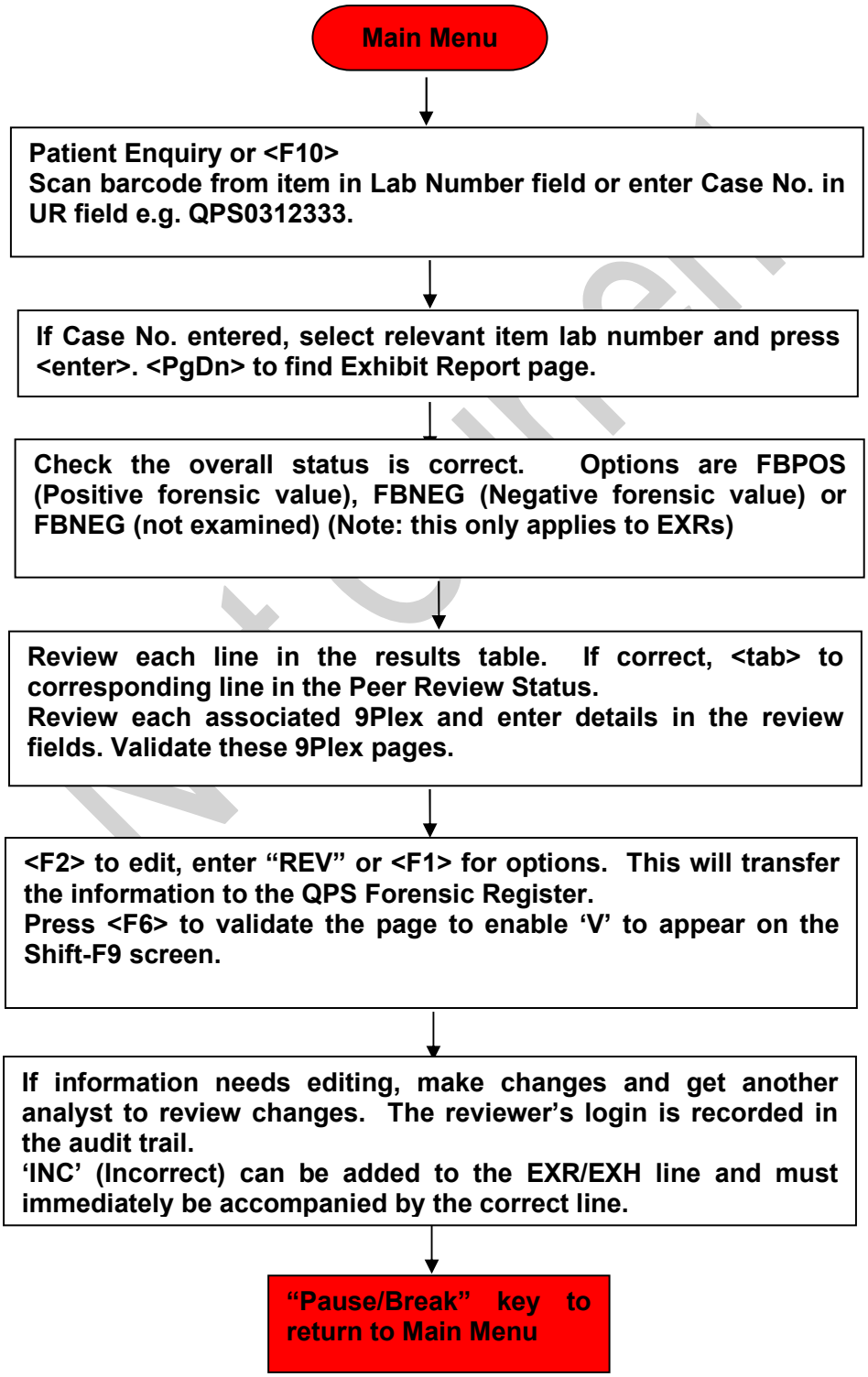
"Pause/Break" key to return to Main Menu

APPENDIX 7

Review of Exhibit Reports

Purpose: An Exhibit report is created for each item as a way of transferring results back to the QPS Forensic Register. Each line of an exhibit report must be reviewed before it can be released and sent to the QPS Forensic Register.

To review an exhibit report:

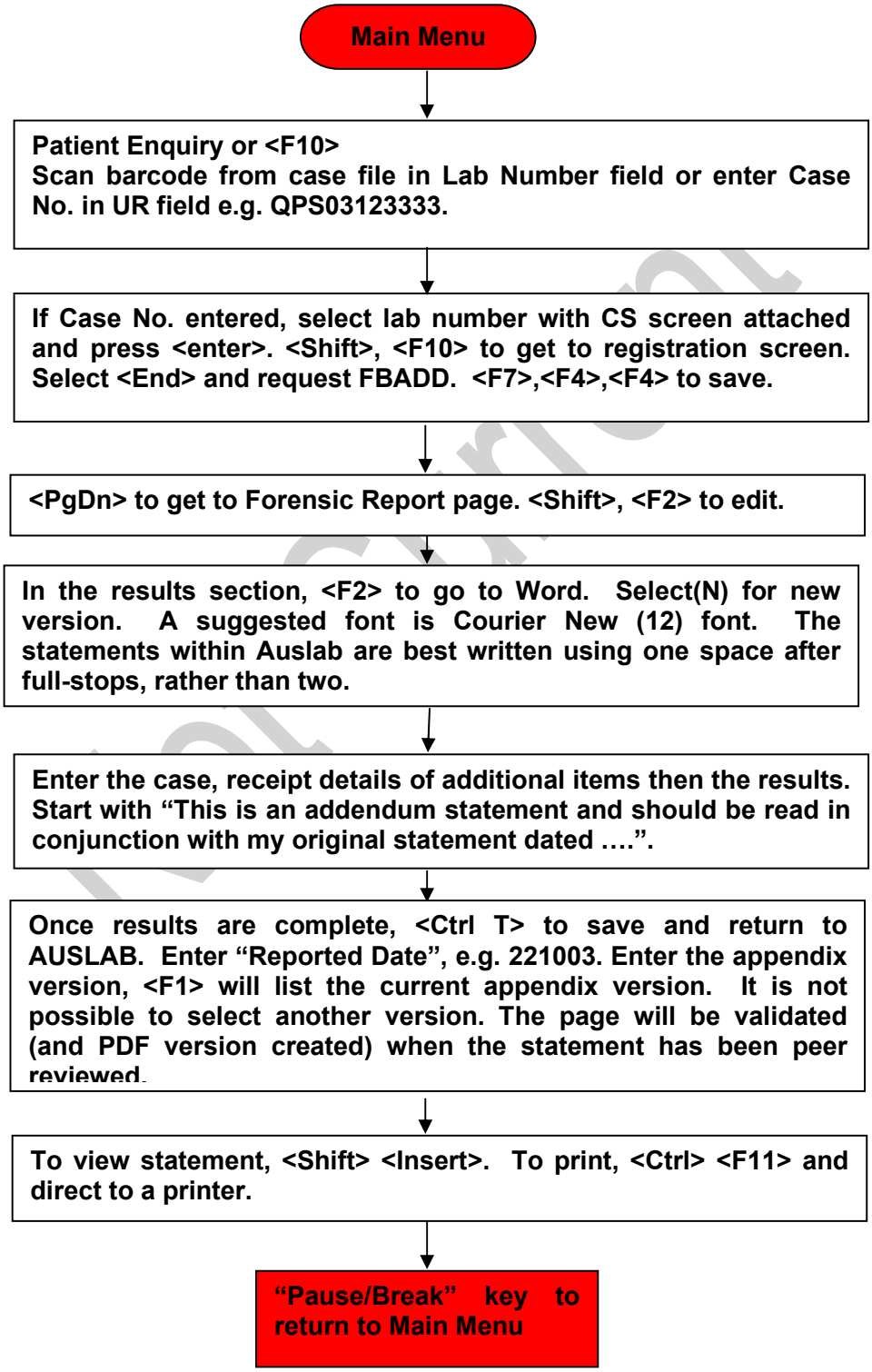


APPENDIX 8

Creating an Addendum Statement

Purpose: The test code FBADD creates a statement without the receipt details automatically entered. All statement test codes include the preamble and appendix details. This format is used for cases where an additional statement is being written.

To create an addendum statement:

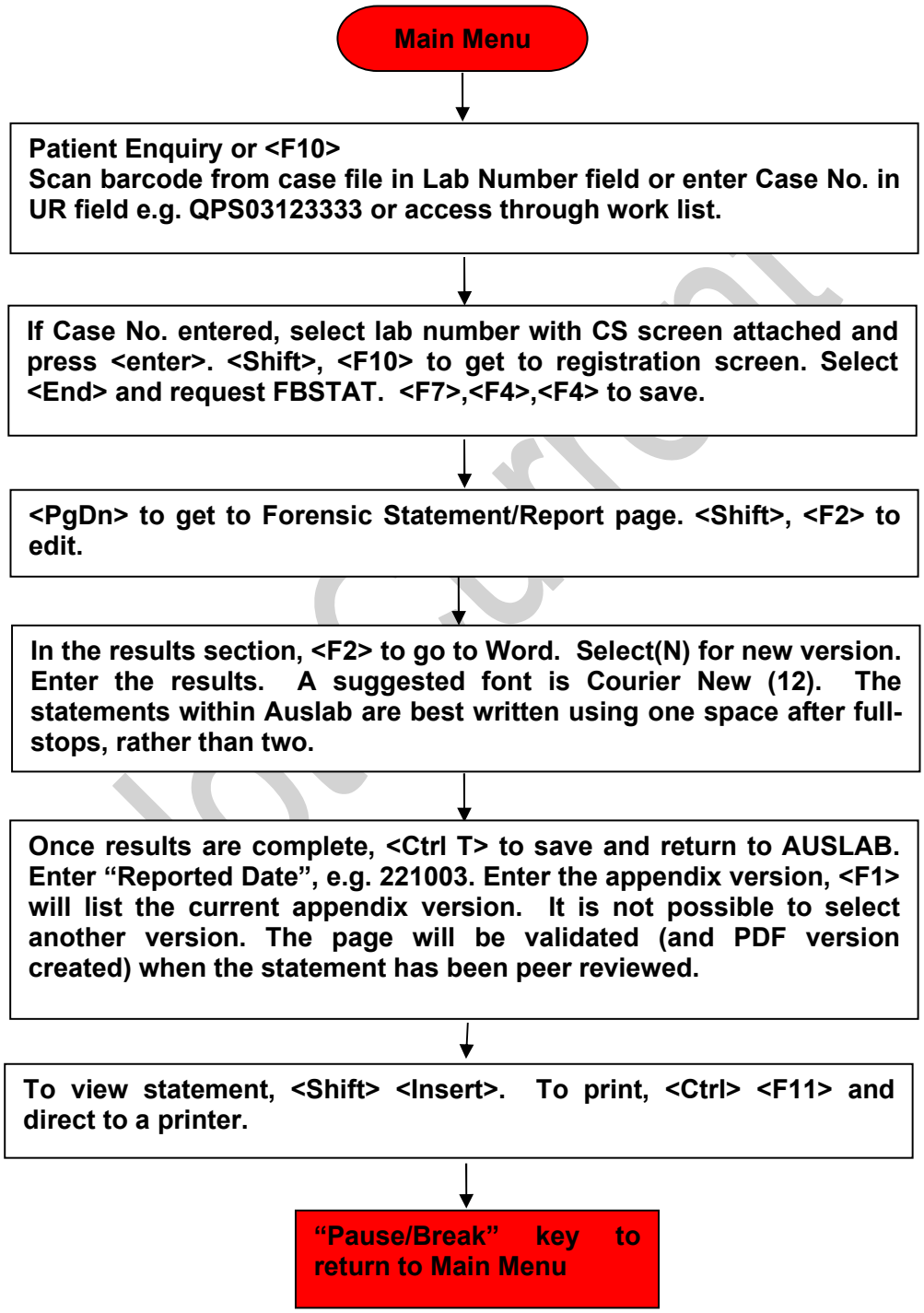


APPENDIX 9

Creating a Statement with Receipt Details

Purpose: The test code FBSTAT creates a statement with the receipt details automatically entered. All statement test codes include the preamble and appendix details.

To create a statement with receipt details:

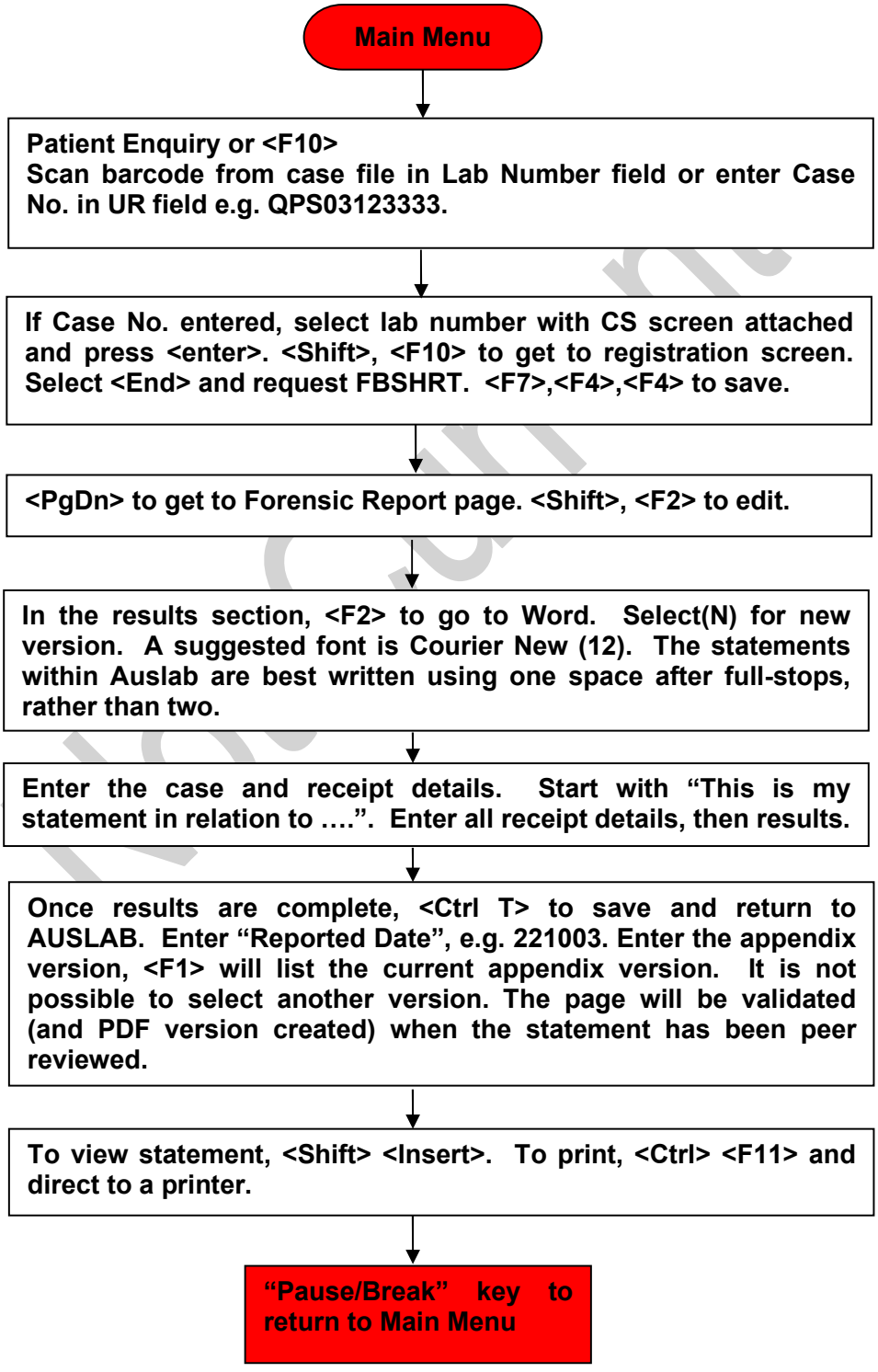


APPENDIX 10

Creating a Statement without Receipt Details

Purpose: The test code FBSHRT creates a statement without the receipt details automatically entered. All statement test codes include the preamble and appendix details. This format is used when statement is being written for more than one case number.

To create a statement without receipt details:



APPENDIX 11

EXR/EXH REPORTING (Sub-Sample No. Rules)

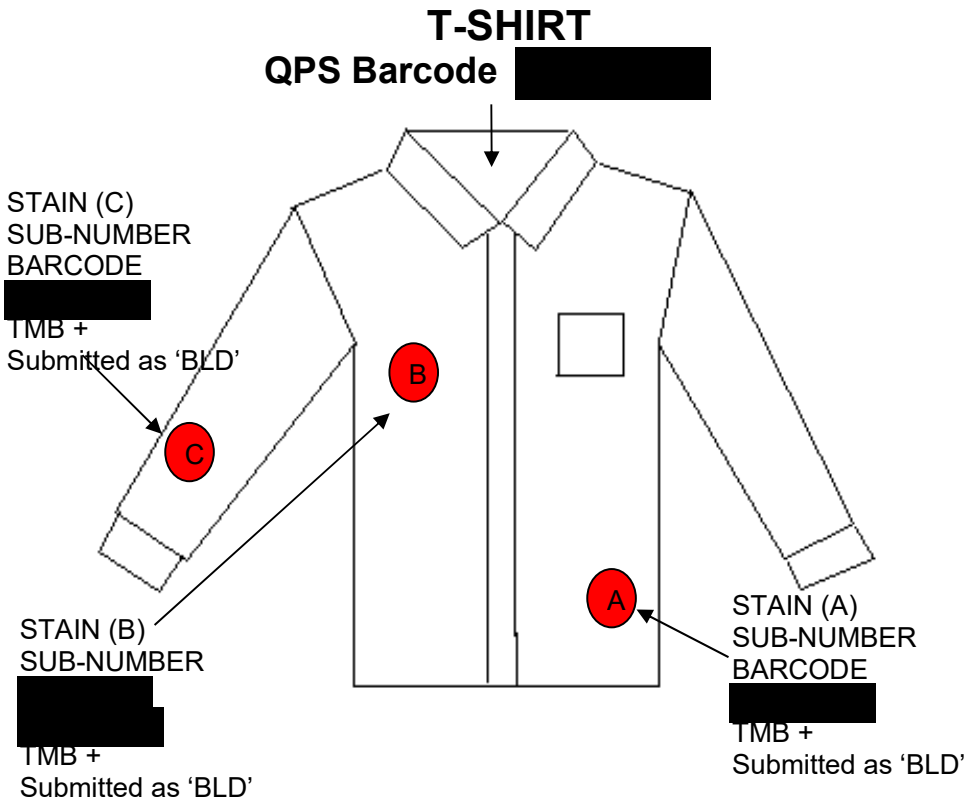
- This appendix is for the process of reporting back of results via EXR/EXHs, to the Queensland Police Forensic Intelligence and Result Management Unit (FIRMU) for individual items by staff of DNA Analysis.

