SUPPLEMENTARY STATEMENT OF PAULA BRISOTTO

I, Paula Michelle Brisotto, care of Queensland Health Forensic and Scientific Service, Team Leader Evidence Recovery and Quality Team, Forensic DNA Analysis, do solemnly and sincerely declare that:

1. I am employed by Queensland Health Forensic and Scientific Service ('QHFSS').

2. I hold a Bachelor of Science from Griffith University, and a Master of Science in Forensic Science from Griffith University.

3. I have previously provided four statements to the Commissioner, dated 9 August 2022, 25 August 2022, 21 September 2022 and 17 October 2022. This is a supplementary statement to those previous statements.

VALIDATIONS

4. In relation to validations of instruments or scientific process changes, I have not performed any validations myself, since mid-2003 when I was promoted to the Volume Crime Team. Prior to that, I worked as a technical officer within the Analytical team and may have been involved in some validations but would not have led the project myself.

5. There have been many validations I have endorsed over the years while employed at QHFSS. I have collated a list of these validations into an excel spreadsheet attached as exhibit **PB138.**

6. Every proposal and final validation report is provided to NATA. NATA do an audit of these proposals and validations every two years where they attend the laboratory on site and view the validation documentation and audit our procedures. They review training modules and SOPs, and they can request further documentation as requested. Kirsten Scott is the main contact for preparing the documents for these audits.

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7. The purpose of endorsing a project proposal is to provide feedback on the proposal and complete a risk assessment in accordance with SOP 22872 *"Project Risk Assessment"*. The purpose of approving the final report is to consider the recommendations put forward by the project team. Depending on the subject of the project, the project may involve the validation or verification of an instrument or system.

8. If the Management Team, as the endorsers of the project, require further information, the document will be returned to the project team for editing before being resubmitted to the Management Team for further consideration.

9. If the recommendations in the final report are endorsed by the Management Team and approved by the Managing Scientist, the recommendations are implemented in accordance with SOP 22871 which is attached to this statement as **PB139**.

10. The relevant SOP 22871 "*Procedure for Change Management in Forensic DNA Analysis"* details the process for project approval and completion. SOP 22871 (version 17) provides that the Management Team must provide feedback on the proposal, complete the risk assessment, provide feedback on the final report and then e-sign the documents as/when requested by the project leader/line manager.

11. The Management Team members are the reviewers/endorsers as detailed in SOP 22871. The project manager is generally the line manager of the team implementing the change. The project officers are staff within the relevant team or with the relevant competencies to undertake the tasks within the projects (for example, Analytical processing). There is also opportunity for staff with the relevant laboratory skills to be mentored by a senior scientist or experienced staff member if they have not previously undertaken a project.

12. A technical reviewer's role is to 'peer view' critical technical aspects of the project (e.g. new instrument programs/settings, new analytical procedures) and/or to review data analysis within the project (e.g. Excel data transformations, formula's and calculations etc.).

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13. A technical reviewer is generally nominated at the project proposal stage by the project manager or the Management Team to 'peer view' critical technical aspects of the project and/or to review data analysis. The technical reviewer should provide a written document to the Management Team that outlines the aspects of the project reviewed and general findings.

14. The staff selected for the project may be chosen by the project manager or by expression of interest, if the project is large. The endorsement of the validations is then conducted by the Management Team.

15. I do not have concerns with validation or endorsement as we do not undertake research, and most of the project work undertaken is not novel. Generally, with instruments or commercial kits, these are developmentally validated by the manufacturer and internally we are assessing how they perform in our hands, and if they are fit for purpose.

16. I do not have any concerns with the validation or endorsement process. I think the documented change management process and associated SOPs are suitable for the purposes of verification and validation within the Forensic DNA Analysis laboratory. That being said, continuous quality improvements are part of the systems and process in place, and processes will continue to improve as we find better ways of doing things. I am of the view that further resourcing, specific staff time allocation and shared knowledge/processes with other forensic laboratories may further improve the process.

NO DNA DETECTED

17. Review and validation of the result line "No DNA Detected" allows the result to be viewed by the QPS in the Forensic Register, with the expanded wording "*This item/sample was submitted for DNA analysis; however no DNA was detected above the limit of detection at the quantitation stage. No further processing was conducted on this item*"; this is in accordance with SOP 34229; version 3 of which is attached to my statement of 21 September 2022 as **PB72**

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18. The task of validating the "No DNA Detected" results is a task allocated to the Analytical team and is undertaken by the scientists within the team who are competent in this task. The process is listed in SOP 34064 "*Miscellaneous Analytical Procedures and Tasks*" at section 9.1; version 3 of SOP 34064 is attached to this statement as **PB140.**

19. The process for validating 'No DNA Detected' results is listed in SOP 34064. The Senior Scientist of the Analytical team, who is currently Luke Ryan, has update responsibility for SOP 34064. Staff within the Analytical team may assist in authoring updates to the SOP, as delegated by Luke Ryan. Any updates to the SOP must be approved by the Managing Scientist.

20. Every SOP undergoes review after a minimum of 18 months. This is a general requirement set in QIS, however, updates to a SOP can occur at any time. For example, if there is a change to the process, the SOP will then undergo review and approval by the Managing Scientist within QIS. Comments can be added against the SOP in QIS, indicating changes required for update in the next revision. This process would apply to SOP 34064.

21. I do not undertake these tasks, however my understanding is prior to the validation of the "No DNA Detected" result, the scientist undertaking the quantification batch performs a number of checks prior to completing the batch. These checks are set out in SOP 34045 "*Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit*" at section 8 "Batch Finalisation". On completion of the batch, the samples become available for validation. Version 7 of SOP 34045 is attached to this statement as **PB141.**

22. The Forensic Register is programmed to assign a Technique and Method based on the quantification result. The scientist who undertakes the quantification process will perform the checks and finalise the batch in accordance with SOP 34045, as outlined above.

23. The scientist performing the validation of the result will ensure that the quantification value is within the range outlined in Table 9 of SOP 34045, save for the DNA

Insufficient range referenced in the table for which the process is contained in the comments for SOP 34045 following the 6 June 2022 decision:

Table 9 Default values for Quant Results page.

Type	Quantification Value	Priority	Technique	Method
CW	<0.001 ng/µL	P1	No DNA Detected	Blank
	≥0.001 – ≤0.0088 ng/µL	P1	Post-Extraction	Microcon PowerPlex21
	>0.0088 ng/µL	P1	STR Amplification	PowerPlex21 3500xl
	>5 ng/µL	P1	Post-Extraction	Dilution
	<0.001 ng/µL	P2 or P3	No DNA Detected	Blank
	≥ 0.001 – 0.0088 ng/µL	P2 or P3	DNA Insufficient	Blank
	>0.0088 ng/µL	P2 or P3	STR Amplification	PowerPlex21 3500xl
	>5 ng/µL	P2 or P3	Post-Extraction	Dilution
	<0.001 ng/µL	QPS ENVM*	No DNA Detected	Blank
	≥0.001 – ≤0.0088 ng/µL	QPS ENVM*	DNA Insufficient	Blank
	>0.0088 ng/µL	QPS ENVM*	STR Amplification	PowerPlex21 3500xl
	>5 ng/µL	QPS ENVM*	Post-Extraction	Dilution
Ref	<0.0088 ng/µL	All	On Hold	Reference Sample Review
	≥0.0088 – ≤0.0176 ng/µL	All	Post-Extraction	Microcon PowerPlex21
	>0.0176 ng/µL	All	STR Amplification	PowerPlex21 3500xl
	>5 ng/µL	All	Post-Extraction	Dilution

Note: QPS ENVM samples will have the well designation highlighted blue.

24. The SOP 17117 "*Procedure for Case Management*" additionally details workflows for "No DNA Detected" results in sections 2, 6.5.4 and 8. Version 21 of SOP 17117 is attached to this statement as **PB142**.

25. Attached to this statement as **PB143** are example screenshots from the Forensic Register.

DNA INSUFFICIENT FOR FURTHER PROCESSING ('DIFP')

26. The Analytical team commenced validating "DIFP" results in the Forensic Register in February 2018 for Priority 2 and 3 samples.

27. After the implementation of PowerPlex 21 for casework samples, the Analytical team reviewed "DIFP" results for Priority 3 samples in AUSLAB until the reversion to Profiler Plus in May 2013.

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28. Review and validation of the result line "DNA insufficient for further processing" allows the result to be viewed by QPS in the Forensic Register, with the expanded wording "*This item/sample was submitted for DNA analysis. Low levels of DNA were detected in this sample and it was not submitted for further DNA profiling. Please contact the DNA Management Section if this sample is requested to be assessed for further processing. Further processing could include concentration of the low levels of DNA obtained, pooling with other samples (where appropriate), resampling of the parent item (where appropriate), or a combination of the processes*" in accordance with the comments for SOP 34229 (version 3).

29. The task of validating the "DIFP" results is performed by the Analytical team and is undertaken by the scientists within the team who are competent in this task. The process is listed in SOP 34064 at section 9.1.

30. The information and material considered when validating 'DIFP' results is the same as that outlined above at paragraph 18 in relation to 'No DNA detected'. I have attached example screenshots of the Forensic Register windows at **PB143**. Staff access the same window when validating 'No DNA detected' and 'DIFP' results.

31. From 6 June 2022, the Analytical staff no longer validate 'DIFP' results. All samples above the limit of detection at quantification proceeded through to DNA profiling for all priority samples, with Priority 1 samples continuing to be auto-microconned.

Reworks

32. Generally, any reworking of crime scene samples is performed by FRIT staff, or other staff trained in the case management of results. I do not undertake the task of validating "No DNA Detected" results, however it is my understanding that reworks would not be routinely requested by the Analytical team unless an issue was detected by the operator during the quantification process, or other analytical processes. These would generally be reported to the Analytical Senior Scientist for assessment before ordering reworks.

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33. I estimate that this would, however, occur rarely. If a batch had an issue observed, it is likely the whole batch would be rerun. If inhibition is observed during the checks performed at the quantification batch finalisation (as per SOP 34045) the operator may order further processing, for example a nucleospin clean-up.

34. The reworking of 'No DNA Detected' results by the Analytical team is not part of its scope of duties as those scientists are not trained in case management/profile data interpretation. Also, my understanding is that the Analytical team does not use the Profile Data Analysis page in the Forensic Register for "*No DNA Detected samples*" which means the information that is used by a case manager to assess a sample is not viewed by the analytical staff.

35. In accordance with section 6 of SOP 17117, the case managing scientist is required to assess the results for reworking:

> "*The purpose of case management is to collate and report any DNA results that have been obtained and to prepare the case file for a statement (if required) or for peer review. To achieve this, the case managing scientist may be required to:*
>
> i. *Assess DNA results to determine whether reworking is required to improve or confirm results.*
> ii. *Enter final Exhibit reports via the Profile Data Analysis (PDA) page in the FR.*
> iii. *Compile case file.*"

SPERM MICROSCOPY AND PROJECT #181

36. I do not know when and how the suspension method was introduced. Abigail Ryan, a scientist in the Evidence Recovery team, has provided me with a file note she prepared after she recently undertook a search for records of how the suspension method was introduced. From her file note, the change appears to have occurred between QIS 17189, version 9 (which was published on 24 July 2007) and QIS 17189, version 10 (which was published on 20 September 2010).

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37. The project plan for Proposal #181 which was prepared by Allan McNevin and/or Emma Caunt and signed by me on 13 October 2016 states:

> *"Given that no formal validation of the making of cell suspensions was recorded at the time the procedure was introduced (possibly around 2008, details not found), an investigation into the effectiveness of current procedures will fill the gap in departmental records."*

38. I have now viewed the comments against SOP 17189, version 10 (which was active from 20 September 2010) to SOP 17189, version 13 (which was active from 29 June 2015). There are no comments that relate directly to the preparation of the suspension procedure. I was not involved in the changes to the suspension procedure and cannot comment on any validations that may have been done between its implementation and 2016.

39. Standard Operating Procedure (**SOP**) 17189 "*Examination For & Of Spermatozoa*" (version 13) was active from 29 July 2015. This is the SOP related to the detection and testing of spermatozoa that was in force as at January 2016. This SOP is attached to this statement as **PB144**.

40. In relation to the process and procedure in January 2016 for testing samples suspected to contain spermatozoa (including the use of preliminary and presumptive testing and policies concerning when the testing should cease) my understanding of the process would have been limited to reviewing the relevant SOP active at the time.

41. In January 2016, I was on maternity leave. I had undertaken theoretical training in the relevant training modules in 2011. As I had not reported on results since that time, I would not have been competent to do so in January 2016.

42. As I was on leave at the time the sperm microscopy issue was first raised I am not aware of the specifics of how the sperm microscopy issue was first raised. If the

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concern was raised by staff within the FRIT team, it generally would have been raised through the line managers.

43. I also cannot comment on when the Management Team first became aware of the issue. Based on the initial request for Proposal #181 dated 2 June 2016, I understand that concerns were raised by the FRIT team. From the Project #181 final report, part 2.1 Background Information indicated the observation was first made in 2015.

44. Management Team minutes from 12 May 2016 (while I was maternity leave) reveal that an issue was raised by Allan McNevin as to the difference between sperm seen on differential lysis extraction slides compared to the Evidence Recovery suspension slide.

45. When I returned from maternity leave in July 2016, Project #181 had already been initiated with an initial request signed by Kirsten Scott on or around 2 June 2016.

46. I returned from maternity leave on 12 July 2016 and usually worked 3 days a week (Tuesday, Wednesday and Friday) until the end of February 2017, after which time I worked 4 days a week (with Wednesdays as a non-work day). I do not recall when I first became aware of the issue on my return from maternity leave. I have located an email I sent to Allan McNevin, the project manager for Project #181, on 19 July 2016[1] requesting an update on the project plan.

47. There were no OQIs or adverse events raised in relation to the sperm microscopy issue. Information relating to the investigation is recorded in the Project #181 documentation and final report. As I was on maternity leave at the time Project #181 was initiated I cannot comment on the reasoning why the project was developed as opposed to an OQI or an adverse event.

[1] FSS.000.0052.8289

Project # 181

48. The final report for Project #181 lists the project team as Allan McNevin, Chelsea Savage, Emma Caunt and Matthew Hunt. I was not involved in drafting the final report. The project team were responsible for drafting this report. The interim reports were drafted by Allan McNevin and Emma Caunt.

49. The project was initiated and approved to proceed to a project in May 2016, with the final report signed in August 2020. The experimental work conducted through the duration of this project is listed in the project report.

50. After my return from maternity leave in 2016, from emails it appears that in July I followed up with Allan McNevin on the status of the initial project plan.

51. My role in Project #181 included reviewing and endorsing the further experiments proposed, being involved in Management Team discussions and voting (by email) on options put forward by the project team as next steps, which would be incorporated in the further project proposals.