- Since 01 July 2008, the bulk of the examinations have been performed by QPS. After their examinations, samples are received by DNA Analysis in tubes. The barcode on the tubes relate to an EXH barcode and as such, the presumptive and final results are reported back on the single barcode.

- Different scenarios have been included in examples given in the following pages. These scenarios relate to reporting on non-in tube cases, and the table format is as per EXH pages (excluding the 'Peer Review' column). The scenarios include:

1. **One Item – multiple stains** = same presumptive result and only one type of extraction requested.
2. **One Item – multiple stains** = different presumptive results and two types of extractions requested.
3. **One Item – multiple stains** = different presumptive results (but with same extraction request) as well as three differing types of extractions requested.
4. **Swabs** – where no sub-sample barcode is required
5. **Cigarette Butts** – where no sub-sample barcode is required
6. **Multiple items received in a bag**- under one QPS barcode.
7. **Sexual Assault Investigation Kits** (SAIK) & clothing
8. **Sexual Assault investigation Kits** (SAIK) – negative results.

EXAMPLE 1.



EXR/EXH TEST CODE is **registered under 12345-6789 (T-SHIRT)**

ONLY SUB-NUMBERS ARE USED TO REPORT BACK PRESUMPTIVE TESTS & FINAL RESULTS

PRESUMPTIVE TESTS

LAB NO.	Result/Status	Linked No.	Warm Linked Name
[redacted]	Presumptive blood test pos. Submitted – results pending		

Note as all three stains were TMB positive, only one presumptive test result needs to be entered. (Any one of the three sub-numbers for the stains can be entered)

FINAL RESULTS

IF ALL THREE DNA PROFILES ARE THE SAME, THEN ONLY ONE RESULT NEEDS TO BE REPORTED BACK. IF THIS IS THE CASE, THEN USE THE SAME SUB-NUMBER AS USED TO ORIGINALLY REPORT BACK THE PRESUMPTIVE TEST RESULTS.

LAB NO.	Result/Status	Linked No.	Warm Linked Name
[redacted]	Presumptive blood test pos. Submitted – results pending		
[redacted]	9 loci DNA profile	20076738	COOPER

OR

IF THE SUB-SAMPLE ORIGINALLY USED IS NOT THE BEST PROFILE, YOU STILL NEED TO REPORT BACK ON IT – BUT YOU WILL ALSO NEED TO ADD THE SUB-NUMBER WHICH DOES GIVE YOU THE BEST PROFILE.

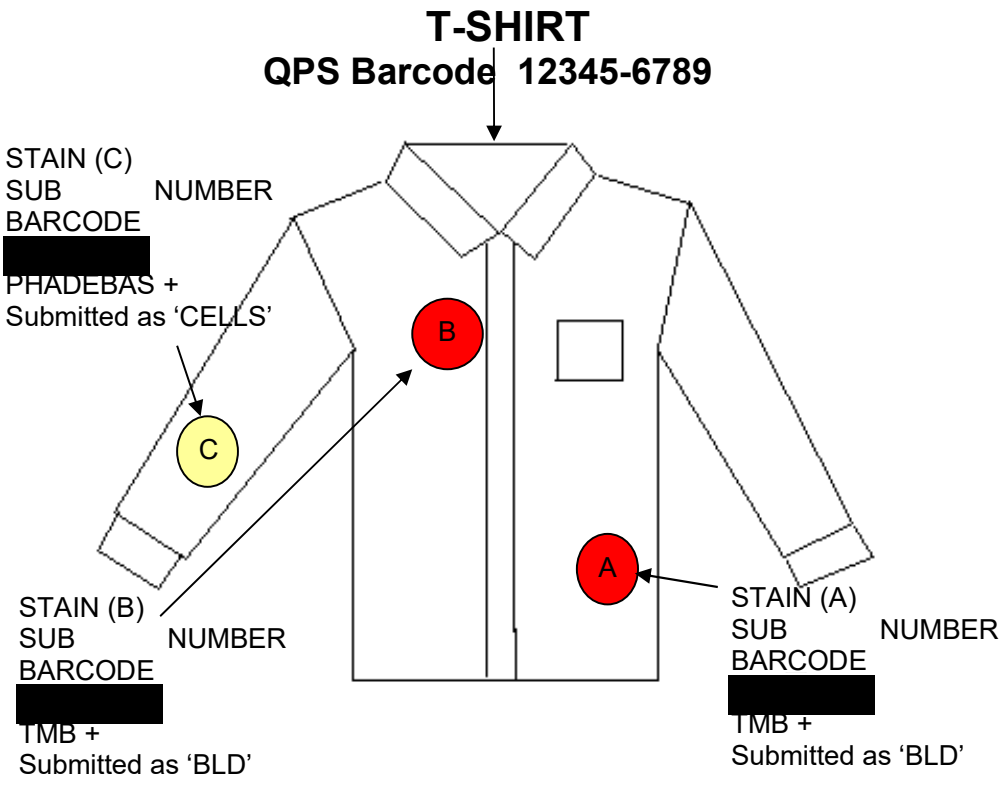
LAB NO.	Result/Status	Linked No.	Warm Linked Name
[redacted]	Presumptive blood test pos. Submitted – results pending		
[redacted]	Partial DNA profile	20076738	COOPER
[redacted]	9 loci DNA profile	20076738	COOPER

OR

IF THERE ARE TWO OR THREE DIFFERING DNA PROFILES RESULTING FROM THE THREE STAINS SUBMITTED FOR ANALYSIS, THEN **REPORT BACK ALL DIFFERING PROFILES** USING THEIR SUB-NUMBER BARCODES (as above).

LAB NO.	Result/Status	Linked No.	Warm Linked Name
[redacted]	Presumptive blood test pos. Submitted – results pending		
[redacted]	9 loci DNA profile	20076738	COOPER
[redacted]	9 loci profile. Uploaded to NCIDD	UKM1	
[redacted]	9 loci profile. Uploaded to NCIDD	UKM2	

EXAMPLE 2.



EXR/EXH TEST CODE is **registered under 12345-6789 (T-SHIRT)**

ONLY SUB-NUMBERS ARE USED TO REPORT BACK PRESUMPTIVE TESTS & FINAL RESULTS.

PRESUMPTIVE RESULTS

LAB NO.	Result/Status	Linked No.	Warm Linked Name
[redacted]	Presumptive blood test pos. Submitted – results pending		
	Presumptive saliva positive. Submitted – results pending		

Note as two stains were TMB positive, you only need one TMB+ presumptive test result to be sent back to QPS FIRMU for this item. Any one of the two sub-numbers for the TMB+ stains can be entered (as above). A second presumptive result is sent back for the Phadebas + result as well.

FINAL RESULTS

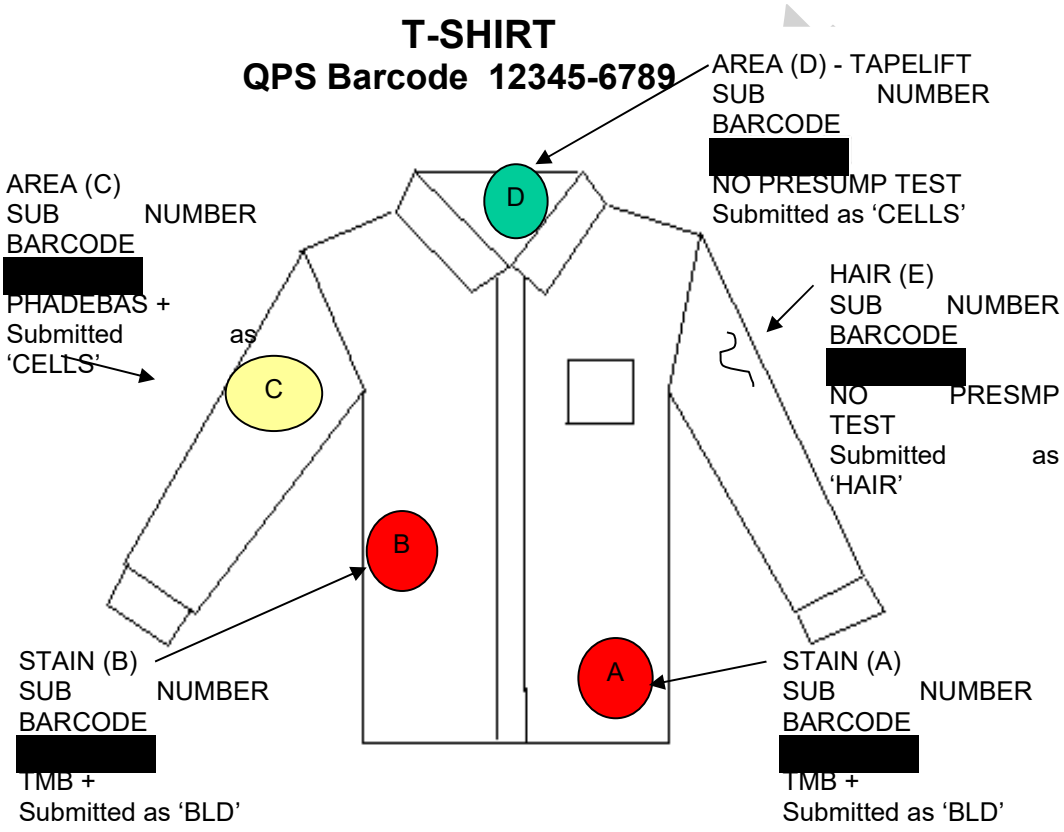
LAB NO.	Result/Status	Linked No.	Warm Linked Name
[redacted]	Presumptive blood test pos. Submitted – results pending		
	Presumptive saliva positive. Submitted – results pending		
	9 loci profile. Uploaded to NCIDD	03091930	ELLIS
	9 loci profile	03091930	ELLIS

[redacted]	9 loci profile. Uploaded to NCIDD	05101929	JEFFREY

AS TWO PRESUMPTIVE RESULTS WERE SENT TO FIRMU INITIALLY, BOTH THE FINAL RESULTS FROM THESE SUB-SAMPLES NEED TO BE REPORTED BACK – REGARDLESS IF THESE PROFILE END UP BEING FROM THE SAME SOURCE. BY DOING THIS FIRMU CAN ASSOCIATE THE RESULTING PROFILES TO A POSSIBLE CELL SOURCE.

IF THE TWO SAMPLES SUBMITTED FOR THE BLOOD EXTRACTION RESULT IN THE SAME DNA PROFILE, THEN ONLY ONE RESULT NEEDS TO BE REPORTED BACK – USE THE SAME SUB-NUMBER AS REPORTED IN THE PRESUMPTIVE TEST RESULTS. IF THE PROFILES DIFFER THEN BOTH ARE REPORTED BACK VIA THEIR SUB-NUMBERS.

EXAMPLE 3



<u>EXR/EXH TEST CODE</u> is **registered under 12345-6789 (T-SHIRT)**

ONLY SUB-NUMBERS ARE USED TO REPORT BACK PRESUMPTIVE TESTS & FINAL RESULTS.

<u>PRESUMPTIVE RESULTS</u>

LAB NO.	Result/Status	Linked No.	Warm Linked Name
[redacted]	Presumptive blood test pos. Submitted – results pending		
	Presumptive saliva positive. Submitted – results pending		

[redacted]		Submitted results pending		
		Hair located. Submitted – results pending		

FINAL RESULTS

LAB NO.	Result/Status	Linked No.	Warm Linked Name
[redacted]	Presumptive blood test pos. Submitted – results pending		
	Presumptive saliva positive. Submitted – results pending		
	Submitted results pending		
	Hair located. Submitted – results pending		
	9 loci DNA profile. Uploaded to NCIDD	22021958	GUGINO
	Partial DNA profile	2021958	GUGINO
	9 loci DNA profile	14041961	ARKIN
	No DNA Profile		
	9 loci DNA profile. Uploaded to NCIDD	UKF1	

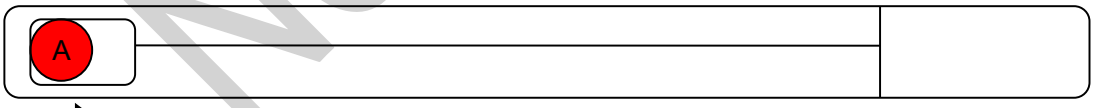
AS FOUR PRESUMPTIVE RESULTS WERE SENT TO FIRMU INITIALLY, ALL FOUR FINAL RESULTS FROM THESE SUB-SAMPLES NEED TO BE REPORTED BACK – REGARDLESS IF THESE PROFILE END UP BEING FROM THE SAME SOURCE. BY DOING THIS FIRMU CAN ASSOCIATE THE RESULTING PROFILES TO A POSSIBLE CELL SOURCE.

IF THE TWO SAMPLES SUBMITTED FOR THE BLOOD EXTRACTION RESULT IN THE SAME DNA PROFILE, THEN ONLY ONE RESULT NEEDS TO BE REPORTED BACK – USE THE SAME SUB-NUMBER AS REPORTED IN THE PRESUMPTIVE TEST RESULTS.

IF THE PROFILES DIFFER THEN BOTH ARE REPORTED BACK VIA THEIR SUB-NUMBERS (as shown above).

EXAMPLE 4.

SWAB - QPS BARCODE 12345-6789 (-001)



STAIN (A)
NO SUB NUMBER BARCODE GIVEN
TMB +
Submitted as 'BLD'

NO SUBNUMBERING REQUIRED FOR THIS ITEM

PRESUMPTIVE AND FINAL EXR/EXH ON SWAB EXR/EXH BARCODE

LAB NO.	Result/Status	Linked No.	Warm Linked Name
[redacted]	Presumptive blood test pos. Submitted – results pending		
	9 loci DNA profile. Uploaded to NCIDD	16031989	ANDREWS

EXAMPLE 5.

CIG BUTT - QPS BARCODE 12345-9999 (-001)

AREA (A)
NO SUB NUMBER
BARCODE
GIVEN
NO PRESUMPTIVE
TEST
Submitted as 'CELLS'

NO SUBNUMBERING REQUIRED FOR THIS ITEM

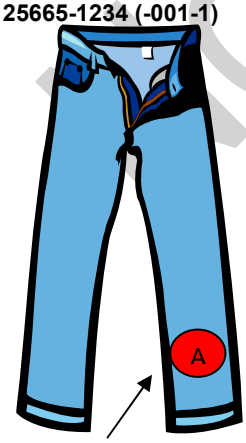
PRESUMPTIVE AND FINAL EXR/EXH ON CIGERETTE BUTT EXR/EXH BARCODE

LAB NO.	Result/Status	Linked No.	Warm Linked Name
[redacted]	Submitted results pending		
	9 loci DNA profile. Uploaded to NCIDD	20071994	CAMERON

EXAMPLE 6.

Bag of clothing (3 items) all received under QPS Barcode 12345-6789 (-001)

Jeans (item) labelled with a sub-number barcode
eg. 25665-1234 (-001-1)

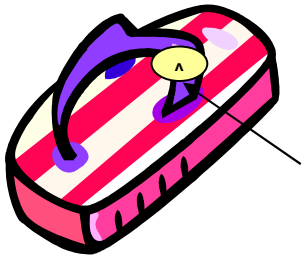


STAIN (A)
SUB NUMBER
BARCODE
2[redacted]
(-001-1-1)
TMB +
Submitted as 'BLD'

Shirt (item) labelled with a sub - number barcode
eg. 25665-4567 (-001-2)

STAIN (A)
SUB NUMBER
BARCODE
[redacted]1
(-001-2-1)
TMB +
Submitted as
'BLD'

T/LIFT AREA
(B)
SUB NUMBER
BARCODE
[redacted]
(-001-2-2)
NO PRESUMP
TEST
Submitted as
'CELLS'



Thong (item) labelled with a Sub Number barcode
eg. 25665-8910 (-001-3)

SWAB OF AREA (A)
SUB NUMBER BARCODE
[redacted]
(-001-3-1)
NO PRESUMPTIVE TEST
Submitted as 'CELLS'

- The **EXR/EXH is registered under** the barcode which QPS has assigned to the **brown paper bag** containing all three items i.e. **EXR/EXH = 12345-6789**

- Whilst **each item** within is given its own barcode, by the case scientist, this barcode is simply to identify the item. Therefore the item is **registered in AUSLAB as an exhibit and given only a FBEXAM test code.**

EG: 12345-6789 (-001) = BAG OF CLOTHING (EXR/EXH)
25665-1234 (-001-1) = JEANS (test code = FBEXAM only)
25665-4567 (-001-2) = SHIRT (test code = FBEXAM only)
25665-8910 (-001-3) = THONG (test code = FBEXAM only)

All results (presumptive and final) for samples submitted for DNA analyses are therefore reported back to FIRMU under the EXR/EXH for the brown paper bag, but **using the sub-sample barcodes of the 9plex requests:** See examples below:

e.g. 25665-0000 (-001-1-1) = TMB+ stain from JEANS 25665-1111 (-001-2-1) = TMB+ stain from SHIRT

EXR/EXH TEST CODE is **registered under 12345-6789 (BAG of CLOTHING)**

ONLY SUB-NUMBERS of THE 9-PLEX REQUESTS ARE USED TO REPORT BACK PRESUMPTIVE TESTS & FINAL RESULTS IN THIS SCENARIO.