52. On 23 January 2017, Cathie Allen emailed Kirsten Scott and I stating:

> *"Paul Csoban has asked Helen Gregg to have a look at Project #181 with a view to ensuring that our NATA accreditation is not a risk. I've given Helen a brief overview and will provide her with the project documentation, but she may ask you some questions.*
>
> *I've limited my discussion with her to Project #181 and the surrounding discussions of the process."*

53. I expect Kirsten Scott was included in the email about this issue as Kirsten coordinated NATA accreditation for Forensic DNA Analysis. I am and was at the time her line manager. I did not respond to Cathie's email and I do not recall being involved in or having any discussions with Helen Gregg and Kristen Scott about NATA accreditation in relation to Project #181.

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54. As mentioned above, when the initial request for Project #181 was signed in May 2016, that approved the progression to a full project plan. According to SOP 22871 "*Procedure for Change Management in Forensic DNA Analysis*", the change management procedure applies to all process changes or projects that involve the validation/verification of technical procedures, are internal projects (minor or major) which impact on sample reporting/processing and significantly alter workflow procedures.

55. My understanding is, as the concern was raised as a potential issue, investigation and experiments needed to be undertaken to assess if there was a difference in sensitivity between the Evidence Recovery slide preparation and that prepared during extraction. This is reflected in the management meeting minutes of 12 May 2016 as:

> *"This has been raised as a potential issue. First step to look at this is to compare a number of Diff Lysis extraction slides against ER suspension slide to see if the trend shows that more are seen on the extraction slides as a norm. This first step project is being monitored by ARM and KDS."*

56. The purpose and scope of Project #181 is recorded in the final report for Project #181, dated July 2020:

> *"The initial aim of the project was to investigate the performance of the current ER (Evidence Recovery Team) microscopy slide preparation process, in terms of relative sensitivity for spermatozoa detection and presumptive testing for seminal fluid...".*

57. On 17 February 2017, I made a diary entry where I recorded "*errors/issues had not yet been identified, and until the project complete and some outcome, we wouldn't know what cases may be affected. We are only at the initial stages of assessment, and if any errors identified, they would be addressed in the outcome/audit*". This is attached to this statement as **PB145.**

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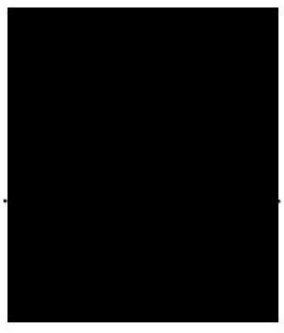

58. These notes were taken months before Part 2 of Project #181 was signed off to proceed (which was in May 2017). It may have even been before the initial experiments were completed, as there appears to be a presentation to Management on 16 March 2017 with "results so far and next steps". The data I refer to showing them in the notes, would have been in my original email to Cathie and Justin on 9 February 2017, which was just the simple table.

59. In May 2017, I approved Proposal #181 Part 2 while acting in the Managing Scientist role. All other proposals were approved by Cathie Allen.

60. On 14 November 2017, I emailed Justin Howes some draft notes titled 'Notes for Paul' I had prepared which included a basic timeline of events leading up to Project #181. I cannot recall with certainty but believe I compiled this information using the initial request, project proposals and reports prepared for Project #181 that were available at the time, as well as SOPs and the draft data analysis report containing the tracked changes made by Matthew Hunt and Luke Ryan to my draft in June and August 2017. I may not have specifically included Kylie Rika's feedback (which she provided to me on 9 June 2017) as I felt that Matthew Hunt had explained, with the wording he provided, what I thought Kylie was saying. When Matthew provided his feedback to me on 1 August 2017, Kylie and Luke were copied in to that email. Also, some of the comments Kylie made I felt were better directed to Project #181.

61. I think this information and email relate to a request made by Peter Culshaw, who was Acting Managing Scientist at the time, for Justin and I to provide him with information about the lead up to Project #181. I do not have a record of or remember the request he made, all I recall that the email shows is that the information was to be provided to Paul Csoban. On 15 November 2017 at 4.23pm, Justin emailed Peter Culshaw with the notes he had "*fleshed out*". I was not involved in any further requests for information from either Peter Culshaw or Paul Csoban relating to Project #181.

62. The final report for Project #181 was approved by Cathie Allen in August 2020. The initial request on 2 June 2016 was approved by Kirsten Scott.

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63. The following recommendations were made at the conclusion of the final report for Project #181:

> *"The following recommendations are made, based on the conclusions above:*
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> *1. Implement the proposed workflow for the examination of all samples submitted for semen testing. The process set out in Part 3 should be incorporated into a standard operating procedure, which should also include the adaptations described in Part 6, including the addition of 400 uL of water to allow for potential presumptive testing. Sample incubation is recommended to be undertaken using a standard hotblock set at 15 mins@~30°C.*
> *P30 testing alone to be used as the standard presumptive screening technique for the detection of seminal fluid in the absence of spermatozoa.*
>
> *2. Cessation of AP testing as a standard presumptive screening technique for the detection of seminal fluid on sub-samples from swabs, fabrics etc., instead maintaining AP screening solely for the purpose of screening whole items (such as clothing or bedding) for the possible presence of semen stains and subsequent sub-sampling."*

64. Project #181 aimed to investigate the differences in sensitivity. At the conclusion of the project, the final report details:

> *"Initial investigations into the possible cause of the notable difference in sensitivity of ER semen microscopy compared to Diff Lysis microscopy were inconclusive and exaggerated differences between ER and Diff Lysis microscopy were not able to be replicated. Despite this, early experimental results did show sperm microscopy conducted at ER to be consistently less sensitive than the same technique conducted at the Diff Lysis stage. Although this finding was not unexpected, it is desirable for microscopy to be optimised for maximum possible sensitivity in order to be able to provide the most informative results."*

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65. A final report for Project #181 was produced in August 2020, concluding the project work. Implementation tasks followed Management Team sign-off of the report, including some ongoing discussions about implementation in November 2020 and also about publication of the project in May 2021.

66. Once the project and changes were implemented, it would be considered completed. Further ethics approval was obtained post completion, for publication purposes.

67. The results of the initial experiments performed during Project #181 showed sperm microscopy conducted at Evidence Recovery was consistently less sensitive than the same technique conducted at the Differential Lysis stage. As Project #181 resulted in a change in process whereby the microscopic detection of spermatozoa was performed on the differential lysis slide, I believe it adequately resolved the sensitivity differences.

68. Matthew Hunt provided a presentation to the Reporting teams regarding Project #181 implementation and summarised the feedback in an email to the Management Team on 26 November 2020 (see exhibit **PB146** attached to this statement). In this email, Matthew stated:

> *"Neither team expressed strong opinions against dropping AP for presumptive screening (except for locating semen stains)"*

69. I was not present at the Reporting team meetings. I did email the project and Management Team on 26 November 2020 with concerns about storage space after Allan McNevin raised a query about how long supernatants should be retained. My email is attached to this statement as exhibit **PB147**.

70. A number of Standard Operating Procedures were changed as a result of the sperm microscopy issue and Project #181. From within my team specifically, there were the following SOPs changed as a result:

a. 17189 Examination For & Of Spermatozoa;

b. 33798 Examination of Sexual Cases;

c. 17186 The Acid Phosphatase screening test for seminal stains;

d. 17185 Detection of Azoospermic Semen in Casework Samples.

71. The SOPs relevant to the change were updated by the Evidence Recovery Team and the Reporting Team. The SOPs were updated to reflect the recommendations from Project #181, and workflow changes relevant to this change. These included Forensic Register enhancements for the Analytical team which were outlined in an email from Luke Ryan dated 23 November 2020 (attached to this statement as exhibit **PB148**).

72. The changes as an outcome of Project #181 were implemented on 30 November 2020.

73. I am not aware of any collation of samples or casefiles that may have occurred prior to my return from maternity leave. My understanding was that QPS submitted a SAIK first, with a view to submitting further samples pending the results from the SAIK.

74. The final report for Project #181 states:

> "*Generally multiple samples are submitted for SAIKs, and often more than one sub-sample is collected from a larger item, thus somewhat reducing the overall risk to case.*"

75. My understanding is that examination strategies in place at the time additionally reduced the overall risk, as progression to extraction did not solely rely on spermatozoa being detected at Evidence Recovery. Examination strategies by the Evidence Recovery team were recorded for all SAIKs and workflows performed were as per SOP 32106.

76. In 2016, the laboratory information system was AUSLAB, which had different workflows than currently exist within the Forensic Register.

77. According to the case management SOP active at the time (17117 version 18), any sub-samples submitted from a SAIK were recorded under the same exhibit, providing

visibility of samples and any presumptive screening by the Evidence Recovery team for the SAIK. My understanding is that case managers and reporting scientists on assessment of results obtained could also submit samples for additional testing, including reworking through the Analytical section (to improve a DNA profile obtained), re-examination of items or a submission for extraction of samples previously not submitted. This could occur when reviewing results obtained from a sample within the case.

78. I am not aware of any workplace culture/environment issues between FSS staff leading up to the project being initiated, as I was on maternity leave from February 2015 until July 2016. I am however aware of an event that occurred during a management meeting prior to my return from maternity leave. This event was with the A/Managing Scientist from the time it occurred, and on my return was being managed at that level.

79. Shortly after my return, I followed up on the project plan for Project #181, which was sent to Management Team to review on 1 September 2016.

80. In March 2017, Allan and Emma presented to the Management Team the outcome of initial testing and proposed next steps. In April 2017, part 2 of Project Proposal #181 was provided to the Management Team for review.

81. During 2016 and 2017, a large process was being undertaken across all teams regarding the implementation of the Forensic Register. Staff across all teams were involved in developing and training for the new system which was implemented in July 2017. This was a significant amount of work.

82. There appears to be some delay with progression of the experimental design for Project #181 Part 2. I was included in several emails with Kylie Rika, Justin Howes and Allan McNevin in January 2018 wherein Allan advised that approval was with Cathie Allen. Justin Howes advised on 18 January 2018 that he would follow up with Cathie.

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83. In the ERQ weekly report dated 4 December 2017, Project #181 states "*still on hold*". In the subsequent ERQ weekly report, Project #181 is listed as " *Part B to start as soon as practicable*". During this time, I was in the A/Team Leader FRIT role. It appears Luke Ryan provided this report when he was in the A/Team Leader ERQ role during this time. I cannot recall what information may have been provided to proceed with this.

84. On 21 May 2019, Justin Howes emailed the Reporting team Senior Scientist asking for assistance as Emma Caunt was on leave. There were subsequent discussions between Justin Howes and Allan McNevin about who was taking over Project #181, and Matthew Hunt and Chelsea Savage (an Evidence Recovery Scientist) joined the project.

85. Work then progressed on part 4 of the project from June 2019 until the project was completed.

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86. I was not involved in the ESR review. I understand Cathie Allen and/or Paul Csoban proposed the review and the purpose was to review the current SOP. The only record I have of the ESR outcome is an email from Cathie Allen at 9:26am on 4 January 2018 sending a report dated 11 April 2017, advising she will discuss.

87. I cannot recall having a discussion with Cathie Allen on 4 January 2018 about the report. It is unlikely I would have seen or read her email and/or the report prior to my meeting with Cathie, as I would have been catching up on my emails following my return from leave from 20 December 2017 until 4 January 2018 according to my timesheet, I commenced work that day at 9am.

88. I do not know who developed and finalised the Terms of Reference for the review as I was not personally involved. I do not know who prepared the documents for the Terms of Reference as I was not involved in that either. I do not know who determined what documents should be provided, however I understand that Cathie Allen or Paul

Csoban were involved. I had no personal involvement in supplying any documents to ESR.

89. As I said on 4 January 2018, Cathie Allen emailed me the report. I cannot recall if I read the report after my meeting with Cathie, as I cannot recall seeing the report until I found the email recently. I cannot recall what, if anything, occurred during any discussion, and I do not know why the report was sent to me in January 2018. From looking at the report presently, it appears to be supportive of the processes in place at the time.

90. From looking at the report now and from QIS2 records, comments by Cathie Allen have been made against SOP 17189 (version 13), 32106 (version 4) and 17185 (version 10) which appear to be in response to the information in the report.

THE VALIDATION AND IMPLEMENTATION OF POWERPLEX21

91. PowerPlex21 (PP21) was introduced at the laboratory in 2012. Project #99 to #107 was completed as part of the project and implementation of PP21 at the time. As part of the Management Team, I was involved in endorsing the PP21 validation. I was also involved in updating the exhibit result lines that were reported to the QPS, as per the response in my previous statement (dated 21 September 2022). This was not directly related to the experimentation within the project. This was related to reporting of the results within AUSLAB for PP21 and STRmix, with the process undertaken with Emma Caunt in consultation with Management Team, project staff and the QPS.

92. I cannot recall specifically how I determined that the validation was completed successfully for this process. My usual process for validations is to read the final report at the time and consider whether I accept the information within the report and the recommendations put forward. I would have had no reason to doubt the conclusions drawn. If I provided feedback, I would check to see that my feedback had been incorporated (if it was more significant the grammatical or wording suggestions) before providing endorsement.

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93. I endorsed the Final report for Project #105 (signed 17 December 2012). I was on maternity leave when the Final report for Project #105 (full volume) was signed (signed 13 March 2013).

94. I do not recall any feedback or advice provided directly to me. Discussion would have been had with the Management Team and project staff, and feedback provided to the team prior to implementation. As the validation recommended the PP21 amplification kit was fit for purpose, I would have seen no issue in endorsing the validation report.

95. I cannot recall if I had any specific concerns with the validation of PP21 and STRmix back in 2012. I cannot locate any emails indicating I had a concern at the time. The work being done was with the whole of the Management Team, and a number of project staff from across the different teams.

96. Half volume and full volume amplifications were both validated in the Project Report under Project #107. Both half and full volume amplifications were also validated in Project #105 (for which Emma Caunt was on the project team for).

97. I do not recall telling Emma Caunt that we should be implementing PP21 at half volume because Cathie Allen had advised us to do this. The final validation report relating to the implementation of PP21 (Project #107) indicated that both full and half volume amplifications were suitable for implementation, and recommended half volume be implemented. This project was signed in December 2012 by the Management Team, consisting of Cathie Allen, Justin Howes, Sharon Johnstone, Amanda Reeves, Emma Caunt, Adrian Pippia, Allan McNevin and Thomas Nurthen. Emma Caunt signed her endorsement of the Project #107 report on 14 December 2012. Attached to this statement as exhibit **PB149** is a copy of the final report for Project #107.