PRESUMPTIVE RESULTS

	Result/Status	Linked No.	Warm Linked Name
[redacted]	Presumptive blood test pos. Submitted –results pending		
	Presumptive blood test pos. Submitted -results pending		
	Submitted results pending		
	Submitted results pending		

FINAL PROFILES

Result/Status	Linked No.	Warm Linked Name
Presumptive blood test pos. Submitted – results pending		
Presumptive blood test pos. Submitted – results pending		
Submitted results pending		
Submitted results pending		
9 loci DNA profile. Uploaded to NCIDD	11091977	GARDAM
9 loci DNA profile	11091977	GARDAM
9 loci DNA profile. Uploaded to NCIDD	20071997	JAMES
9 loci DNA profile	200719970	JAMES

IN THIS PARTICULAR SCENARIO, THE EXR/EXH IS REGISTERED UNDER THE 'BAG OF CLOTHING' NOT THE PARTICULAR ITEMS. THEREFORE, THERE IS NO WAY FIRMU WILL KNOW WHICH PRESUMPTIVE RESULT RELATES TO WHICH INDIVIDUAL ITEM. FIRMU WILL RING EITHER THE CASE

SCIENTIST OR THE DNA ANALYSIS RESULT MANAGEMENT SECTION TO RESOLVE SUB-SAMPLE BARCODE IDENTIFICATION, IF THAT INFORMATION IS REQUIRED BY THEM.

EXAMPLE 7.

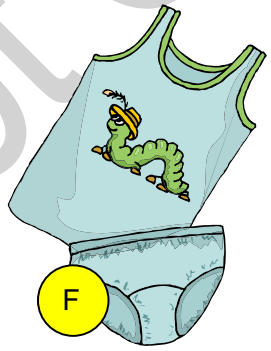
SAIK = QPS BARCODE 12345-6789

SAIK CONTAINS FIVE SWABS and TWO CLOTHING ITEMS (NOT BARCODED BY QPS)

A	SUB-BARCODE – [redacted] AP POS MICRO POS FOR SPERMATOZOA	HV SWAB
B	SUB-BARCODE – [redacted] AP POS MICRO POS FOR SPERMATOZOA	LV SWAB
C	SUB-BARCODE – [redacted] AP NEG MICRO NEGATIVE FOR SPERM	VULVAL SWAB
D	SUB-BARCODE – [redacted] AP NEG MICRO NEGATIVE FOR SPERM	PERIANAL SWAB
E	SUB-BARCODE – [redacted] AP NEG MICRO NEGATIVE FOR SPERM	RECTAL SWAB

TWO CLOTHING ITEMS:

SUB-BAROCDE: 25665-6666
ITEM: UNDERPANTS
AP POSITIVE
MICRO POS FOR SPERM



SUB –BARCODE: 25665-5555
ITEM: SINGLET TOP
AP NEGATIVE

Reporting back on SAIK via EXR/EXH registered under barcode 12345-6789.

PRESUMPTIVE RESULTS

	Result/Status	Linked No.	Warm Linked Name
[redacted]	Micro positive for sperm. Submitted results pending		
	Semen not detected.		
	Micro positive for sperm. Submitted results pending		

NOTE: ONLY THE HIGH VAGINAL SWAB IS REPORTED BACK TO QPS OUT OF THE FIVE SWABS SUBMITTED. IN THIS EXAMPLE: THREE SWABS SHARE THE SAME POSITIVE RESULTS AND TWO SWABS ARE NEGATIVE. THE EXR/EXH TO QPS IS REPORTED BACK ON THE MOST PROBATIVE OF ALL THE POSITIVE SWABS – THE HIGH VAGINAL SWAB.

THERE IS NO NEED TO REPORT BACK THE NEGATIVE SWABS RESULTS AS THESE RESULTS DO NOT ADD ANY INFORMATION NEEDED BY QPS AT THIS STAGE.

BOTH ITEMS OF CLOTHING ALSO HAVE THEIR PRESUMPTIVE RESULTS REPORTED BACK VIA EXR/EXH TO QPS.

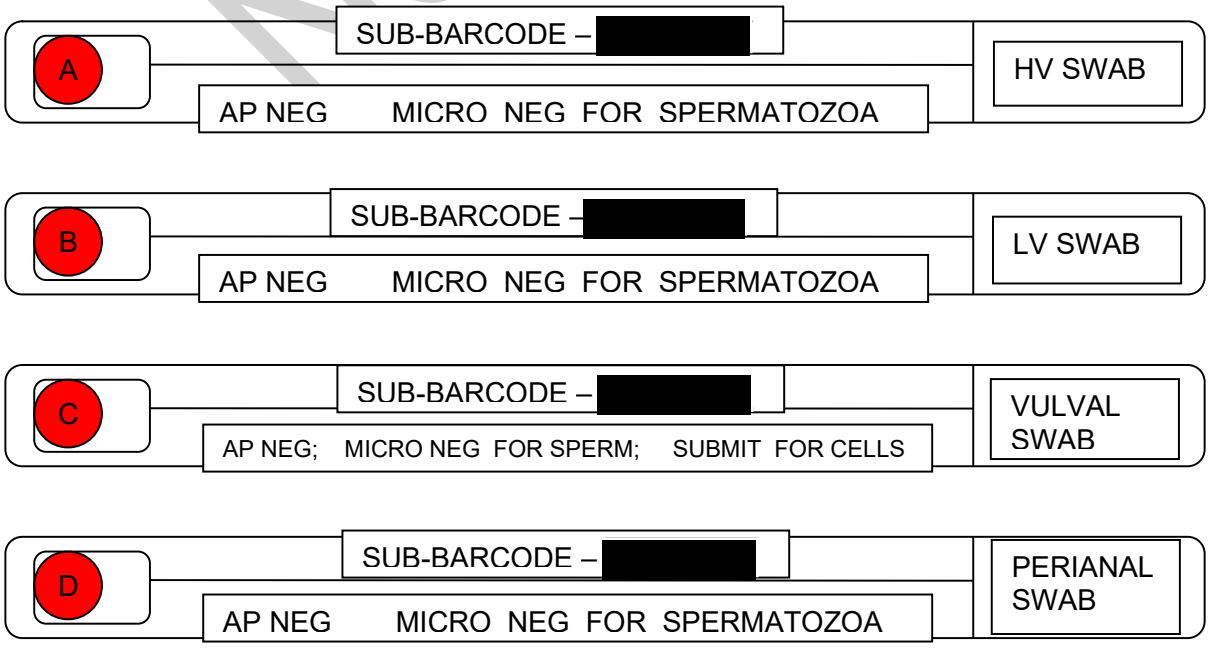
FINAL PROFILES

	Result/Status	Linked No.	Warm Linked Name
[redacted]	**Micro positive for sperm. Submitted results pending**		
	Semen not detected.		
	Micro positive for sperm. Submitted results pending		
	Mixed profile, major component uploaded to NCIDD.	195000101	DEVO
	Mixed profile, partial minor component.	20062654	CHILDS
	Mixed DNA profile, conditioned on.	20062654	CHILDS
	Mixed DNA profile. Remaining profile after conditioning.	1950000101	DEVO
	Mixed part profile. No major/minor. Unable to load to NCIDD.		

EXAMPLE 8.

SAIK = QPS BARCODE 12345-6789

SAIK CONTAINS FIVE SWABS (NOT BARCODED BY QPS)

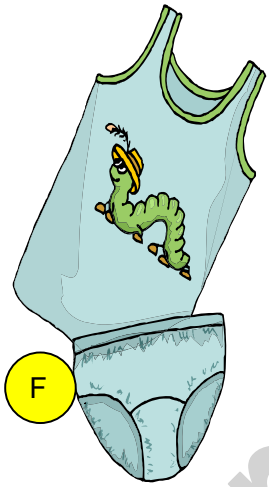


E | SUB-BARCODE – [redacted] | RECTAL SWAB

AP NEG MICRO NEG FOR SPERMATOZOA

TWO CLOTHING ITEMS:

SUB –BARCODE: [redacted]
ITEM: SINGLET TOP
AP NEGATIVE



SUB-BAROCDE: [redacted]
ITEM: UNDERPANTS
AP NEGATIVE

Reporting a negative SAIK

Reporting back on a SAIK via EXR/EXH registered under barcode 12345-6789.

PRESUMPTIVE RESULTS

	Result/Status	Linked No.	Warm Linked Name
[redacted]	Semen not detected.		
	Submitted as cells		
	Semen not detected.		
	Semen not detected.		

NOTE: ONLY THE HIGH VAGINAL SWAB AND VULVAL SWABS ARE REPORTED BACK TO QPS OUT OF THE FIVE SWABS SUBMITTED. IN THIS EXAMPLE: THE FIVE SWABS WERE ALL NEGATIVE FOR (AP) AND MICROSCOPY. THE TWO ITEMS OF CLOTHING ARE ALSO REPORTED BACK AS NEGATIVE TO QPS. HOWEVER, EVEN THOUGH THE VULVAL SWAB WAS ALSO NEGATIVE TO ALL PRESUMP TESTING, IT WILL STILL BE SUBMITTED FOR A 'CELL' EXTRACTION. DNA ANALYSIS IS REQUESTED FOR THE VULVAL SWAB AS A LAST DITCH EFFORT – GIVEN BOTH THE SAIK AND CLOTHING ARE NEGATIVE FOR ALL TESTING PERFORMED.

FINAL PROFILES

	Result/Status	Linked No.	Warm Linked Name
[redacted]	Semen not detected.		

[redacted]	Submitted as cells		
	Semen not detected.		
	Semen not detected.		
	No DNA profile		

APPENDIX 12

COMPLETE CASEWORK PREAMBLE – EXAMINATIONS BY QHFSS

11. The following information is provided to assist in the understanding of the contents of this statement.

Forensic Biology

As a Forensic Biologist, it is my role to report on the examination of items submitted in relation to this case for the presence of possible biological material. If identified, a sample of the biological material is analysed in an attempt to obtain a DNA profile. Any DNA profiles which are obtained from these samples are then compared with the DNA profiles obtained from an individual's reference sample.

As a representative of the laboratory, I am only able to comment on the processes performed within DNA Analysis.

Examinations

The examinations described in this Statement of Witness were carried out by colleagues. The notes, which have been referred to in the preparation of this report, were made at the time of examination. All examinations were carried out in accordance with Standard Operating Procedures.

DNA Profiling

DNA is a complex chemical found in almost all cells in the human body. It carries genetic information which determines the physical and chemical characteristics of a person. The DNA system used at Queensland Health looks at 10 regions of DNA, 9 of which contain Short Tandem Repeats (STRs). The tenth region gives an indication as to the gender of the donor.

A DNA profile obtained from biological material such as blood, semen, saliva or hair can be compared with the DNA profile obtained from a reference sample from any person. If the DNA profiles are different then that person can be excluded as a possible source of the biological material. If the DNA profiles are the same, then that person together with anyone else who has the same DNA profile, can be considered as a potential source of the biological material. The evidential significance of a match can be evaluated in relation to the probability of obtaining such a match by chance.

If less than the ten regions of DNA are seen in a DNA profile, this will be referred to as a partial or incomplete DNA profile. When more than one person has contributed to a DNA profile, this is referred to as a mixed DNA profile.

Mixed DNA profiles

When more than one person has contributed DNA to a sample, the DNA profile obtained is referred to as a mixed DNA profile. The mixture of DNA can happen in an infinite number of ways, however, the resultant DNA profile can often be explained in terms of major and minor profiles.

Major / minor mixed DNA profile – this occurs when one person contributes more DNA to a sample than another person. The individual contributions to the mixed DNA profile are referred to as major profiles and minor profiles.

Even mixtures – this occurs when two (or more) people contribute DNA to a sample in approximately equal proportions. It is not always possible to determine individual contributions to these types of mixed DNA profiles.

Complex mixtures - this is when the DNA profile may contain an unknown number of contributors, and / or be partial in nature and therefore only provide a limited amount of information. In some cases it may be possible to compare the reference DNA profile of a person with the DNA components within these complex mixtures. If there is a possibility that that person could have contributed some of the DNA components observed in the complex DNA profile then this person can be described as not being excluded / eliminated as a potential contributor to the DNA. In other cases the mixed DNA profile may be so complex or incomplete that it may not be possible to draw any conclusions as to whether a person could have contributed to the DNA. In these instances, the complex DNA profile is deemed unsuitable for comparison purposes.

Touch DNA/ Transfer of DNA

When a person touches a surface it is possible for cellular material from that person to be transferred onto the surface that they have touched. This cellular material can be recovered onto a swab, tape lift or cutting depending upon the nature of the surface in question and this sample can then be subjected to DNA profiling.

The amount of cellular material transferred, and therefore the resulting DNA profile, will depend on many factors. These include the nature of the surface being touched and the amount of cellular material that person has available to transfer.

The persistence of any transferred cellular material on the surface will depend largely upon the nature of the surface and the conditions under which it has been kept in the time between deposition and recovery. For example, cellular material could be lost from the surface by washing.

It therefore follows that the absence of a DNA profile from a touched surface does not necessarily mean that that person has not come into contact with that surface, since it is possible for a person to come into contact with a surface without a detectable amount of their DNA being transferred or recovered.

Blood stains

Blood stains are located in the laboratory by means of their visual appearance and the use of a chemical test (Tetramethylbenzidine – TMB). A positive result with the chemical test is a good indication that blood is present, but it does not provide proof as other substances can very occasionally give the same result. The appearance of blood can also be very distinctive.

Semen Stains

The presence of semen on an item can be indicated by the use of a presumptive chemical test which detects a major constituent of seminal fluid (Acid Phosphatase – AP). This constituent does, however, exist in other body fluids, such as vaginal secretions. Additional presumptive chemical testing (Prostate Specific Antigen – PSA) can be undertaken and a positive reaction to both AP

and PSA makes it highly likely that seminal fluid is present. The presence of semen can be confirmed via the microscopic identification of spermatozoa (sperm heads).

Where reference has been made to a Differential Lysis Extraction, this means that the sample has undergone a process that aims to separate Spermatozoa and Epithelial/ Cellular fractions. This separation is not always completely effective, and carry-over between fractions can occur to some degree. This may be referred to as 'cellular carry-over'.

Semen staining on items

The presence of semen on an item of clothing would normally be the result of either direct ejaculation or by contact with an item wet with semen. Any semen which may have been transferred can subsequently be lost by actions such as washing.

Persistence of semen in the vagina

The presence of semen in the vagina would normally be the result of vaginal intercourse with internal ejaculation. The likelihood of detecting semen on a vaginal swab depends upon a number of factors such as the efficiency of the sampling process and the delay between the act of vaginal intercourse and the taking of the swabs during the medical examination.

The greater the delay, the less chance there is of finding semen. Semen is likely to be found on vaginal swabs if they were taken 1-2 days after the act of vaginal intercourse. It is sometimes found on swabs taken between 2-7 days afterwards, but it is highly unlikely to be detected after 7 days.

This is due to a number of factors which can include the following:
- Drainage of semen from the vagina
- Loss of semen by bathing or washing, this would affect the external sites in particular
- Degradation of the spermatozoa

Saliva

The laboratory test (Phadebas) used to detect the presence of saliva exploits the enzyme activity of a constituent of saliva called amylase. Amylase is usually present in relatively high amounts in saliva, though concentrations may be variable in human saliva. Amylase is also present in other body fluids, such as sweat and vaginal and anal secretions, however, this would generally be at much lower concentrations.

If an area of the body is sucked or licked, the saliva may be transferred onto the skin and subsequently onto any items of clothing worn on this area of the body. Saliva staining, in the form of amylase may then be detected on skin swabs or items of clothing as long as the clothing or skin has not been washed. Cellular material will be shed, to varying degrees, with the saliva and as such, it may be possible to obtain a DNA profile from an area of saliva staining.

The Use of Queensland Caucasian Data

The Queensland DNA Analysis Laboratory routinely uses the Queensland Caucasian data for statistical calculations. It is policy of the laboratory to use the Queensland Caucasian data unless the alleged incident occurred off the Queensland mainland, in which case the Queensland Caucasian and Queensland Aboriginal data would both be quoted.

We do not make any assumptions as to the ethnic origin of the DNA obtained from alleged crime scenes, in which case the Queensland Caucasian population being the largest sub-population in Queensland, is used for statistical calculations. Having said this, calculations using Queensland Aboriginal and Asian data can be provided upon request.

Validity of the Caucasian Data

The data used for statistical interpretations in the laboratory have been validated for use by external Forensic Statisticians Simon J WALSH and Dr John S BUCKLETON. The report of their findings is held in the laboratory and is available upon request.

12. The results of the scientific examinations conducted in this laboratory are as follows:

APPENDIX 13

COMPLETE CASEWORK PREAMBLE - EXAMINATIONS BY QPS AND QHFSS

11. The following information is provided to assist in the understanding of the contents of this statement.

Forensic Biologist

As a Forensic Biologist, it is my role to:

1. Report on the examination of items submitted in relation to a case for the presence of possible biological material. If identified, a sample of the biological material is analysed in an attempt to obtain a DNA profile.

2. Report on the DNA profiles obtained from samples submitted by the Queensland Police Service (QPS) in relation to a case.

Any DNA profiles which are obtained from these samples are then compared with the DNA profiles obtained from an individual's reference sample.

As a representative of the laboratory, I am only able to comment on the processes performed within DNA Analysis.

Examinations

Unless otherwise stated, the examinations of items for biological material were conducted by officers within the QPS. Sub-samples from these items were forwarded to Queensland Health Forensic and Scientific Services (QHFSS) for the purposes of conducting DNA analysis.

It is my understanding that QPS are responsible for item prioritisation, sample selection, selection of screening/sampling methods, anti-contamination procedures and the application of Standard Operating Procedures on work undertaken on the items/samples prior to submission to the QHFSS DNA Analysis laboratory. As such, Forensic Biologists may not be able to provide information or opinion on possible biological origin of DNA profiles that may be obtained from these samples.

Some items may be submitted to this laboratory for the purposes of both examination and DNA profiling. This occurs at the discretion of the QPS. These examinations are performed in accordance with the Standard Operating Procedures (SOPs) of this laboratory. For these items, notes are made at the time of the examination by the examining scientist and form part of the casefile.

DNA Profiling

DNA is a complex chemical found in almost all cells in the human body. It carries genetic information which determines the physical and chemical characteristics of a person. The DNA system used at Queensland Health looks at 10 regions of DNA, 9 of which contain Short Tandem Repeats (STRs). The tenth region gives an indication as to the gender of the donor.

A DNA profile obtained from biological material such as blood, semen, saliva or hair can be compared with the DNA profile obtained from a reference sample from any person. If the DNA profiles are different then that person can be excluded as a possible source of the biological material. If the DNA profiles are the same, then that person together with anyone else who has the same DNA profile, can be considered as a potential source of the biological material. The evidential significance of a match can be evaluated in relation to the probability of obtaining such a match by chance.

If less than the ten regions of DNA are seen in a DNA profile, this will be referred to as a partial or incomplete DNA profile. When more than one person has contributed to a DNA profile, this is referred to as a mixed DNA profile.