98. The Minor Change register shows that on 4 February 2013, Justin Howes made an entry to "cease half-volume amp profiling". On 22 February 2013, Justin Howes made a further entry in the Minor Change register stating, "amplifications at full volume PP21 started for routine analysis." Screenshots of these entries are below:

[redacted]

Implementation Date	Details	Project Leader	Area Affected
4/02/2013	Cease half-vol amp profiling. Report SS, Complex unsuit, No DNA and DNA insufficient profiles. Full-volume reactions to be assessed.	JAH	

Implementation Date	Details	Project Leader	Area Affected
22/03/2013	As of 2:30pm, no longer doing EB checks routinely. They can be performed for FGM batches if need be.	JAH	

99. An email from me was sent on 4 February 2013 to indicate half volume amplifications were ceasing. A further email from Justin Howes on the same day indicates both full and half volume amplifications would cease. I was on maternity leave from 11 February 2013 and can therefore not comment on discussions had prior to the second entry on 22 February 2013.

100. From records I have been able to locate, it appears the decision was made with the Management Team based on feedback from the Analytical and Reporting teams regarding profile interpretation issues. Analytical team meeting minutes on 4 February 2013 indicated several issues were being experienced with PP21 (including stochastic events, artefacts, peaks) that was making case management (DNA profile interpretation) difficult. The meeting minutes indicate PP21 amplifications were on hold, while data was being assessment and other work was being undertaken to assist.

QIASYMPHONY (PROJECT #192)

101. As part of the management team, I was involved in endorsing the QIAsymphony Bone/Teeth validation (Project #192).

102. Feedback on Project #192 and repeatability and reproducibility discussions in relation to other projects (as per my statement of 17 October 2022) led to additional experiments being undertaken, however this did not impact on my endorsements of the final reports when this work was incorporated.

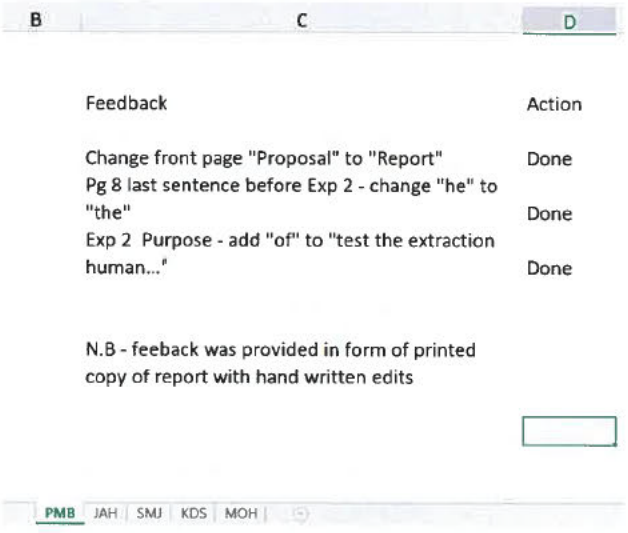
103. I cannot recall specifically how I determined that the validation was completed successfully for this process. My usual process for validations is to read the final report at the time and consider whether I accept the information within the report and the

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

recommendations put forward. If the results indicate that the acceptance criteria were met, and the technical review was performed, I would have had no reason to doubt the conclusions drawn. If I provided feedback, I would check to see that my feedback had been incorporated (if it was more significant the grammatical or wording suggestions) before providing endorsement.

104. I cannot now recall what feedback I provided in relation to Project #192. I also cannot recall having any specific concerns with the validation process at the time.

105. I have located a spreadsheet of feedback provided in relation to this project which is annexed to this statement as **PB150.** This document records my feedback as follows:



B	C	D
	Feedback	Action
	Change front page "Proposal" to "Report"	Done
	Pg 8 last sentence before Exp 2 - change "he" to "the"	Done
	Exp 2 Purpose - add "of" to "test the extraction human..."	Done
	N.B - feeback was provided in form of printed copy of report with hand written edits	

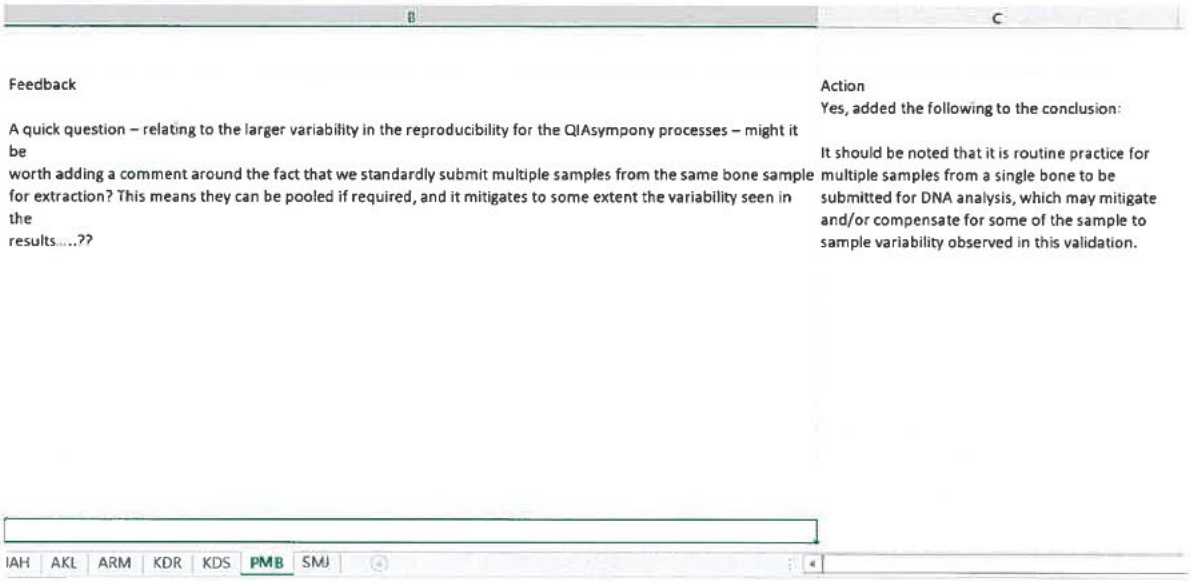
PMB | JAH | SMJ | KDS | MOH

106. Project #192 contained a supplementary experiment, which I endorsed. I have similarly located the collated feedback for this supplementary experiment, which records my feedback as follows:

[redacted]

Paula Michelle Brisotto

Witness [redacted]



B	C
Feedback	Action
A quick question – relating to the larger variability in the reproducibility for the QIAsympony processes – might it be worth adding a comment around the fact that we standardly submit multiple samples from the same bone sample for extraction? This means they can be pooled if required, and it mitigates to some extent the variability seen in the results.....??	Yes, added the following to the conclusion: It should be noted that it is routine practice for multiple samples from a single bone to be submitted for DNA analysis, which may mitigate and/or compensate for some of the sample to sample variability observed in this validation.

IAH | AKL | ARM | KDR | KDS | PMB | SMJ

VALIDATION OF STRMIX (PROJECT #105 AND #151)

107. As part of the Management Team, I was involved in endorsing STRmix Project #105 (signed 17 December 2012), however I was on maternity leave when the final report for full volume was signed off in 13 March 2013).

108. Again, I cannot recall specifically how I determined that the validation was completed successfully for this process. My usual process for validations is to read the final report at the time and consider whether I accept the information within the report and the recommendations put forward. Similarly, as with PP21 above, I would have no reason to doubt the conclusions drawn. If I provided feedback, I would check to see that my feedback had been incorporated (if it was more significant the grammatical or wording suggestions) before provided endorsement.

109. I am unable to recall now what specific feedback I provided at the time in relation to the validation of STRMix. I also cannot locate any written feedback that I may have provided at the time to refresh my memory.

110. I cannot recall now whether I had any issues or concerns at the time concerning the validation of STRMix. If there were any issues or feedback provided at the time, I assume the issues must have been addressed for the project to have been implemented.

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

111. In December 2014, a new version of STRmix (Version 2.0.6) was required to be implemented following issues noted with Version 2.0.1. Project # 160 was proposed and initiated to cover the verification of the changes in the new version of the software. I was an endorser for Project # 160. There were no issues or concerns that I was aware of, nor any issues or concerns that I was aware of at the time, prior to endorsing the project.

VALIDATION OF QUANT STUDIO 5 (PROJECT # 185)

112. As part of the Management Team, I was involved in endorsing the Quant Studio 5 validation (Project #185). I do not recall having any issues or concerns in relation to Project #185 at the time. Any issues or concerns from FRIT staff relating to this project were directed to Justin Howes.

113. I cannot recall specifically how I determined that the validation was completed successfully for this process. My usual process for validations is to read the final report at the time and consider whether I accept the information within the report and the recommendations put forward. If the results indicate that the acceptance criteria were met, and the technical review was performed, I would have had no reason to doubt the conclusions drawn. If I provided feedback, I would check to see that my feedback had been incorporated (if it was more significant the grammatical or wording suggestions) before provided endorsement.

114. Feedback during Project #185 led to additional experiments being undertaken, however this did not impact on my endorsements of the final reports when this work was incorporated.

115. Experiment 3 of Project Proposal #185 was an additional experiment, which was included in the final report which I endorsed.

116. In 2022, an issue arose concerning the NIST standards that had been used during Project #185 outside of the expiry date. An OQI was requested to be raised (OQI

56218) by Thomas Nurthen. As a result of this, new NIST standards were ordered, and a minor trial was run.

117. On 9 September 2022, Tara Prowse, one of the Analytical Scientists, sent by email, a PDF version of the Proposal for Minor Change – Comparison of NIST Standards 2372 and 2372a. These were sent to me, Luke Ryan and Kirsten Scott for signing. The proposal was forwarded to Thomas Nurthen by Kirsten Scott on the same date for feedback, as the initiator of the OQI (see **PB151** attached to this statement). I do not have any further correspondence relating to feedback on this proposal. I understand that the OQI is still underway and feedback from Thomas Nurthen has not yet been provided.

118. A risk assessment was also performed by Chelsea Savage, co-author of the Proposal for Minor Change document. This risk assessment was signed by Luke Ryan on 9 September 2022. See attached exhibit **PB152**.

VALIDATION OF HAMILTON STARLET A (PROJECT #173)

119. I was on maternity leave at the time of the project proposal and as such I was not an endorser on the project proposal for STARlet A (Project #173). As part of the management team, I was involved in endorsing the final report for the STARlet A validation.

120. I cannot recall specifically how I determined that the validation was completed successfully for this process. My usual process for validations is to read the final report at the time and consider whether I accept the information within the report and the recommendations put forward. If the results indicate that the acceptance criteria were met, and the technical review was performed, I would have had no reason to doubt the conclusions drawn. If I provided feedback, I would check to see that my feedback had been incorporated (if it was more significant the grammatical or wording suggestions) before provided endorsement.

[redacted]

Paula Michelle Brisotto

Witness [redacted]

TAKEN AND DECLARED before me at Brisbane in the State of Queensland this 18th day of October 2022.

[redacted]

.....

Paula Michelle Brisotto

[redacted]

.....

Witness

Caitlin Fletcher

[redacted]

.....

Paula Michelle Brisotto

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Witness

[redacted]

EXHIBIT INDEX

Exhibit	Document Title	Pages
PB138	Validations	27-33
PB139	SOP 22871 "Procedure for Change Management in Forensic DNA Analysis" (version 17)	34-71
PB140	SOP 34064 "Miscellaneous Analytical Procedures and Tasks (version 3)	49-71
PB141	SOP 34045 "Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit" (version 7)	72-106
PB142	SOP 17117 "Procedure for Case Management" (version 21)	107-142
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PB146	Email from Matthew Hunt to the Management Team dated 26 November 2020	164-165
PB147	Email to Allan McNevin on 26 November 2020	166-170
PB148	Email from Luke Ryan dated 23 November 2020	171-173
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PB150	Feedback spreadsheet for Project #192	Attached separately
PB151	Email from Kirsten Scott dated 9 September 2022 with Proposal for Minor Change – Comparison of NIST Standards 2372 and 2372a	246-255
PB152	Risk assessment dated 9 September 2022	256-269

[redacted]

Paula Michelle Brisotto

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

Witness

PB138

Project number	Project name	Implementation date	Comments	Endorsed by PMB
238	Evaluation of DBLR v1.2	ongoing		
237	Reduction in Physical Case file creation	Sep-21		
236	Exhibit Result Line Revision	Nov-21		
235	2021 FR version upgrade	ongoing		
234	Process mapping of Interpretation and reporting	closed		
233	Bone sampling and demineralisation protocol	on-hold		
232	Paternity calculations for mixed DNA samples	closed		
231	Verification of STRmix v2.8	Jun-21		*
230	Implementation of 3500xL PP21 Casework	Feb-21		Endorsed implementation plan (no final report with this project)
229	Paternity Index Distributions by Locus in PP21	closed		
228	Review of current baseline thresholds 3130xl using PP21	closed		
227	Baseline method trial	ongoing		
226	Collection of sperm from pubic hair	on-hold		
225	Evaluation of DBLR	not implemented		*
224	Evaluation of FaSTR DNA	not implemented		
223	DCS v4.0	Apr-22 (3500xL B)		*
222	Profiling of Spermatozoa from microscopy slides	ongoing		
221	Impact of magnetic fingerprint powders on bead-based trace DNA extraction (collab with QPS)	closed		
220	Verification of commercial H & E stains	Q1-21		*
219	Verification STRmix 2.7 for 3500xL	Mar-21		*
218	Verification of BSD600 Ascent A2	Nov-20		*
217	Verification of Maxwell FSC Instruments	Jun-20		*
216	Validation of Ion Chef & S5	ongoing		
215	STRmix v2.7 - comparison of LRs for 5p mixtures	on-hold		
214	Validation of STRmix v2.7	Feb-20		*
213	Verifiler Plus	ongoing		* Analytical reports endorsed. Reporting and Interpretation projects ongoing.
212	Storage Transition into FR	Aug-19		*
211	Streamlining of DNA profile result reporting workflow	on-hold		
210	Verification of GeneMapper v1.6	Jan-20		*
209	Verification of SPEX 6775 Freezer Mill	Q4-19		*
208	Verification of STRmix v2.6.2	Jun-19		*
207	Verification of Pro K	Aug-19		*
206	Y Filer Plus	ongoing		
205	Post implementation review of STRmix v2.6.0	nil		
204	Diamond dye collaboration with QPS	not implemented		*
203	Number of alleles for SS LR greater than 100billion with STRmix v2.6	closed		
202	Validation STRmix v2.6.0	Jan-19		*
201	QIAsymphony QSL3 Verification	Feb-19		*
200	Statement format and wording revision	on-hold		
199	Proflex	Jan-22		Proposal only endorsed. Absent for signature on final report.
198	Assessment of OSD reworking on Intelligence Reference samples	closed		
197	Interpretation of 4 person mixtures using STRmix v.2.0.6	Aug-18		*
196	Verifiler Plus Trial	nil		*
195	Testing of Quant and Amp reagent stability at room temperature	Apr-18		*