Mixed DNA profiles

When more than one person has contributed DNA to a sample, the DNA profile obtained is referred to as a mixed DNA profile. The mixture of DNA can happen in an infinite number of ways, however, the resultant DNA profile can often be explained in terms of major and minor profiles.

Major / minor mixed DNA profile – this occurs when one person contributes more DNA to a sample than another person. The individual contributions to the mixed DNA profile are referred to as major profiles and minor profiles.

Even mixtures – this occurs when two (or more) people contribute DNA to a sample in approximately equal proportions. It is not always possible to determine individual contributions to these types of mixed DNA profiles.

Complex mixtures - this is when the DNA profile may contain an unknown number of contributors, and / or be partial in nature and therefore only provide a limited amount of information. In some cases it may be possible to compare the reference DNA profile of a person with the DNA components within these complex mixtures. If there is a possibility that that person could have contributed some of the DNA components observed in the complex DNA profile then this person can be described as not being excluded / eliminated as a potential contributor to the DNA. In other cases the mixed DNA profile may be so complex or incomplete that it may not be possible to draw any conclusions as to whether a person could have contributed to the DNA. In these instances, the complex DNA profile is deemed unsuitable for comparison purposes.

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When a person touches a surface it is possible for cellular material from that person to be transferred onto the surface that they have touched. This cellular material can be recovered onto a swab, tape lift or cutting depending upon the nature of the surface in question and this sample can then be subjected to DNA profiling.

The amount of cellular material transferred, and therefore the resulting DNA profile, will depend on many factors. These include the nature of the surface being touched and the amount of cellular material that person has available to transfer.

The persistence of any transferred cellular material on the surface will depend largely upon the nature of the surface and the conditions under which it has been kept in the time between deposition and recovery. For example, cellular material could be lost from the surface by washing.

It therefore follows that the absence of a DNA profile from a touched surface does not necessarily mean that that person has not come into contact with that surface, since it is possible for a person

to come into contact with a surface without a detectable amount of their DNA being transferred or recovered.

Blood stains

Blood stains are located in the laboratory by means of their visual appearance and the use of a chemical test (Tetramethylbenzidine – TMB). A positive result with the chemical test is a good indication that blood is present, but it does not provide proof as other substances can very occasionally give the same result. The appearance of blood can also be very distinctive.

Semen Stains

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Where reference has been made to a Differential Lysis Extraction, this means that the sample has undergone a process that aims to separate Spermatozoa and Epithelial/ Cellular fractions. This separation is not always completely effective, and carry-over between fractions can occur to some degree. This may be referred to as 'cellular carry-over'.

Semen staining on items

The presence of semen on an item of clothing would normally be the result of either direct ejaculation or by contact with an item wet with semen. Any semen which may have been transferred can subsequently be lost by actions such as washing.

Persistence of semen in the vagina

The presence of semen in the vagina would normally be the result of vaginal intercourse with internal ejaculation. The likelihood of detecting semen on a vaginal swab depends upon a number of factors such as the efficiency of the sampling process and the delay between the act of vaginal intercourse and the taking of the swabs during the medical examination.

The greater the delay, the less chance there is of finding semen. Semen is likely to be found on vaginal swabs if they were taken 1-2 days after the act of vaginal intercourse. It is sometimes found on swabs taken between 2-7 days afterwards, but it is highly unlikely to be detected after 7 days.

This is due to a number of factors which can include the following:
- Drainage of semen from the vagina
- Loss of semen by bathing or washing, this would affect the external sites in particular
- Degradation of the spermatozoa

Saliva

The laboratory test (Phadebas) used to detect the presence of saliva exploits the enzyme activity of a constituent of saliva called amylase. Amylase is usually present in relatively high amounts in saliva, though concentrations may be variable in human saliva. Amylase is also present in other

body fluids, such as sweat and vaginal and anal secretions, however, this would generally be at much lower concentrations.

If an area of the body is sucked or licked, the saliva may be transferred onto the skin and subsequently onto any items of clothing worn on this area of the body. Saliva staining, in the form of amylase may then be detected on skin swabs or items of clothing as long as the clothing or skin has not been washed. Cellular material will be shed, to varying degrees, with the saliva and as such, it may be possible to obtain a DNA profile from an area of saliva staining.

The Use of Queensland Caucasian Data

The Queensland DNA Analysis Laboratory routinely uses the Queensland Caucasian data for statistical calculations. It is policy of the laboratory to use the Queensland Caucasian data unless the alleged incident occurred off the Queensland mainland, in which case the Queensland Caucasian and Queensland Aboriginal data would both be quoted.
We do not make any assumptions as to the ethnic origin of the DNA obtained from alleged crime scenes, in which case the Queensland Caucasian population being the largest sub-population in Queensland, is used for statistical calculations. Having said this, calculations using Queensland Aboriginal and Asian data can be provided upon request.

Validity of the Caucasian Data

The data used for statistical interpretations in the laboratory have been validated for use by external Forensic Statisticians Simon J WALSH and Dr John S BUCKLETON. The report of their findings is held in the laboratory and is available upon request.

12. The results of the scientific examinations conducted in this laboratory are as follows:

APPENDIX 14

COMPLETE PATERNITY PREAMBLE

10. The following information is provided to assist in the understanding of the contents of this statement.

Forensic Biology

As a Forensic Biologist, it is my role to report on the examination of items submitted in relation to this case for the presence of possible biological material. If identified, a sample of the biological material is analysed in an attempt to obtain a DNA profile. Any DNA profiles which are obtained from these samples are then compared with the DNA profiles obtained from an individual's reference sample.

Examinations

The examinations described in this Statement of Witness were carried out by colleagues. The notes, which have been referred to in the preparation of this report, were made at the time of examination. All examinations were carried out in accordance with Standard Operating Procedures.

DNA Profiling

DNA is a complex chemical found in almost all cells in the human body. It carries genetic information which determines the physical and chemical characteristics of a person. The DNA system used at Queensland Health looks at 10 regions of DNA, 9 of which contain Short Tandem Repeats (STRs). The tenth region gives an indication as to the gender of the donor.

Parentage testing and Statistical calculations:

DNA is inherited from our biological mother and father.
In a disputed paternity matter, DNA profiles are obtained from the foetus/child, the biological mother and the putative father(s). Based on the assumption that the mother is indeed the biological mother of the foetus/child, it is possible to determine which DNA components within the DNA profile of the child could have originated from her. The remaining DNA components within the DNA profile of the foetus/child must have originated from the biological father, and are called *obligate paternal alleles*.

If the DNA profile of a putative father **does not** contain the obligate paternal alleles in at least two of the DNA regions tested, then that person is **excluded** as a potential biological father of the foetus/child.

If the DNA profile of a putative father **does** contain the obligate paternal alleles at each of the DNA regions tested, then that person is **not excluded** as a potential biological father of the foetus/child. This means that this putative father could indeed be the biological father.

Statistical analysis is then conducted to aid in the understanding of the strength of the evidence. The Paternity Index (PI) is a likelihood of two probabilities conditional upon different competing hypotheses;

1. The alleged father contributed the obligate paternal alleles observed in the DNA profile of the foetus/child

2. Another man chosen at random contributed the obligate paternal alleles observed in the DNA profile of the foetus/child.

The PI reflects how many times more likely it is to see the evidence (ie. Set of alleles) under the first hypothesis compared to the second hypothesis. The generally accepted minimum standard for an inclusion of paternity is a PI of 200 or greater (NATA Paternity Testing Technical Advisory Group, 2004).

(Adapted from Butler, J.M. (2005) Chapter 23, *Kinship and Parentage Testing in* Forensic DNA Typing, Biology, Technology, and Genetics of STR Markers, 2 Ed. Elsevier Academic Press: Burlington, MA 01803, USA.)

11. The results of the scientific examinations conducted in this laboratory are as follows:

Reference Samples

nn: XX - mother
nn: XY - suspect
nn: CC - child

DNA profiles were obtained from these reference samples. These DNA profiles were different to each other.

Information was observed within the DNA profile of CC, supporting the assumption that XX is indeed the biological mother of CC.

The DNA profile obtained from the reference sample from XY was compared to the DNA profiles obtained from the reference samples of XX and CC in order to assist in the determination of the possible paternity of CC.

XY possesses all of the obligate paternal alleles. In my opinion, it is possible that XY is the biological father of CC given that XX is the natural mother. The following statistical weighting has been calculated in support of this opinion:

The DNA profile from CC is n times more likely to have occurred if CC was the offspring of XX and XY rather than if CC was the offspring of XX and a random man unrelated to XY <population data set>.

APPENDIX 15

QUALITY PARAGRAPHS

NB. These paragraphs are required to be entered into the preambles of statements containing results from DNAIQ extractions on the Automated Platforms during the period October 2007- July 2008. The particular category will depend on the investigation outcomes of each case.

Quality

Category A

Testing for this case has been conducted in a period where some results were the subject of an adverse event. An adverse event includes a deficient operation or function identified to have occurred within the automated extraction process utilised in testing during the period October 2007 to July 2008. Testing for this case was not the subject of any adverse result. An adverse result is a result which has been affected by an adverse event, whose integrity cannot be verified. This conclusion has been reached by conducting a review of the results and assessing a number of factors, including, but not limited to, the comparison of all other results from samples processed alongside this result, to detect whether the integrity of each sample can be verified. Retesting has been conducted on identified samples which have confirmed information in the original results.

Category B

Testing for this case has been conducted in a period where some results where the subject of adverse events. An adverse event includes a deficient operation or function identified to have occurred within the automated extraction process utilised in testing during the period October 2007 and July 2008. Within this case, the adverse event is demonstrated to have affected a result or results and the integrity cannot be verified. There was no remaining sample for retesting to be conducted. These results have therefore been reported as follows 'these samples did not pass our Quality System requirements at the DNA analysis stage and therefore the DNA profiling results relating to these samples cannot be reported'.

Category C

Testing for this case has been conducted in a period where some results were the subject of adverse events. An adverse event includes a deficient operation or function identified to have occurred within the automated extraction process utilised in testing during the period of October 2007 and July 2008. Testing for some samples within this case has been the subject of an adverse event. The cause of the adverse event was identified to have occurred within the automated extraction process. Portions of the sample remained available for further testing. Retesting has been conducted, using an alternative manual extraction method and all quality assurance checks were satisfactory. These samples have been reported as they have been assessed as no adverse event having been detected and the results have passed all quality assurance checks.

APPENDIX 16

EVIDENTIARY CERTIFICATE WORKFLOW

Receive written request and clarify which samples require to be included

⇓

Ensure that there is adequate time to complete audit trails and issue certificate at least 10 days before court date – negotiate with IO/DPP if required

⇓

Request Quality Team assistance to create spreadsheet of all batches involved in case (for those specified in written request)

⇓

The Quality Team will compile batch numbers for the specified samples and save the document to G:\ForBiol\Quality Assurance and NATA\Audit Trails\Evidentiary Certificates\Case #.
The Quality Team will also print out the storage audits for all specified samples.

⇓

Create Evidentiary checklist using master G:\ForBiol\AAA Forensic Reporting & Intel\Audits-Evidentiary Certificate\ Evidentiary Certificate Checklist.xls.
Copy batch numbers (from the table created by the Quality Team) into separate worksheets in this checklist (removing duplicates).
Note Portal batches do not need to be included as not an actual batch – it simply exists for 'holding samples' in Auslab

⇓

Work through each batch type, looking at audit trail in Auslab to see if any issues have been raised. eg sample removed as not transferred yet, pos control is partial profile, failure of batch due to controls not working/OQI

There are three outcomes:
1. No issues and audit trail ok.
2. Issue raised in audit trail (such as point from curve removed), but batch has proceeded to be completed and results released. (OK)
3. Issue raised in audit trail that results in Quality control failure and results not able to be released.

Each outcome needs to be noted on Checklist for that batch. If there is an issue with the batch (option 2 or 3) print audit trail to place in Evidentiary Certificate file and note outcome of issue.

⇓

Remember to check extraction positive and negative controls are all ok and fill this in checklist. See Note 3 at bottom of document.

⇓

Check storage audit trail for each sample, noting that there should be two final locations for most samples – one for spin basket and one for final amplified product. Ensure that throughout the audit trail, sample has been added and removed from particular location as it works its way through the process.
Note on printout if audit trail has been checked and ok and update

spreadsheet.

⇓

Check Amplification worksheets, CE/Genescan Worksheet and Genotyper reader sheets to ensure all pages are signed, corrections signed and dated and calculations legible and present

⇓

If this is not the case, get original paperwork correction signed and dated or whatever is required. If this staff member is on holidays, or no longer working in department, get their HP4/HP5 to sign and date on their behalf.

⇓

Remember to check ladder, positive and negative controls are all ok and fill this in on Genotyper batches page in checklist.

⇓

Compile Evidentiary Checklist file, with printouts of each batch type separated, storage audits of samples/receipts and any further paperwork involved.

⇓

Print out checklist and add to front of file with associated documentation including written request for certificate.

⇓

Create Evidentiary Certificate from template: G:\ForBiol\AAA Forensic Reporting & Intel\Reporting guidelines\Evidentiary Certificate - Section 95A - Template.doc

⇓

Fill in scientist name, qualifications, everything delivered for case, specific samples, dates from beginning (date of first item received) to end of case (date of statement review and release) and footer Note: the format is different to standard statements.

⇓

Create cumulative results table containing only samples included in Evidentiary Certificate and cross out footer section “Page 1 of 1” to make last page of certificate documentation.

⇓

Have Evidentiary Certificate Peer Reviewed - only Administrative Review is required to check transcription of samples to certificate and to cross check with written request.

⇓

Issue and Scan into Auslab. Keen paperwork within casefile.

Extra Notes:

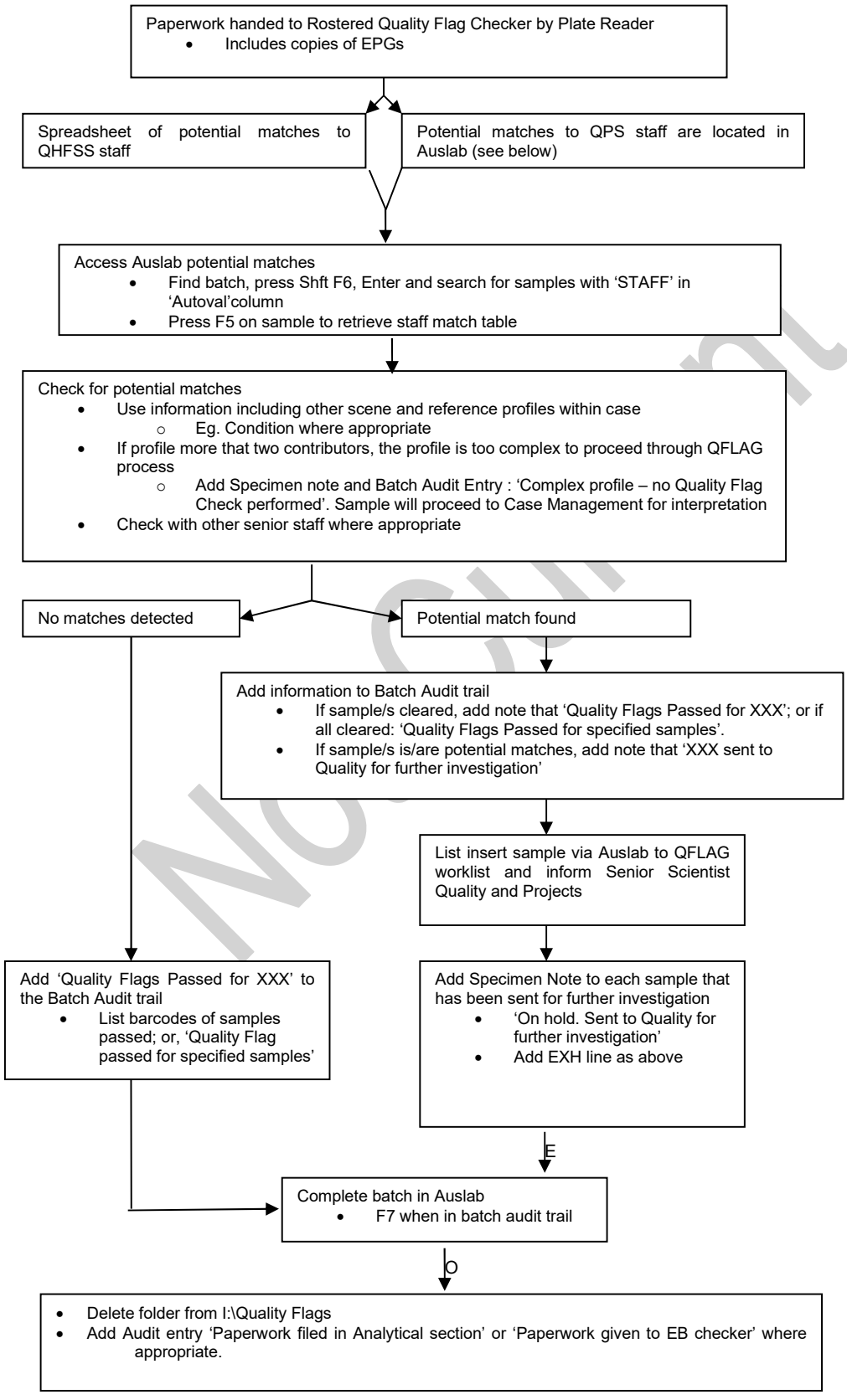
1. Make sure to change page number at bottom of page for Storage Audit printouts (to Page 1 of 1 on printout)

2. If there is no issue with pos/neg controls on batch, do not need to print out their individual audit trail and Genotyper/Genemapper. This is only required if there is a partial profile or quant value in the negative control, or a mixed profile/not matching full or partial profile in a positive control. If a positive control is a partial profile, but the alleles that are called match what is expected, this batch should have been released and this just needs to be noted in the Checklist only.

3. On all checklist pages there is a column for audit trail missing. This is not required if it is determined that your case has not been processing during times when audit trails are known to have not been complete. However, it doesn't hurt to have this column there to ensure that this check has been done. If there is part of the audit trail missing, a 'paper trail' needs to be created for this sample and will need to speak to Quality about this.