194	Verification of QIAsymphony SPAS	Apr-18		*
193	Verification of STRmix v2.5.11	not implemented		*
192	QIAsymphony Bone Teeth	Apr-18		*
191	Effects of HCl on DNA persistence and profiling	nil		
190	Research Project - MPS	nil		
189	Y Filer plus implementation	ongoing		Project finalisation report endorsed. Project under #206
188	Verification of Maxwell for Retain Supernatant	Jun-18		*
187	Verfication of STRmix v2.0.6 for use with the 3500	closed		
186	Analysis of Casework PP21 samples using 3500xL A	not implemented at this time		*
185	Validation of QS5	Feb-19		*
184	Evaluation of the efficacy of Microcons	Feb-18		Project proposal only
183	Implementation of NCIDD-IFA (bonaparte)	Q3-19		*
182	PP21 WEN CW 3500xL Validation	closed		
181	Sperm microscopy sensitivity	Nov-20		*
180	Use of STRvalidator for validation or verification	not implemented		
179	DNA sequencing at D18S51 locus	closed		
178	Verification and implementation of STRmix v2.4.03	not implemented		
177	3500 CW PP21-WEN	closed		
176	Investigation of ICMP protocol	closed		
175	Hamilton ID STARlet - CE	May-19 (Starlet C)		*
174	Verification of ARTEL (PCS and MVS)	retrospective	MVS introduced to laboratory in 2009, PCS introduced 2013	Project plan only endorsed. I was on leave for the final report.
173	Hamilton ID STARlet - Pre PCR	Jan-17 (A) and Jul-17 (B)		*Final reports endorsed. On leave for project proposals
172	Phadebas testing from suspension in ERT	closed		
171	PP21 Verification of new ILS and Matrix	Q3-16		
170	Reassessment of in-house stutter thresholds and stutter file	not implemented		
169	Verification of swab suspension at RT	closed		
168	Validation of QIAsymphony	Nov-16		* Final report endorsed. On leave for project proposal.
167	Verification and implementation of STRmix V2.3	closed		
166	Verification of TMB Screening Test for Blood	retrospective		
165	Verification of Phadebas paper	retrospective		
164	Case Management improvements	Jan-15		
163	Assessment of results obtained from auto-microcon samples	closed		
162	OSIRIS Freeware for Profile Viewing	closed		
161	FTP processing on OSD plate	Mar-15		
160	Verification of STRmix V2.0.6	Jan-15		*
159	M-VAC trial by QPS	not implemented		
158	Statement in Table Format	closed		
157	Quant Standard Data Mining	closed		
156	Verification of 7500A after thermal cycling block change	Aug-14 returned to service		*
155	Verification of software for 3130s	closed		
154	Verfication of software for 7500	Did not proceed		
153	Verification of Trigene Advanced	retrospective		Endorsed proposal only. On leave for final report.

152	Validation of Quantifiler Trio and Y-Filer Plus	Nov-2015 (quantifiler)	Y-filer: additional work is required to complete validation prior to use	Endorsed proposal for Quant trio only. On leave for Y-Filer proposal and Final Quant trio report
151	Verification and implementation of STRmix 2.0.1	Jul-14		*
150	Suitability of combining wet and dry swabs from SAIKs	closed		
149	Development of guidelines for number of contributors	Mar-15		
148	Cleaning bone processing equipment	Jul-19		Endorsed proposal. On leave for final report.
147	Quantifiler re-validation after manufacturing changes	Aug-14		*
146	Globalfiler validation	closed		
145	3500 validation	Mar-15 (A), Jan-16 (B)		Endorsed Plan for Reference samples. Signed Direct Reference Final report only. Was on leave for EREF and Casework.
144	Christmas Tree Staining	closed		
143	Foreign DNA on Semen Negative SAIK swabs	Jan-14		
142	Concentration of large items	not implemented		
141	PowerPlex optimisation program	closed		*
140	GlobalFiler Express Kit FTA sensitivity study	closed		
139	Extraction negative tube	Not implemented		
138	Batch Case Management	Dec-13		
137	Accepting partial Amel	Mar-14		
136	Frozen AP	Jul-14		*
135	Verification of an additional Thermalcycler	Mar-14		
134	Number of alleles for SS LR greater than 100billion	Jul-14		*
133	QPFREG - AUSLAB upgrade	Closed		
132	Mantis verification	Nov-15		
131	PP21 post implementation review	nil		
130	GlobalFiler_testing	Not implemented		
129	Reference profile interpretation (EXH lines)_Combined with #126	All information under #126		
128	Trial of QIAGEN Investigator Quantiplex Kit	Not implemented		
127	Verification of GM-IDX software upgrade	Jun-15		
126	STRmix)	May-13		
125	AUSLAB All Incomplete requests	Jan-14		
124	Generic Instrument interface	Sep-13		
123	Verification of Maxwell DNA Extraction from Bone	Not implemented		
122	Verification ofMaxwell DNA Extraction from Tissue	Not implemented		
121	Verification of new Pro K and DTT	Mar-13		*
120	Verification of new Taq in Profiler Plus kits	Q4-12		*
119	Validation of Extracting DNA from Concrete	Not implemented		
118	Validation of Extracting DNA from Soil	Not implemented		
117	Creation of animal semen repository	Folder empty		
116	Verification of a New Size Std for GeneScan	Mar-13		*
115	Verification of a new membrane for M'con	Jan-13		*
114	Change of SAIK booklet and kit	Aug-12		
112	Evaluation of continued competence	closed		
111	Sexual assault reassessment	closed		
110	AUSLAB hardware replacement cutover	Jun-12		
109	Maxwell C & D verification	Jun-12		*

108	Pipette disposal	Feb-12	
107	PowerPlex 21 Implemen	Ref Sep-12, CW Dec-12	*
106	PowerPlex 21 NCIDD	Ref Sep-12, CW Dec-12	
105	PowerPlex 21 Reporting and STRmix	Ref Sep-12, CW Dec-12	*
104	PowerPlex 21 Concordance	Ref Sep-12, CW Dec-12	
103	PowerPlex 21 Mixture	Ref Sep-12, CW Dec-12	
102	PowerPlex 21 Thresholds	Ref Sep-12, CW Dec-12	
101	PowerPlex21 Population	Ref Sep-12, CW Dec-12	
100	PowerPlex 21 Sens	Ref Sep-12, CW Dec-12	
99	PowerPlex 21 program	Ref Sep-12, CW Dec-12	
98	Page numbering of statements	Jan-12	
97	BSD 200uL	Q3-12	*
96	Statement Appendix 5	Feb-12	
95	P30 addendum	Not implemented	
94	ESI Pro kit	Not implemented	
93	European Loci AUSLAB changes	Not implemented	
92	Efractions in SAIKS	Feb-12	
91	Maxwell Pre lysis	Jul-13	
90	Population dataset	closed	
89	New Change management	May-12	
88	European population dataset	closed	
87	Autoclave use	Nov-11	
86	Maxwell Hair and Fingernail	Jan-12	*
85	Maxwell Diff Lysis	Feb-12	*
84	Maxwell Paper & Gum	Sep-11	*
83	Maxwell Fabric	Sep-11	*
82	Capillary Regeneration	Mar-14	
81	Volume Case management	Jul-11	
80	Volume Undetermined	Jul-11	*
79	Maxwell Tapelifts	Sep-11	*
78	ABA Cards	Jul-11	*
77	GeneAmp 9700 B & D	Mar-11	*
76	Manual Staining	Dec-12	*
75	QA pend	Apr-11	
74	Destructions	Nov-11	
73	GeneAmp 9700 C & E	Mar-11	*
72	GeneAmp 9700 verification	Feb/Mar-11	*
71	BSD Series II	Feb-11	*
70	Maxwell	Q2/3-11	
69	Sensitivity Amp Vol Euro Loci	closed	
68	New Loci	closed	
67	Tube FBX testcodes	Jun-13	
65	CAPIT-ALL decapper	Nov-10	*
64	Modified Off-deck lysis	Not implemented	
63	England Finder	Q1/Q2-11	
62	Re-implement of auto DNAIQ	Aug-09	
61	Theta in Reporting Stats	Dec-12	
60	Change to retention of receipt	Dec-09	*
59	Commercial cell line	Not implemented	
58	Half vol P+ reactions	Not implemented	