APPENDIX 17

QUALITY FLAG CHECKING WORKFLOW

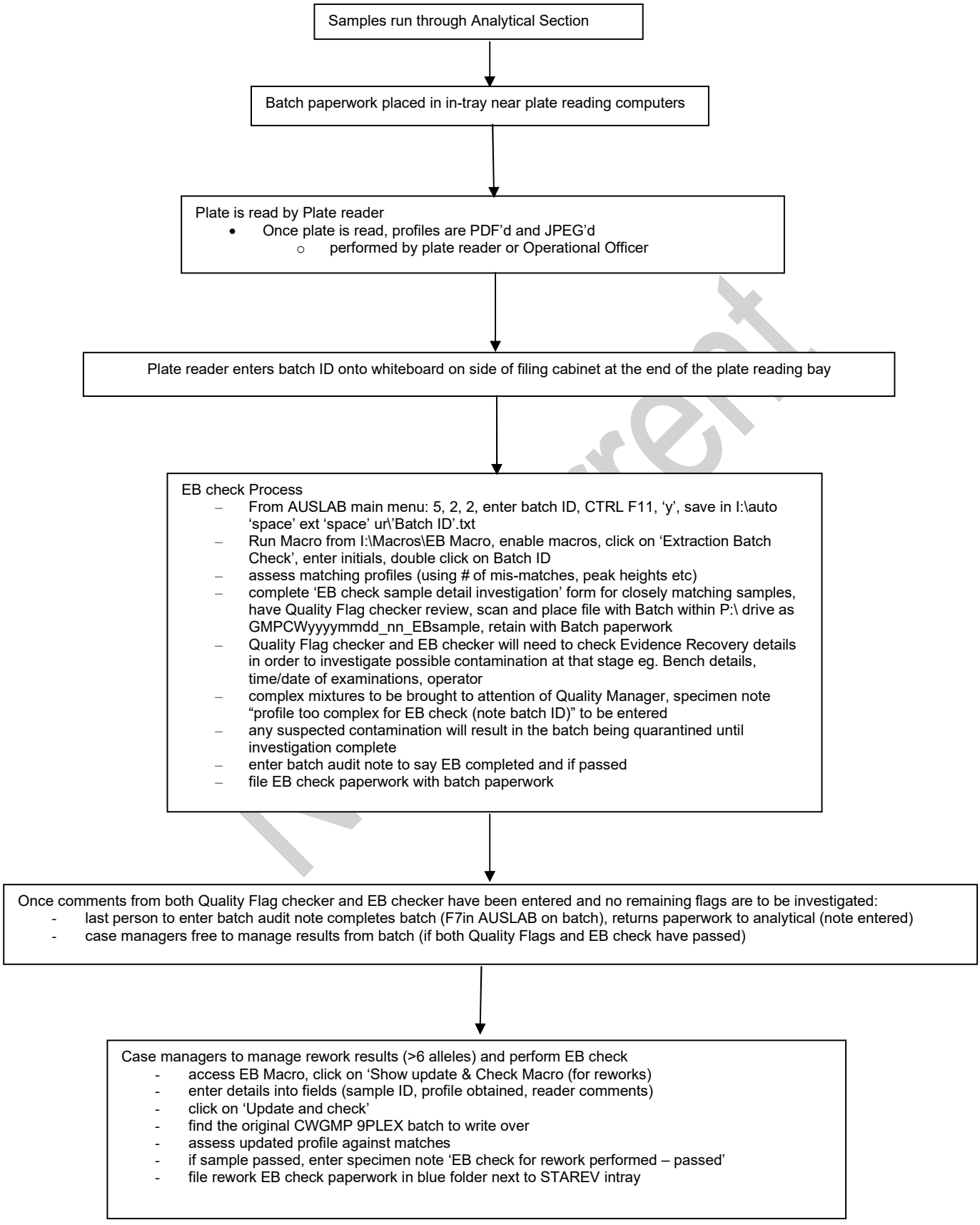


NB.

- Quality Flag Checking is to detect gross contamination that could have occurred at collection or during processing of the sample.
    - This includes single-source profiles, distinct major or minor profiles, or remaining contributions if the mixed profile has been conditioned.
- Due to the complexity of some DNA profiles, profiles that indicate at least three contributors (and therefore an unknown number of contributors) are not suitable for Quality Flag checking for the following reasons:
    - The number of contributors is not known and the often partial nature adds complexity.
    - At most, a person may not be able to be excluded as potential contributors and this interpretation may not be useful to the client as we cannot evaluate the significance of a possible inclusion by adding statistical weight.
- When profiles of at least three contributors are obtained, the Quality Flag Checker should note in the Batch Audit and the Specimen Notes:
    - 'Complex profile – no Quality Flag check performed'
        - The profile will then proceed to case management for interpretation with case context.
- The macro that is applied to detect potential Quality Flags has a stringency of 13 alleles. This means crime scene profiles with less than 13 alleles detected will not go through the macro and therefore will not be Quality Flag checked.

APPENDIX 18

EXTRACTION BATCH CHECKING WORKFLOW (EB Checking)



NB.

- Macro includes profiles greater than 6 alleles.
- Macro has separate tabs for single source and mixtures.
- Macro displays matching profiles in groups. The profile highlighted in white is the profile that the profiles underneath have matched to.
- Macro displays loci of matching profiles in different colours: green is for at least 2 alleles matching, yellow is for 1 mismatch, light orange is for 2 mismatches, bright orange is for a mismatch at Amelogenin.
- EB check to be performed at desk, single profile to be displayed on screen or printed and checked against matching profiles flagged by the macro.

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Investigating Adverse Events in DNA Analysis

Contents:

1. PURPOSE

The purpose of this document is to provide guidelines around standard investigation protocols and result acceptance criteria for adverse events in the DNA Analysis Unit. The procedure outlines the key considerations in an investigation, the required actions and the necessary documentation for issues that may interfere with the quality of results within the DNA Analysis Unit.

These guidelines have been developed in complement to the OQI process (QIS 13965), and the Procedure for Quality Practice (QIS 17154).

2. SCOPE

This procedure applies to all staff within the DNA Analysis Unit. This document has attempted to cover key quality issues that may arise from adverse events in sample preparation, in screening of exhibits for biological fluids and in DNA profiling; however it can not cover all possible adverse events. Where an event occurs which is outside the scope of this document, consult Senior Scientists and Team Leaders for guidance. This document does not cover adverse events that relate to workplace health and safety.

3. DEFINITIONS

For a comprehensive list of abbreviations refer to QIS 23849 Common DNA Analysis Terms and Acronyms.

Adverse Event: Any event or occurrence which brings into question a procedure or result

AI: Allelic imbalance

AP: Acid phosphatase

CE: Capillary electrophoresis

DNA Profiling techniques: All procedures, analytical instruments and consumables used in the process of obtaining a DNA profile (including extraction, quantification, amplification, capillary electrophoresis and profile interpretation).

EB Check: Extraction batch check completed by reporting staff as a quality check for adverse events occurring during the automated DNA extraction process.

EFTA: Extraction FTA sample

OQI: Opportunity for quality improvement

ReGs (Re-CE): Sample or batch is re-prepared and analysed again on the 3130*x*/

SD: Standard deviation

TMB: Tetramethylbenzidine

QEXH: Case management list to hold quarantined samples

QPS: Queensland Police Service

4. EVIDENCE RECOVERY – PRESUMPTIVE TESTING QUALITY CONTROL

4.1. Tetramethylbenzidine (TMB) presumptive screening

Tetramethylbenzidine (TMB) is a presumptive test for blood used within the DNA Analysis Unit. Before the reagent can be used for casework, both positive and negative controls must pass quality control criteria.

The positive TMB control is a known blood sample (Refer to QIS 17190 for testing methodology). A positive control pass is the appearance of the blue-green colour developing in <5 seconds. A colour change in 5-20 seconds should be considered inconclusive and the test repeated, if after repetition it is still inconclusive this should be considered a fail (refer below). A positive control fail is the absence of the blue-green colour change, or the appearance of the blue-green colour developing >20 seconds. If a colour change occurs after the addition of TMB only (without hydrogen peroxide) it is also a failed test.

The negative control for TMB (Refer to QIS 17190 for testing methodology) is performed on a substrate that does not react to TMB (e.g. clean filter paper). A negative control pass is the absence of a blue-green colour developing in 10 seconds, with a negative control fail being the development of a blue-green colour within 10 seconds.

If the positive or negative controls fail, the TMB and hydrogen peroxide reagents should be re-prepared and the controls re-tested (Refer to QIS 17190 for methodology). If the new reagent preparation has passing TMB controls, the chemical test is acceptable for use. If the newly prepared reagents fail the quality criteria for TMB positive and negative controls, notify the Senior Scientist Evidence Recovery. New reagents may need to be purchased and new positive controls prepared for testing.

4.2. Acid Phosphatase (AP) presumptive screening

Acid phosphatase (AP) is a presumptive test for seminal fluid used within the DNA Analysis Unit Laboratory. Before the reagent can be used for casework, both positive and negative controls must pass.

The positive AP control is a known seminal fluid sample (Refer to QIS 17186 for testing methodology). A positive control pass is the appearance of a purple colouration within 5 seconds. A positive control fail is purple colouration developing >5 seconds or the absence of the purple colour change after 5 seconds.

The negative control for AP (Refer to QIS 17186 for testing methodology) is performed on a substrate that does not react to AP (e.g. clean filter paper). A negative control pass is the absence of a purple colouration within 2 minutes. A negative control fail is the development of a purple colouration within 2 minutes.

In cases where the positive or negative controls fail, the AP reagent should be re-prepared and the controls retested (Refer to QIS 17186 for methodology). If the new reagent preparation has passed AP controls, the reagent is acceptable for use. If the newly prepared reagent fails the quality criteria for AP positive and negative controls, notify the Senior Scientist Evidence Recovery. New reagents may need to be purchased, and new positive controls prepared for testing.

4.3. Phadebas presumptive screening

Phadebas is a presumptive test for saliva used within the DNA Analysis Unit. The laboratory utilises both a supernatant (liquid), and a paper based testing procedure (Refer to QIS 17193 for methodologies). Positive and negative controls must both pass for the test results to be accepted and reported.

The phadebas positive control is a known saliva sample (obtained from staff), and the negative control is a Nanopure water only sample. The positive and negative controls for the supernatant test are different from those used in the paper based test, refer below for details.

Phadebas paper test:

A positive control pass is the development of pale blue zones on the blank side of the phadebas paper at 40 minutes. On the spotted/treated side of the paper the blue spots appear dissolved or smudged (Refer to QIS 17193 for methodology). A positive control fail would be the absence of the pale blue zones on the blank side of the paper, and/or the absence of the dissolved/smudged blue spots on the treated side at 40 minutes.

A negative control pass is indicated by no colour change on either side of the paper (at 40 min.), with a negative control fail occurring if the phadebas paper develops pale blue zones or dissolved/smudged areas on the treated side of the paper at 40min. (Refer to QIS 17193 for methodology).

For phadebas paper based testing, the positive and negative controls should be processed prior to use on casework samples, as a control failure would constitute an unacceptable risk to the exhibit.

If a positive control fails for the phadebas paper, the paper should be retested with the saliva of a different staff member used as the positive control (Refer to QIS 17193 for methodology). If the second control passes, the results can be accepted (as individual staff may have differing levels of amylase). If the second positive control does not pass, notify the Senior Scientist Evidence Recovery, as new phadebas paper may need to be purchased.

If the negative control for the phadebas paper test fails (Refer to QIS 17193 for methodology), fresh nanopure water should be obtained and the negative control retested. If the retested negative control passes, the phadebas test can be performed on casework samples. If the negative control still fails an investigation will be required. The investigation should consider the area in which the test was performed (e.g. laboratory bench), the equipment (spray bottles) and the water used for possible contribution of amylase and/or the function of the phadebas paper. Casework samples are not able to be processed until both the positive and negative controls pass.

Phadebas supernatant test:

A positive control pass is indicated by a blue coloured supernatant in the positive control sample after processing (Refer to QIS 17193 for methodology), with a positive control fail indicated by the absence of a blue colouration in the supernatant.

A negative control pass is indicated by a clear and colourless supernatant in the negative control sample after processing, and a negative control fail occurring if the supernatant is blue in colour (Refer to QIS 17193 for methodology).

In the phadebas supernatant testing procedure a positive and negative control are processed prior to casework samples being tested, this ensures that the reagents are suitable for use (i.e. reagent controls).

If the positive control fails for the phadebas supernatant test, it should be retested with the saliva of a different staff member (Refer to QIS 17193 for methodology). If the second control passes, the results can be accepted (as individual staff may have differing levels of amylase). If the second positive control does not pass, in consultation with the Senior Scientist Evidence Recovery an investigation may be required and/or new phadebas tablets may need to be purchased.

If the negative control for the phadebas supernatant test fails (Refer to QIS 17193 for methodology), in consultation with the Senior Scientist Evidence Recovery and Senior Scientist Quality and Projects an investigation should be initiated. The investigation should examine the environment, processing procedure, labware and reagents used in testing for possible sources of amylase. Until the positive and negative reagent controls pass, no casework samples can be processed.

If the first set of controls pass (reagent controls), the positive and negative controls are re-run with the casework samples (as methodology controls). On completion of the batch the pass/fail status of the controls determines if the casework phadebas results can be accepted and reported.

4.4. ABAcard p30 – seminal fluid presumptive screening

ABAcard® p30 test (Abacus Diagnostics Inc.) detects p30 and is a presumptive test for seminal fluid used within the DNA Analysis Unit. The ABAcard® device has two result areas within the device window; the control "C" area and the test "T" area.

On completion of the test, a pink line in the "C" area is a positive control pass, and indicates that the test is functional. On test completion a pink line in the "T" test result area is a positive test result i.e. presumptive positive for seminal fluid. The absence of a pink line in the "T" test area is a negative test result i.e. presumptive negative for seminal fluid.

For valid use of the ABAcard® test, the positive control line must be apparent on completion of the test, and the test must not be used after the expiration date. If there is no pink line visible in the "C" control area of the test, it is inconclusive and the test should be repeated. If the second ABAcard® test fails (i.e. no pink line visible in the control area) notify the Senior Scientist Evidence Recovery. New test kits may need to be purchased.

5. SAMPLE PREPARATION/PROCESSING - ADVERSE EVENT INVESTIGATIONS

Adverse events can occur during sample preparation and/or processing. This procedure is not able to provide a comprehensive coverage of all possible adverse occurrences, but will outline the three most critical types of events which may occur and would require investigation. These include:

Incorrect labelling (Refer section 5.1)
Sample cross contamination (Refer section 5.2)
Incorrect use of reagents (Refer section 5.3)

Minor adverse events, adverse events which do not require corrective actions and/or adverse events which do not require investigation must be detailed in specimen notes (e.g. mis-storage of an exhibit, a sample being dropped during handling) or a batch audit entry if required. Significant adverse events, or adverse events for which corrective action is needed will require an investigation to be completed (an OQI may also be required) in addition to the specimen notes.

5.1. Incorrect labelling event

Where there are labelling discrepancies on samples delivered to the DNA Analysis Unit from QPS, an investigation by DNA Analysis Unit staff is not required; as these labelling issues are reported back to QPS (by the validation of the "Labelling discrepancy" EXH line during examination) for their investigation. Prior to processing the sample, an Evidence Recovery scientist/senior scientist needs to ensure that the sample within the packaging is

in fact the correct sample (check details on forensic register, contact QPS sample management unit). After QPS have investigated these occurrences, they communicate any additional required action/s back to the DNA Analysis Unit. Any communication received by DNA Analysis Unit from QPS must be put into AUSLAB (e.g. emails scanned to AUSLAB, phone conversations added as a UR Note to the relevant case).

Where labelling discrepancies have occurred during the processing of exhibits, sub-samples or DNA tubes within the DNA Analysis Unit, an investigation is required. Labelling discrepancies may occur as a result of incorrect data entry, barcode misprinting or from the application of an incorrect barcode to a tube. The Senior Scientist Evidence Recovery, Senior Scientist Analytical or the Senior Scientist Quality and Projects must be notified of any instances of labelling discrepancies. Investigations into these occurrences will depend on the nature of each event, however strategies and considerations for an investigation into mislabelling should include:

- An examination of the AUSLAB audit trail to determine when the affected samples labels were printed, and the staff member that printed it (information on samples processed simultaneous, or samples processed by a single person - can be obtained from AUSLAB extended enquires for the purpose of investigation). Using the information from AUSLAB audit trails, from discussions with staff, and from worksheets or examination notes, it should be possible to determine the number of potential labelling errors that may have occurred. The information may be of use to determine how the mislabelling happened. For example: if a mislabelling occurred during examination, other samples processed by that sampling scientist, or other sample barcodes printed at the same time could potentially be affected.
- A review of the documentation which relates to the processing of the sample is required (e.g. examination notes, analytical worksheets, AUSLAB batch audit entries) to see if the correct identity of the sample can be established.
- A confirmation of sample type should be completed as an identity check, and/or to provide additional information to an investigation. For example if examination notes indicate that sample barcode 123456789 should be associated to a swab, but on retrieving the sample it is noted to contain a cigarette butt, a sample/barcode switch should be investigated.
- An assessment of the AUSLAB tracking of the sample may be informative. In situations where barcode labels have been switched (between two items), mis-printed, or duplicate labels printed, evidence on the time at which this occurred may be obtained from AUSLAB storage records.

Corrective actions and documentation:

In **all** cases of mislabelling, specimen notes must be added to all affected samples. Specimen notes must record the adverse event and if applicable the corrective action (e.g. OQI). The investigation must be detailed in an OQI, in specimen notes, the teams' events register, and/or in I:\Quality & Projects\Investigations (Refer to section 8.2.3).

If the sample can be positively identified as a result of the investigation, the result may be reported after the completion of corrective actions and documentation as described above. If the sample can **not** be positively identified, in consultation with the Senior Scientist Quality and Projects or a Team Leader the sample (or sub-sample) may be failed. Where a sample is failed, if it is possible - it should be re-sampled/re-extracted. If it is not possible to positively identify the sample, or to reprocess the sample, the sample must indicate a quality failure (with an EXH and/or by communications to QPS). Communications to QPS

on sample failures will only occur in consultation with the Senior Scientist Quality and Projects or a Team Leader. An Intel letter may be required for this communication (Refer to QIS 24015).

5.2. Sample cross contamination

Sample cross-contamination can occur between exhibits, between DNA samples, or from staff to exhibits/samples. The type of contamination that has occurred will determine how/when the contamination is detected and how it will be investigated. Detection of cross contaminations are usually identified after profiling.

Detection of staff contamination of samples can be identified at plate reading by:
- the staff match macro (for casework and reference samples) which identifies potential matches between samples and DNA Analysis staff, prior to result upload to AUSLAB.
- the AUSLAB staff match function (for casework samples only) which identifies potential matches between samples and QPS staff, after result upload to AUSLAB

Detection of sample-to-sample contaminations can be identified by:
- The extraction batch (EB) check (performed on auto-extraction batches processed on the MPII)
- Case management and reporting processes
- Link creation/confirmation
- Incorrect profile in positive or negative controls

Detection of staff to sample or sample-to-sample contaminations may also be identified from quality searches (as performed by the Quality Team)

Where a cross contamination event is suspected, in consultation with the Senior Scientist Evidence Recovery, Senior Scientist Analytical or Senior Scientist Quality and Projects the following actions should be considered:

- If a possible **contamination event of an exhibit/DNA sample by a staff member** is identified by the staff match macro or by a quality search, AUSLAB records including audit trails, and/or FBX fields should be reviewed to establish if the staff member has contacted the exhibit/DNA during processing i.e. during examination, DNA extraction etc. If there is no evidence that the staff member has contacted the sample, an analytical investigation is required (Refer to section 8). For environmental monitoring samples which contain a possible staff match: Refer to QIS 23602 for required actions.

- If a **contamination between exhibits is suspected**, AUSLAB records should be reviewed (by extended enquiry function, user audits and/or audit trails) to establish who has handled the exhibits, when they were processed/moved, and where the exhibits have been located/examined. It may also be useful to refer to Forensic Register records (from QPS). This information should enable any potential cross contamination events - due to physical proximity (time/place/staff handling) to be identified. The possibility of transfer of DNA from exhibit to equipment (e.g. tweezers) and equipment on to the next exhibit should also be considered (swabbing and profiling the equipment may assist an investigation). If there is no evidence of physical proximity of the exhibits under investigation, an analytical investigation will be required (Refer to section 8).

- If a **contamination between DNA samples is suspected**, an analytical investigation is required (Refer to section 8).

Corrective actions and documentation:

In **all** cases of cross-contamination, specimen notes must be added to all affected samples. Specimen notes must record the adverse event and if applicable the corrective action (e.g. OQI). The investigation must be detailed in an OQI, in specimen notes, the teams' events register, and/or in I:\Quality & Projects\Investigations (Refer to section 8.2.3).

If after investigation it is determined that the sample/s have not been contaminated the results may be reported after the completion of the investigation documentation as described above. If after the investigation it is determined that the sample has been contaminated, in consultation with the Senior Scientist Quality and Projects or a Team Leader the sample may be failed. When a sample (or sub-sample) is failed, if it is possible - the item should be re-sampled/re-extracted and profiled. If it is not possible to reprocess the item, the item/sample must indicate a quality failure (with an EXH and/or by communications to QPS). Communications to QPS on sample failures will only occur in consultation with the Senior Scientist Quality and Projects and a Team Leader. An Intel letter may be required for this communication (Refer to QIS 24015).

5.3. Incorrect use of reagents

The incorrect use of reagents during the preparation of samples, or in the completion of a presumptive screening test, has the potential to detrimentally impact on further presumptive testing, DNA extraction and/or profiling results. If incorrect reagent usage is suspected, an investigation is required and the Senior Scientist Evidence Recovery, Senior Scientist Analytical or Senior Scientist Quality and Projects should be advised. The investigation into incorrect reagent usage should include:

- A check of the labelling on the reagents used for sample processing. Ensure that the correct reagent has been used, and that the reagent has not expired.
- Review all the reagents that have been used for the processing of the sample - as shown in the AUSLAB consumables audit trail. A check of other samples processed with the same reagent/s, is required to determine if the reagent has functioned (as expected) on previously tested samples.
- If the reagent is specific to a presumptive test, repeat the presumptive test with the suspected incorrect reagent (and if possible a known functional reagent) with the presumptive tests positive and negative controls (Refer to section 4). The function of the test on the controls - may provide information on the correct function of the reagents and/or the presumptive test.
- Note any unusual test results or test performance issues
- Ensure that the correct procedure has been used (refer to active QIS document as applicable)

Before any further testing is conducted, reagents should be re-prepared (if applicable), purchased (if applicable), and/or retested with positive and negative controls. All quality controls (positive and negative) must pass the criteria as outlined in section 4, section 8 and/or section 9 before further testing can be conducted on casework/reference samples.

Corrective actions and documentation:

In **all** cases of incorrect reagent usage, specimen notes must be added to all affected samples. Specimen notes must record the adverse event and if applicable the corrective action (e.g. OQI). The investigation must be detailed in an OQI, in specimen notes, and/or in I:\Quality & Projects\Investigations (Refer to section 8.2.3).

If after investigation it is determined that the sample/s have not been adversely affected, the results may be reported after the completion of the investigation documentation as described above. If after the investigation is complete it is determined that the sample has been adversely affected by incorrect use of reagents, but still has some evidentiary value, the impact of the event of the sample should be described in specimen notes.

If the sample is no longer suitable for reporting due to the adverse event, in consultation with the Senior Scientist Quality and Projects and a Team Leader the sample may be failed. Where a sample (or sub-sample) is failed, if it is possible - the item should be re-sampled/re-extracted and profiled. If it is not possible to reprocess the item/sample, the sample must indicate a quality failure (with an EXH and/or by communications to QPS). Communications to QPS on sample failures will only occur in consultation with the Senior Scientist Quality and Projects and a Team Leader. An Intel letter may be required for this communication (Refer to QIS 24015).

6. CASEWORK EXTRACTION AND AMPLIFICATION BATCHES: RESULTS ACCEPTANCE CRITERIA

Microcon and nucleospin batches contain a negative control only. For microcon and nucleospin batches, the negative control must pass (refer to Figure 2) for the batch to pass. If the negative control is not No Sizing Data (NSD), Analytical staff re-CE the plate to confirm the profile is reproducible, before initiating investigation processes.

All other casework extraction and amplification batches contain a minimum of one positive control and one negative control. If the positive control within a batch is not the expected full profile, and/or the negative control is not No Sizing Data (NSD), Analytical staff re-CE the plate to confirm the profile is reproducible, before initiating investigation processes. For an extraction or amplification batch to pass, **both the positive and negative controls must pass** as determined by the quality criteria indicated in Figure 1 (positive control criteria) and Figure 2 (negative control criteria).

In some rare circumstances, where the amplification batch positive control fails, it may be possible to use a sample as a "positive control", if the sample has been previously profiled and the profile results for the sample in this batch match its' previous profile (this approach requires consultation with the Team Leader, Senior Scientist Quality and Projects, or Senior Scientist Analytical).

Where the positive and/or negative control profile is not ideal (i.e. expected full profile for the positive control, and NSD for the negative control) there are specific actions which must be completed, and batch details assessed before the batch can be passed (Refer to Figure 1 and Figure 2). Figure 1 and Figure 2 outline the required actions and batch considerations which must be made prior to passing or failing a batch. The actions and considerations are dependant on the control profile/s results (i.e. partial profile, excess, or a mixture profile). **The required actions and batch check details in Figure 1 and Figure 2 are brief, for full details of requirements for each action refer section 8.**

7. QUANTIFICATION BATCHES: RESULTS ACCEPTANCE CRITERIA

Quantification batches have several quality criteria which need to be assessed to determine if the batch is passed or failed (Refer to Figure 3). In circumstances where quality criteria/thresholds are not met, the batch requires review and is to be discussed with the Senior Scientist Analytical (or Team Leader/Senior Scientist Quality and Projects) to determine batch outcome.

An initial evaluation of the extraction negative controls occurs during the quantification process (refer to section 6, and Figure 2 for additional information on extraction control quality guidelines). The quality control criteria and actions for quantification values in extraction negative controls are also detailed in the Quantification of Extracted DNA (19977) standard operating procedure.

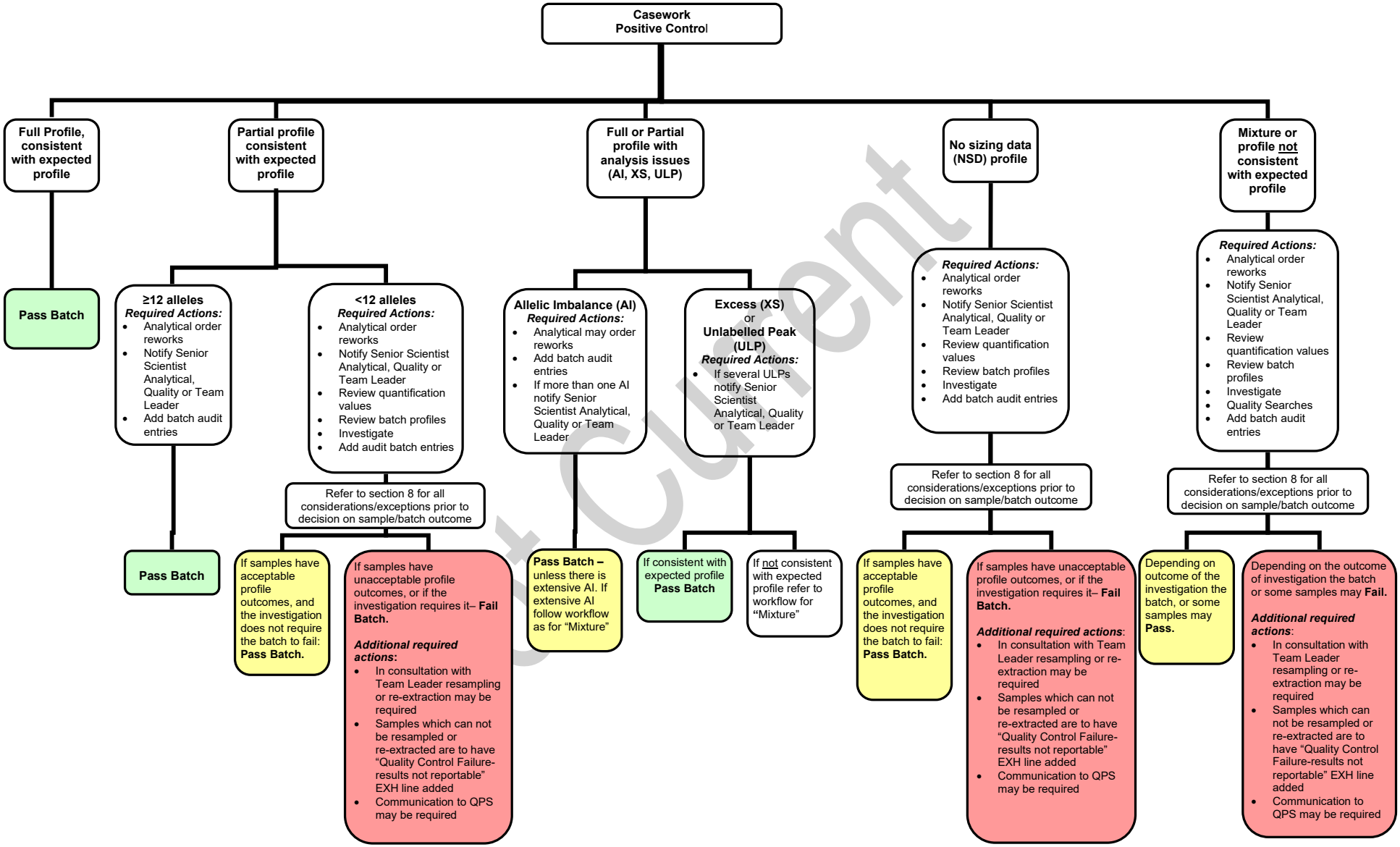

Figure 1: Casework Positive Control Workflow (Extraction and Amplification Batches). *Required actions and batch check details are brief - refer to section 8 for full details of requirements.*

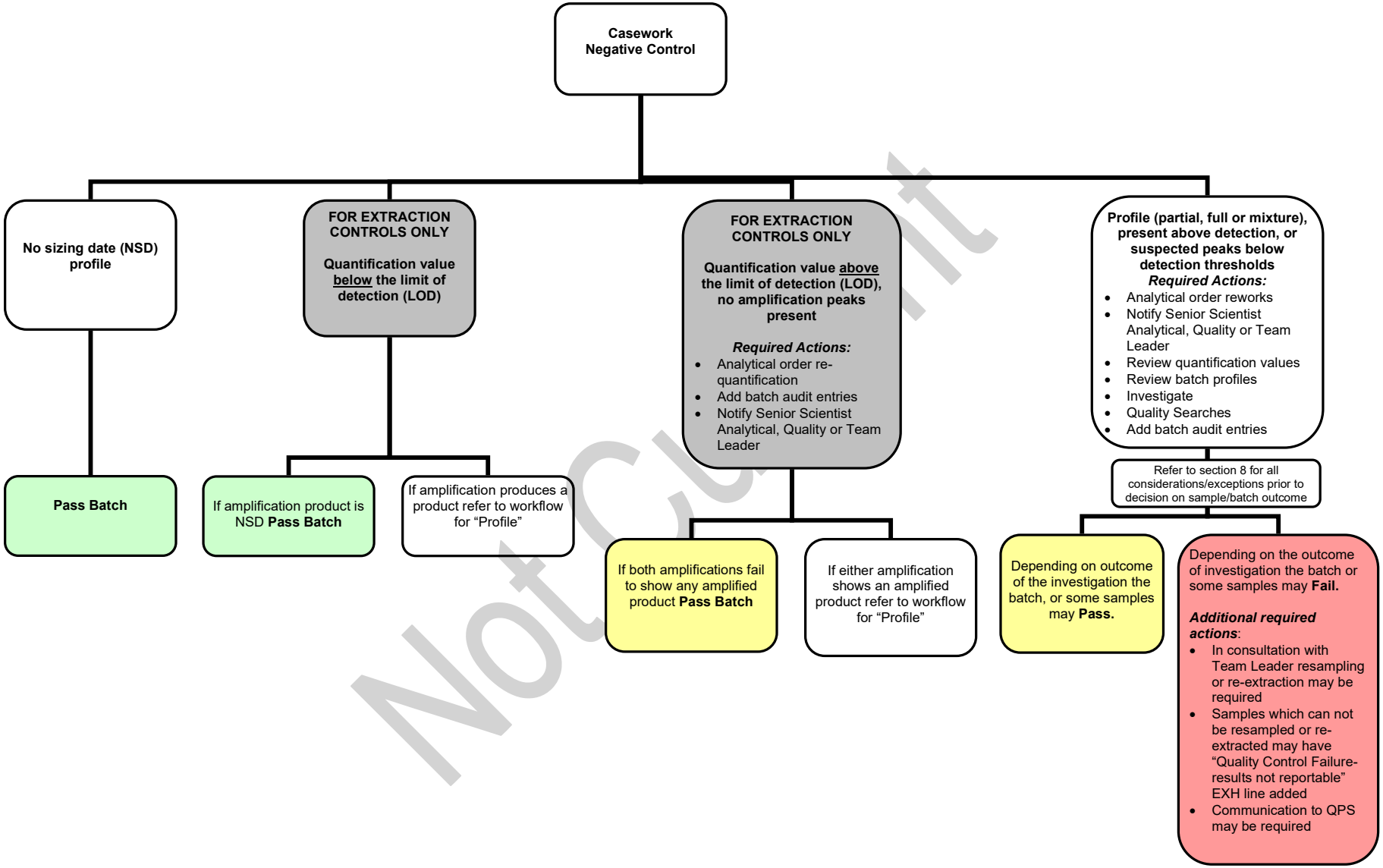

Figure 2: Casework Negative Control Workflow (Extraction and Amplification Batches). *Amplification negative controls are not processed through quantification - quantification values do not apply. Required actions and batch check details are brief - refer to section 8 for full details of requirements.*

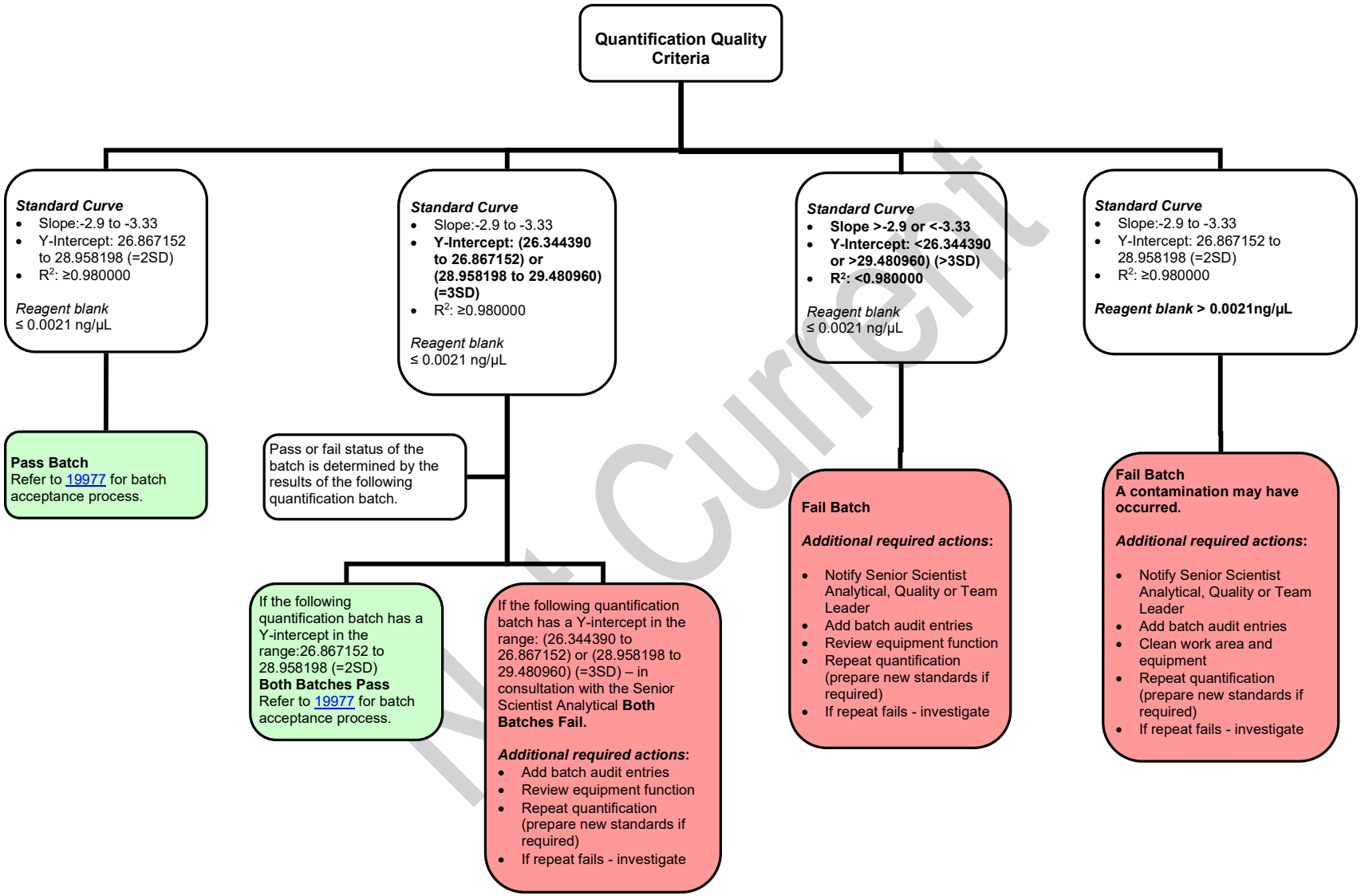

Figure 3: Quantification quality criteria and required actions. *Required actions and batch check details are brief - refer to section 8 for full details of requirements.*

8. INVESTIGATIONS INTO ADVERSE ANALYTICAL EVENTS: INCLUDING CONTROLS OUTSIDE ACCEPTANCE CRITERIA

In cases where unexpected profile/s are obtained from positive controls, DNA is detected in negative controls (from extraction or amplification batches), or a laboratory processing event has occurred which has the potential to cause a DNA contamination event, an investigation into the adverse event is required. Investigations into potential contamination events will be conducted in consultation with the Senior Scientist Analytical and the Senior Scientist Quality and Projects. Where possible, all results from batches under investigation should be placed on-hold until the outcome of the investigation is complete. Investigations should include the actions as described in sections 8.1-8.7.

8.1. Repetition of CE results prior to investigation

If the positive control within a batch does not pass (shown in yellow/red) in Figure 1, and/or the negative control within a batch does not pass (shown in yellow/red) in Figure 2, Analytical staff will re-CE the plate to confirm the profile outcome (i.e. is it reproducible), before an investigation is initiated (also consider CE carry-over as a possible source of unexpected alleles - particularly negative controls). Other adverse events may also require re-CE to confirm the adverse event is reproducible, before an investigation is initiated.

Analytical staff will order reworks on controls as per standard operating procedures QIS 24012 and QIS 17130 where it is indicated as necessary by the workflows in Figure 1 and Figure 2. Analytical staff should consider quantification values of the controls, the quantification values of the samples on the batch and profiling results before ordering the rework/s. Where an investigation is required, additional reworks may be requested by the staff that are completing the investigation (refer to section 8.7)

8.2. Investigation process

8.2.1 Results management

For samples or batches under investigation, where it is possible - **no results should be released until the investigation deems it suitable to do so** (part or all of the results may be released after the investigation – depending on the results). This may require the following actions:

- Where possible do not upload to AUSLAB results until the investigation is complete. If the investigation results in the batch failing – Do not upload failed batch results
- Add potentially affected samples/batches to the QEXH list, and remove them from case management lists.
- "DO NOT USE" may be added to 9PLEX or 9FTAR result (if results have already been uploaded to AUSLAB but not yet reported).
- Adding specimen notes and batch audit entries immediately. Batch audit entry should indicate that an investigation is required. For analytical investigations refer also to QIS 24012 for additional information on specimen and batch notes.
- If results have been reported discuss required actions with Senior Scientist Quality and Projects and the Team Leaders.
- Senior Scientist should email details of the investigation to the Management Team.

8.2.2 Required actions and considerations in investigations

Investigations should include the following steps:

- Batch audit entries must be used to detail the investigation process (refer to section 8.3).
- Review of batch audit entries, specimen notes and worksheet to evaluate if there have been any processing issues which may have affected samples or batches that are under investigation.
- Review the controls that relate to the sample/batches under investigation to ensure they meet quality criteria as detailed in Figure 1 - 5 as applicable.
- Review the batch profiles (refer to section 8.5) and quantification values if useful (refer to section 8.4).
- It may be useful in some circumstances, to check the function/programming of the equipment that was used (e.g. was the correct program used on the thermal cycler, was the performance of the 3130xl Genetic Analyzer suitable for interpretation).
- A check of the controls, chemicals/kits and reagents that have been used may be useful (information located on worksheets and AUSLAB material audit history) including:

  - correct control for the batch (e.g. the correct FTA control card punched)
  - expiry date of reagents/kit
  - has the reagent/kit functioned on a previous and a subsequent batch
  - in cases of contamination, consider reagents/chemical as a possible source

- In consultation with Team Leaders and Senior Scientist Quality and Projects order reworks (e.g. microcons, re-amplification, re-extractions etc) if they will provide additional information to the investigation. Refer to section 8.7 for rework strategies for investigation purposes. However before reworks are ordered the amount of sample available for testing should be carefully considered. Additional quality searches and batch checks may be required on reworked samples.
- Complete quality search if applicable (refer section 8.6). There may be instances where a quality search is completed at the beginning of an investigation and then repeated after rework results have been obtained.
- Raising an OQI should be considered, particularly in instances of a significant or reoccurring adverse event. If an OQI has been raised – the findings of the investigation will be recorded within QIS.

8.2.3 Documentation of investigation

On completion of the investigation, detailed batch audit entries (refer section 8.3) and/or specimen notes should be completed for all affected samples.

Where results are released to AUSLAB for interpretation (and there have been unexpected processing issues or profiling results for the sample/batch) notes should include: a description of the adverse event, the investigation that was completed, the corrective actions completed (if applicable), the impact of the event on the sample/s, and the considerations that are required for the interpretation of the profile/s as a result of the issue.

Where results are not suitable for release notes should include: a description of the adverse event, the investigation that was completed, the corrective actions completed (if applicable) and the impact of the event on the sample/s. A clear statement that the results are not suitable for interpretation or reporting should be made.

Failed samples/batches will need to be repunched/reworked (if they are reference samples), or reworked/re-extracted/resampled or failed (if they are casework). Failure of samples will occur only in consultation with a Team Leader/Senior Scientist Analytical/Senior Scientist Quality and Projects, and may require additional communications

with the QPS. If needed, supporting data and information for investigations into adverse events can be stored to network drive I:\Quality & Projects\Investigations or the the teams' events register. Issue/s and findings (including OQIs) may also be discussed in relevant team meetings, to alert staff to quality issue/s.

8.3. Batch audit entries

Batch audit entries must:

- Be entered in a timely way.
- Should be added progressively if the batch is under investigation. For example if a batch is on hold pending the results of an investigation, the batch audit entry must state that the batch is under investigation. As reworks are ordered for the purpose of investigation, the details of the reworks and the implications of the findings of the rework/s should be stated in the batch audit entry.
- For analytical investigations refer to QIS 24012 for additional information on specimen and batch notes.
- Clearly state if the batch fails, passes (with no quality issues) or passes (but has been affected by one or more quality issues which are detailed in the batch audit entries)
- If there is a quality issue with the batch – the batch audit entry must clearly state what the issue is, the action/s taken (i.e. investigation details), and the outcome of the actions/investigations.
- If there is a quality issue with the batch – the batch audit entry must be accompanied by specimen notes on all samples on the batch; the specimen note must refer to the batch audit entry e.g. the specimen note would state: "Refer to batch audit entry CWGMP2012XXXXXX_XX, in addition to having "*See specimen note*" entered into the comment field for each 9PLEX or 9FTAR page (as applicable).
- Where the negative control on a batch has a quantification value, the batch audit entry must state the quantification value obtained from the negative control, and state if that value is >or< the limit of detection (LOD), or limit of reporting (LOR).
- If an OQI is raised as a result of findings/investigations, the batch audit entry, and specimen notes (for all samples on the batch) should have the OQI number entered into the notes.

8.4. Review quantification values (controls and samples)

Reviews of quantification values of individual samples and/or a batch is beneficial when:

- a quantification batch is under investigation - due to the controls not meeting the quality criteria as described in Figure 3
- a negative control has a quantification value (particularly >LOD)
- an adverse event has occurred that impacts significantly on DNA yield
- an adverse event has occurred and the quantity of DNA in the samples adversely affected would inform the investigation.
- to determine if reworks should be ordered for investigation purposes

A review of a quantification batch, requires a scientist to make an assessment of the expected quantification values (based on sample type and previous quantification results) in comparison with the quantification values obtained from the sample/batch under investigation.

8.5. Review batch profiles (controls and samples)

Where an unexpected profile has been obtained in a control, or there has been an adverse event on a batch which has required investigation, a check of the profiles from the other samples and controls in the batch is required. This check is usually completed in Genemapper ID-X (not Auslab), so that below reporting threshold peaks can be reviewed/assessed. All controls that related to a batch and/or samples under investigation should be reviewed to ensure they meet acceptable quality criteria (refer to Figure 1-5 as applicable).

For casework samples that have been processed on an automated platform this batch check may include an Extraction Batch (EB) check at lowered thresholds (refer to QIS 17119).

For FTA reference batches - the batch review should include a visual inspection of the plate to ensure the correct location and number of spots is present in each well (Refer to section 11 for FTA investigations).

The purpose of the batch review is to:

- Identify any additional quality issues on the batch/plate (if present)
- Establish possible sources of unexpected alleles within a control/sample that may have sourced from within the batch/es in which the sample/controls have been processed (if applicable).
- To assess if an event has impacted on some or all of the controls/samples (e.g. poor amplification)

Examples of batch reviews:

- Where a negative control contains a part or full profile, the review of the batch would aim to determine if any samples from within that batch could have contributed to the alleles that have been observed in the negative control.
- If a positive amplification control was NSD the batch check would determine if it is the control only, or the entire batch that failed to amplify.
- If an FTA or FTA control produced a mixture profile, the batch review would be searching for the source of the additional alleles from FTAs processed on the same batch/plate.

Instances where adverse events impact on casework samples are more difficult to investigate, and may require mixture interpretation to determine if cross-contamination within batches has occurred.

8.6. Quality searches

Quality searches are to be performed when the source of an unexpected profile is not able to be determined (e.g. a profile in a negative control that does not match a sample on a batch). Quality searches can only be completed by the Managing Scientist or the Senior Scientist Quality and Projects. If a quality search is required, a copy of the profile requiring a search will be required. A quality search consists of a search against DNA Analysis staff, QPS staff (if applicable), the unknown profiles database, and a search against all casework and all reference samples that have been processed within DNA Analysis. The quality search may identify possible sources of the unknown profile, and can inform investigations.

In cases where the source of an unknown profile involved in an investigation is not able to be determined, the unknown profile will be uploaded to the “Unknown profile” database that is

maintained by the quality team. This will ensure that any future occurrences of this profile can be identified.

8.7. Rework strategies for investigation purposes

Reworks including microcons, nucleospins or re-amplifications should be requested if they will provide additional information to the investigation. However before reworks are ordered the amount of sample available for testing should be carefully considered. Examples of the use of reworks for investigations include:

Improving profiles for quality searches/match purposes:

- A microcon may be ordered to increase the number of alleles present in a partial/below threshold profile
- Reamplifications at higher DNA concentrations to increase available alleles

Reworks to establish time/source of contamination/s:

- A re-preparation/CE may establish if a contamination occurred at/prior to amplification (if the result is reproducible) or occurred during CE (if the result is not reproducible)
- A re-amplification may establish if a contamination occurred at/prior to extraction (if result is reproducible) or occurred during amplification (if result is not reproducible)
- A re-extraction/re-punch may establish if a contamination occurred during extraction

The quantification values for samples under investigation should be considered. Samples with low quantification values may not produce uniform profiling results - due to the stochastic effect of PCR. Samples with high quantification values should profile consistently. Additional quality searches and batch checks may be required on reworked samples.

9. FTA REFERENCE BATCH CONTROLS: RESULTS ACCEPTANCE CRITERIA

FTA reference batches (this does not include EFTA batches – Refer to section 10 for EFTA samples) contain two positive controls (1 spot control, 2 spot control) and a negative control. If the positive control/s within a batch are not the expected full profile, and/or the negative control is not No Sizing Data (NSD), Analytical staff re-CE the plate to confirm the profile is reproducible, before investigation processes are initiated. For an FTA batch to pass, **both a positive and negative control must pass** as determined by the quality criteria indicated in Figure 4 (positive control criteria) and Figure 5 (negative control criteria). Given that each FTA batch contains two positive controls, the best of the two control profiles is assessed in the Figure 4 workflow to determine required actions for the batch (i.e. if one of the positive controls passes the batch can be accepted, even if the second positive control may not meet required quality criteria).

In some rare circumstances, where the batch positive control fails, it may be possible to use a sample as a "positive control", if the sample has been previously profiled and the profile results for the sample in this batch match its' previous profile (this approach requires consultation with the Team Leader and Senior Scientist Quality and Projects).

Where the positive and/or negative control profile is not ideal (i.e. expected full profile for the positive control, and NSD for the negative control) there are specific actions which must be completed, and batch details assessed before the batch can be passed (Refer to Figure 4 and Figure 5). Figure 4 and Figure 5 outline the required actions and batch considerations which must be made prior to passing or failing a batch. The actions and considerations are dependant on the control profile/s results (i.e. partial profile, excess, or a mixture profile). **The required actions and batch check details in Figure 4 and Figure 5 are brief, for full details of requirements for each action refer section 8 and section 11.**

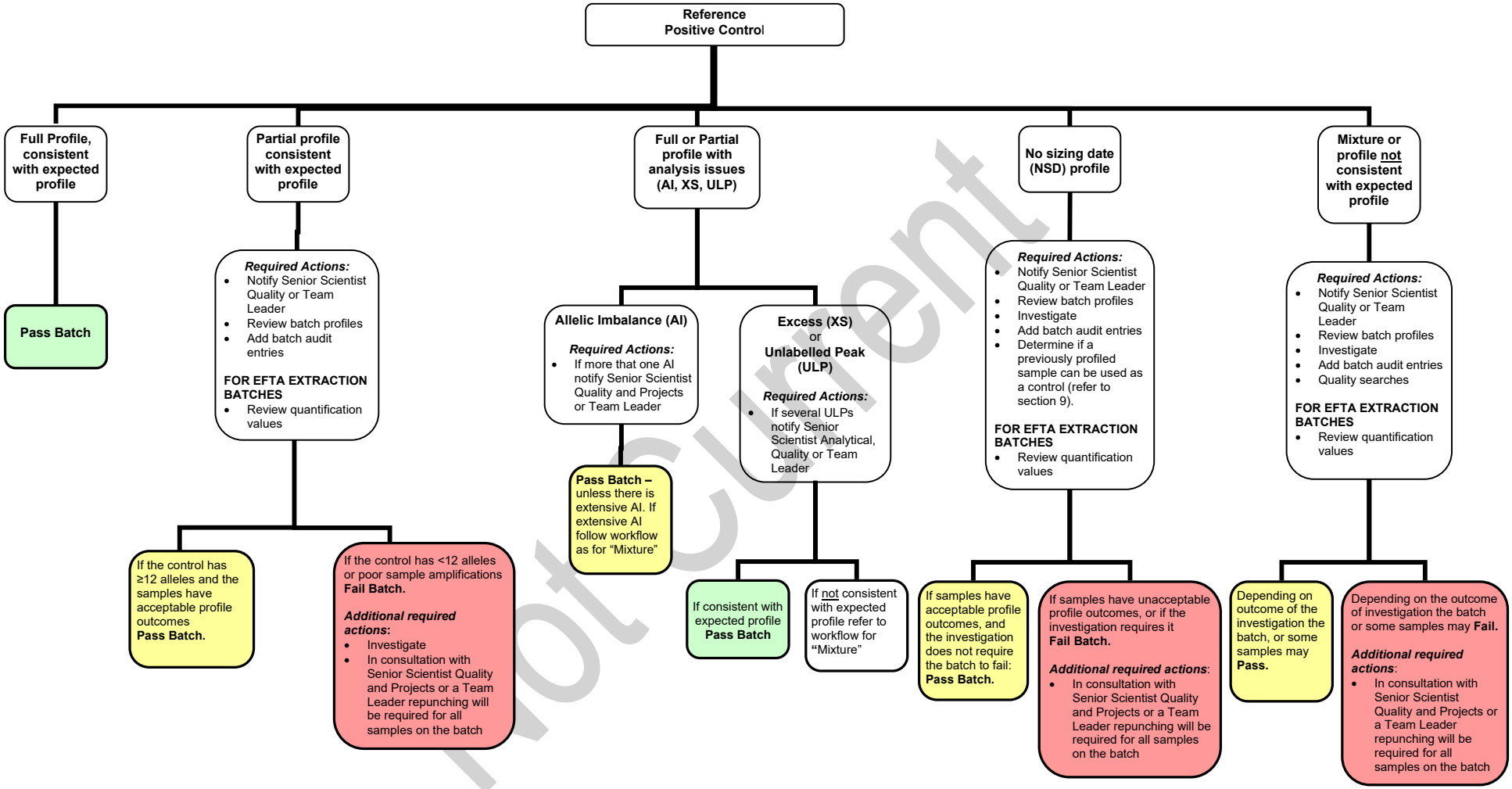

Figure 4: Reference Positive Control Workflow (EFTA Extraction, EFTA Amplification and FTA Batches). *FTA batches have two positive controls, the best of the two control profiles is assessed in this workflow to determine required actions. Refer to section 8 for full details of required actions.*

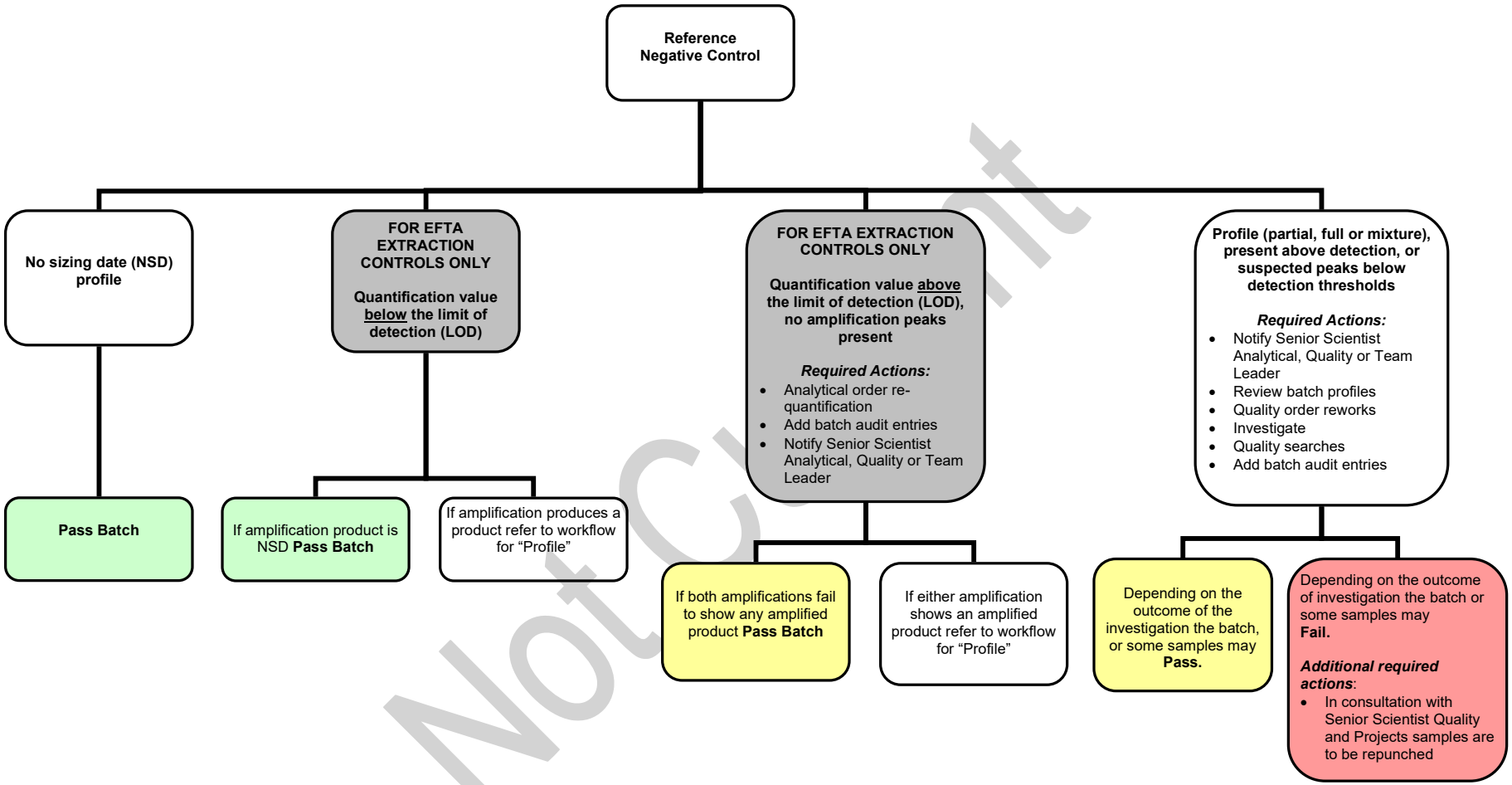

Figure 5: Reference Negative Control Workflow (EFTA Extraction, EFTA Amplification and FTA Batches). *Amplification negative controls are not processed through quantification - quantification values do not apply. Required actions and batch check details are brief - refer to section 8 for full details of requirements.*

10. EXTRACTION FTA (EFTA) BATCH CONTROLS: RESULTS ACCEPTANCE CRITERIA

FTA samples which are processed through a DNA IQ extraction process - are Extraction FTA samples (EFTA). EFTA punch batches contain a negative control only, and do not contain a positive control on the batch. EFTA differ from standard FTA processing, as the samples are processed through an extraction batch, a quantification batch and an amplification batch - each of which have specific controls. EFTA sample quality guidelines and required actions for each step of processing are listed in Table 1. In circumstances where an EFTA batch fails (as a result of not meeting the quality criteria) adequate batch audit entries and specimen notes are required, and an investigation should be initiated (Refer section 8 and section 11). Where a significant or reoccurring quality issue is identified an OQI should be raised.

Table 1: Extraction FTA (EFTA) batches: quality criteria and required actions.

Batch Type	Control	Actions
Punching batch	Negative Control	EFTA negative control pass/fail batch status and required actions are the same as those for standard FTA Negative Controls **Refer to Figure 5** for workflow
Extraction Batch	Positive Control	EFTA positive control pass/fail batch status and required actions are the same as those for standard FTA Positive Controls **Refer to Figure 4** for workflow
	Negative Control	EFTA negative control pass/fail batch status and required actions are the same as those for standard FTA Negative Controls **Refer to Figure 5** for workflow
Quantification Batch		EFTA batch control pass/fail batch status and required actions – Refer to **Figure 3** for workflow.
Amplification Batch	Positive Control	EFTA positive control pass/fail batch status and required actions are the same as those for standard FTA Positive Controls Refer to **Figure 4** for workflow
	Negative Control	EFTA negative control pass/fail batch status and required actions are the same as those for standard FTA Negative Controls Refer to **Figure 5** for workflow

11. INVESTIGATION PROCESSES FOR ADVERSE EVENTS IN FTA PROCESSING

In cases where a mixed profile results from an FTA sample, an unexpected profile is obtained from a positive control, DNA is detected in a negative control, or a laboratory processing event has occurred which has the potential to cause a DNA contamination an investigation into the adverse event is required. In each case, the adversely affected control/sample should undergo a re-CE to confirm the adverse event is reproducible, before an investigation is initiated.

Investigations into potential contamination events will be conducted in consultation with the Senior Scientist Quality and Projects or Team Leader. Where possible, all results from batches under review should be placed on-hold until the outcome of the investigation is complete.

For EFTA batches: If an EFTA extraction, quantification or amplification batch fails – only the samples on the affected batch/es will be failed. These failed samples will likely be distributed over several plate reading batches. For failed EFTA batches partial plate

reading batches may thus need to be uploaded (i.e. with the failed samples removed). This differs from FTA plates which are processed together during punching, amplification and plate reading.

Investigations should include the following actions:

- **Ensure results are not incorrectly utilised or reported.** Refer to Section 8.2.1 for results management guidelines
- Check all positive and negative control samples meet quality guidelines (Refer to Figure 4 and Figure 5).
- Review batch audit entries, specimen notes and worksheet to evaluate if there have been any processing issues which may have affected samples or batches that are under investigation.
- For FTA batches visually inspect the plate (not applicable to EFTA batches) for the correct number of punch spots in each well.
- Batch profiles must be checked - refer to section 8.5. For EFTA batches a review of quantification results may also be required (refer to section 8.4).
- It may be useful in some circumstances, to check the function and or programming of the equipment that was used (e.g. was the correct program used on the thermal cycler, was the performance of the 3130xl Genetic Analyzer suitable for interpretation).
- A check of the controls, chemicals/kits and reagents that have been used may be useful (information located on worksheets and AUSLAB material audit history) including:
  - correct control for the batch (e.g. the correct FTA control card punched)
  - expiry date of reagents/kit
  - has the reagent/kit functioned on a previous and subsequent batch
  - in cases of contamination, consider reagents/chemical as a possible source
- For EFTA investigations order reworks (e.g. microcons, re-amplification etc.) if they will provide additional information to the investigation. Refer to section 8.7 for rework strategies for investigation purposes. Additional quality searches and batch checks may be required on reworked samples.
- Complete quality search if applicable (refer section 8.6). There may be instances where a quality search is completed at the beginning of an investigation and then repeated after rework results have been obtained.
- Raising an OQI should be considered, particularly in instances of a significant or reoccurring adverse event. If an OQI has been raised – the findings of the investigation will be recorded within QIS.
- All investigation findings must be documented as per section 8.2.3
- A photocopy of the failed batch/plate paperwork to be given to Quality Team, as it will be filed in the “FTA Investigations” folders.
- On completion of the investigation: ensure all affected reference samples have been reprocessed, such that reportable results are available.

12. INVESTIGATION INTO REFERENCE SAMPLE MIXTURE PROFILES

Reference samples are expected to be single source samples. In cases where a mixed profile is obtained from an FTA sample, an investigation is required. The investigation will aim to determine if a DNA contamination has occurred within the DNA Analysis Unit

Laboratory or if the sample that was submitted to DNA Analysis (as a reference sample) was not a single source specimen.

A mixture in a reference sample may result from occurrences such as: an FTA card contamination (pre or post delivery to DNA Analysis), BSD punch carryover, FTA spots moving within a plate, labware contamination, reagent contamination, cross contamination during washing/extraction/quantification/amplification, or in very rare circumstances it may be the correct profile for a person.

Before an investigation is initiated the adversely affected sample should under go a re-CE to confirm the adverse event is reproducible. Investigations into potential contamination events will be conducted in consultation with the Senior Scientist Quality and Projects. Where possible, all results from batches under review should be placed on-hold until the outcome of the investigation is complete.

If the re-CE confirms that the result is reproducible, the following ***initial investigation steps are required:***

- Review batch audit entries, specimen notes and worksheet to evaluate if there have been any processing issues which may have affected the sample.
- For FTA batches visually inspect the plate (not applicable to EFTA batches) for the correct number of punch spots in each well.
- Batch profiles must be checked - refer to section 8.5.
- Check all positive and negative control samples meet quality guidelines (Refer to Figure 4 and Figure 5).
- Complete quality search (refer section 8.6).

12.1. Investigation actions for FTA samples with a reproducible mixture

If on completion of the initial investigation actions above (section 12) it is determined that there are multiple quality issues with the plate (i.e. multiple samples on the plate contain mixtures, control failures) the plate should be **failed**. For a failed batch refer to section 11 for investigation processes and required actions.

If there is only one sample on the plate/batch that is a mixture, but the source of the mixture is not able to be determined, after completion of the initial investigations actions above (section 12), a REPUNCH of the sample which has produced the mixture profile should be requested. The plate/batch on which the mixture sample was processed should be placed on hold pending the results of the RPUNCH rework.

If the REPUNCH of the sample confirms the mixture profile, and there are no additional mixtures or analysis issues identified during the batch profiles check (refer to section 8.5), in consultation with Senior Scientist Quality and Projects - the batch may be **passed**. All investigation findings must be documented as per section 8.2.3. The batch can be passed as the mixture has been confirmed as the correct profile for that FTA card, and not as a result of a sample processing issue. However, due to the FTA card producing a mixture profile, it is not suitable as a reference sample and as such a Team Leader should also be advised as a new FTA sample needs to be requested from QPS.

If the REPUNCH is single source in consultation with Senior Scientist Quality and Projects the batch - should be **failed**, as a contamination event has occurred. For a failed batch refer to section section 11 for investigation processes and required actions.

12.2. Investigation actions for EFTA samples with a reproducible mixture

If a mixture profile in an EFTA sample is reproducible (after re-CE) but the initial investigation actions above (section 12) are not able to determine the source/cause of the mixture profile the following actions are required:

Order a re-extraction (EFTA) of the mixture FTA samples (under a connected barcode) and a re-amplification from the initial EFTA sample to determine if the contamination has occurred prior to extraction, during extraction or during amplification. Where possible the batches on which the mixture sample was processed should be placed on hold pending the rework results.

If the re-extraction and re-amplification confirm the mixture profile (and there are no additional quality issues identified during the initial investigation actions above (section 12), in consultation with Senior Scientist Quality and Projects - the batch may be **passed**. All investigation findings must be documented as per section 8.2.3. A Team Leader should also be advised as a new FTA sample may be required.

If the re-extraction and re-amplification is single source (or there are quality issues with the batch/es) in consultation with Senior Scientist Quality and Projects - the batch should be **failed**. For a failed batch refer to section 11 for investigation processes and required actions. The results from the re-extraction and re-amplification will provide information to the investigation on the likely time/process at which the contamination occurred.

If an EFTA extraction, quantification or amplification batch fails – only the samples on the affected batch/es will be failed. These failed samples will likely be distributed over several plate reading batches. For failed EFTA batches partial plate reading batches may thus need to be uploaded (i.e. with the failed samples removed).

13. INVESTIGATING A SAMPLE WHICH HAS DIFFERENT PROFILING RESULTS

Where a casework or a reference sample is profiled twice, and the two resulting profiles do not match, Re-CE of both amplified profiles (Refer to QIS 17130) should be ordered (Note: alleles present in a mixture may vary between amplifications). After Re-CE:

If it is confirmed that both sample profiles are the same:

- Advise Senior Scientist Quality and Projects
- Investigate incorrect CE result (refer to section 8 for casework, section 11 for reference samples)
- Ensure that no incorrect profiles have been reported.

If it is confirmed that the sample profiles are different:

- Advise Senior Scientist Quality and Projects
- Order re-extraction (casework) or re-punch (reference) of the sample.
- Investigate incorrect CE result (refer to section 8 for casework, section 11 for reference samples)
- View samples to ensure they have been correctly labelled
- Ensure that no incorrect profiles have been reported.

All investigation processes, actions and reporting in relation to this type of adverse event will be as described section 8 for casework, section 11 for reference samples.

14. RECORDS

AUSLAB batch audit entries, specimen notes and UR notes (as appropriate) will detail results from adverse events, adverse event investigation/s and outcomes of investigations.
OQIs within QIS may used: particularly in instances of a significant adverse event.
If needed, supporting data and information for investigations into adverse events can be stored to network drive I:\Quality & Projects\Investigations.

Where investigations into reference batch failures have been completed a photocopy of the plate/batch paperwork should be given to Quality to be filed in the "FTA Investigations" folders.

15. ASSOCIATED DOCUMENTATION

QIS: 10001 Quality Information System
QIS: 13965 Opportunities for Quality Improvement (OQIs) Management Procedure (CaSS)
QIS: 17119 Procedure for the Release of Results
QIS: 17130 CE Quality Check of Samples from the ABI Prism 3130xl Genetic Analyzers
QIS: 17154 Procedure for Quality Practice in DNA Analysis
QIS: 17155 Procedure for Errors, Major Concerns, System Breakdowns
QIS: 17186 The acid phosphatise screening test for seminal stains
QIS: 17190 Tetramethylbenzidine screening test for blood
QIS: 17193 Phadebas test for saliva
QIS: 19976 Amplification of Extracted DNA using the AmpFℓSTR® Profiler Plus® Kit or AmpFℓSTR® COfiler® Kit
QIS: 19977 Quantification of Extracted DNA using the Quantifiler™ Human DNA Quantification Kit
QIS: 24015 Procedure for Intelligence Reports and Interstate/Interpol Requests
QIS: 24012 Miscellaneous Analytical Section tasks
QIS: 24823 FTA Processing and Work Instructions.

16. REFERENCES

ABAcard® p30 Test For The Forensic Identification of Semen. Technical Information sheet. Abacus Diagnostics, Inc.

17. AMENDMENT HISTORY

Version	Date	Updated By	Amendments
1	5 April 2012	K Scott	First Issue

18. APPENDICES

Nil

CaSS | Forensic and Scientific Services
A CLINICAL AND STATEWIDE SERVICE

Agenda

QPS

Chairperson:	CJA	**Date and Time:**	31/07/08 11am
Venue:	Conference Room 102	**Contact:**	CJA
Attendees:	GShaw, EJC, MK, TC, TSO		
Apologies:	GSmith		
Guests:			

1.0 AGENDA

Outline events
(6 batches over past 6 months where profiles in neg controls (4 CW, 2 Ref); each event extensively investigated however root cause unable to be isolated. Decision made to not report results from these batches as the controls didn't meet documented quality requirements. Audit of the process identified number of issues and suggestions for improving – still has not definitively determined root cause – suggest a combination of syringe wear and tear and programming of pipetting of instrument. Validation was extensively – Promega asked for a copy of the report as we had validated aspects they had not)

Discuss cases affected (Total: 113; Major: 27, Volume: 86)
Refer to hand out

Strategies for reported results & statements
One statement had been issued – updated EXR results, retracted & replaced statement, system approach to retracting previously reported results via EXR (based on court dates in the first instance). One Link affected due to casework profile being an affected batch – however other result available therefore nil affect, however may be others. Reference samples – quarantined on NCIDD, some samples to be repeated.

Audit of all results from automated extraction platforms
Currently auditing all DNA results generated from automated platforms where controls passed, to ensure that current quality controls are adequately monitoring the extractions.

Strategies for automated platforms
Liquid handling platform specialist currently on-site, reviewing pipetting and programming of extraction platforms, will conduct more validation/verification, run number of soccerball, checkerboard and zebra plates. Begin with reference samples, then migrate back to casework. Investigating purchase of additional piece of equipment to check pipetting accuracy more easily.

Throughput of Analytical section
Decreased capacity, down to approx 50% (same as prior to implementation of platforms), anticipate this situation for 3-4 weeks at least, however, urgent cases can be processed during that period with prior communication

2.0 NEW BUSINESS

3.0 CLOSURE

From: Cathie Allen
To: Allan McNevin; Iman Muharam; Justin Howes; Paula Taylor; Thomas Nurt...
Date: 12/18/2008 4:30 pm
Subject: Fwd: RE: Visit to the Lab

Hi Everyone,

Ross Martin, from DPP would like to tour the lab and get clarification on the recent adverse events that we've had.

I will let him know that if he wants to see the robots working, then he'll need to hand over some DNA.

Iman - can you please organise for a demo on the robot & the seal we used to use.

Tom - if you can have the paperwork handy so I can go through it with him.

Cheers,
Cathie

>>> "Ross Martin" [redacted] 17/12/2008 5:56 pm >>>
Thanks Cathie. I was aiming for Monday at about 10.00. I specifically am interested in having available the details of the matters we discussed at the meeting recently at the DPP offices. I am hoping to see the machines affected, a demonstration of what it is believed went wrong, and a look at relevant paperwork so I can form an understanding upon which to base our response.

Ross Martin SC
Special Counsel (Major Prosecutions)
[redacted]

From: Cathie Allen [redacted]
Sent: Tuesday, 16 December 2008 6:33 PM
To: Ross Martin
Subject: Visit to the Lab

Hi Ross,

We would be pleased to host a visit to the laboratory at any time. Please email me with a time and day that is suitable to yourself (and any others you would like to include) and I will set up a tour for you.

Cheers,
Cathie.

Cathie Allen BSc MSc (Forensic Science)
A/Managing Scientist
DNA Analysis (formerly Forensic Biology)
Queensland Health Forensic & Scientific Services
[redacted]

For Exhibit TN-42 please refer to IT file for this document