57	7500	Jun-10	Approval of request for change form
56	Re-implement of Auto DNAIQ	Aug-09	
55	2uL for CE	Oct-11	
54	400HD ROX	Sep-12	Impact and risk assessment only
53	Artel	See project #174	
52	New Software & interp	Proposal not approved	
51	Paperless in-tube cases	Q3/Q4-09	
50	Nuc clean-up double elution	Not implemented	
49	Recal of Quant control ranges	May-10	
48	Fingerprint techniques & DNA	Not implemented	
47	DNAIQ clean-up	Not implemented	
46	Modified chelex from blood & cells	Mar-09	
45	Kinship Stage 3	Jan-09	Impact and risk assessment only
44	NCIDD Bulk upload	Feb-09	
43	Assessing the success rate of buccal cell controls spotted on FTA indicating paper	Jul-08	
42	Kinship Stage 2	Jan-09	Impact and risk assessment only
41	3130 upgrade	Jul-08	
40	Batch uploading to NCIDD	Jul-09	
39	Semen	Project abandoned	
38	GM ID-x	Feb-09	
37	Tapelift	Project abandoned	
36	Hair	Project abandoned	
35	Quant DUO	Not implemented	
34	Kinship Stage 1	Jan-09	
33	Pk Ht RFU & AI	Feb-09	
32	Off-deck DNAIQ Super retention	Mar-08	
31	One tube testing	Not implemented	
30	RSID Saliva	Not implemented	
29	Swab Submission Improvement	Jul-08	
28	RSID semenogelin	Not implemented	
27	Additional PSA verification	In-house study	
26	Barcodes on receipts	Jul-08	
25	7500 verification	May-08	
24	Supplier change PSA	Not implemented	
23	FTA Destruction checklist	Dec-07	
22	Implementation of the Crime-lite	May-07	
21	Packaging destruction	Jun-08	
20	Upgrade 3130	Feb-08	
19	Tech Admin redesign	Closed	
18	Statement preblurbs	Oct-06	
17	SAIK Improvement	Closed	
16	Cut off limit for statstics and Fst	Closed	
15	Xmas Tree Staining	Not implemented	
14	Statement appendix version 4	Oct-06	
13	Quantifiler Singlicates and Promega Standard	Jun-06	
12	BSD punching for chelex extraction	Apr-08	
11	Blood Clothing test code and list	Dec-06	
10	Digital imaging	Closed	
9	Staff movements	Closed	

8	Move Genotyper Comments Field	Closed
7	Examination form updates	May-06
6	Shaking vs Twirling - Cell Extraction	Closed
5	Movement of DVI/Skeletal Analysis to Mortuary Environment	Mar-06
4	Validation	Feb-06
3	Inclusion/Review of all Profiles	Aug-05
2	Court Notification List (AUSLAB)	Oct-05
1	Change Management	Nov-05
	Projects without a project number	
Date of report		
Jul-11	Adhesive DNA Collector Trial Report for QPS	Not implemented
May-10	Oral Swab Submissions - Detecting Foreign Particles	Information for workflow purposes only
May-10	DNA Profiling of Hair Exhibits	Jun-11
Nov-09	Differential Extraction using the Iprep ChargeSwitch Extraction Chemistry and Instrument	Not implemented
Jun-09	PSA kits: Analysis of sensitivity and high-dose hook effect	Jun-09
May-09	A comparison of DNA recovery and profiling from 4N6 versus rayon swabs: using chelex, Nucleospin and DNAIQ DNA extraction techniques	May-09
Oct-08	Effectiveness of Nucleospin clean-up where the 9PLEX profile is no sizing data	Information for workflow purposes only
Nov-07	Phadebas Supernatent Trial: TNE Extraction of Amylase	Mar-08
Apr-May 2004	Quantifiler initial validation conducted	Jun-04
	3100 - Validation of Casework samples on the ABI 3100 Genetic Analyser	Validation approval date: Jan-03
	3100 Validation (Reference Samples)	Validation approval date: Jan-04
	FTA PFTA Protocol	Validation approval date: Jan-04
	377/3100 Low level DNA study	Validation approval date: Jan-04
	STATS - BRB Stats Validation	Validation approval date: Feb-04
	Investigation into DNA quantitation using Quantifiler sustem (with inhibition data)	Validation approval date: Jan-05
	Quantifiler Report - Victorian Police	Validation approval date: Apr-05
	Review of Petricevic Report	Validation approval date: Jun-05
	STATS - BRB Stats population Data (Caucasion, Aboriginal, Asian)	Validation approval date: 2005
	NCIDD Validation and Test protocols (4.0.0)	Validation approval date: Mar-06
	Extended Internal Prospective Valiation of the ABI Prism 7000/Quantifiler system (Forensic Biology) Including external reports	Validation approval date: Jul-06
	Extended Internal Retrospective Validation of the ABI Prism 7000/Quantifiler System (Forensic Biology)	Validation approval date: Aug-06
	STATS - BRBStats v1.23	Validation approval date: Apr-07
	STATS - BRBStats v 1.26	Validation approval date: Apr-07
	PSA Validation	Validation approval date: Apr-07
	I3	Not implemented

Automation Project

Project 1	Report on the Verification of automated Quantifiler™ Human DNA Quantification Setup using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform	Feb-07
Project 2	Report on the Verification of automated AmpFℓSTR® Profiler Plus® and COfiler® amplification reaction setup using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform	Feb-07
Project 3	Report on the Verification of Automated 3100 Setup using the MultiPROBE® II PLUS HT EX Platform	Refer to #15 and #19
Project 4	Validation of AUSLAB for Analytical Workflow	Feb-07
Project 5	Implementation of AUSLAB enhancements (Batch functionality)	Feb-07
Project 6	Implementation of Pre-PCR platform	Feb-07
Project 7	Implementation of Post-PCR platform	Not implemented
Project 8	Report on the Validation of automated FTA® Processing using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform	Not implemented
Project 9	Report on the evaluation of commercial DNA extraction chemistries	Refer to #11 and #13
Project 10	Implementation of FTA Processing on Multiprobe II	Not implemented
Project 11	Report on the validation of a manual method for extracting DNA using the DNAIQ system	Jun-09
Project 12	25ul Rxn	Not implemented
Project 13	Report on the Verification of an Automated DNA IQ™ Protocol using the MultiPROBE® II PLUS ht ex with Gripper™ Integration Platform	Oct-07
Project 14	Implementation of Extraction Chemistries on the MultiPROBE II	Oct-07
Project 15	Report on the Verification of Automated Capillary Electrophoresis Setup using the MultiPROBE® II PLUS HT EX Platform	Not implemented
Project 16	Mock sample cleaning: Comparing TriGene™ and bleach and its efficiency in removing DNA.	Nov-06
Project 17	Report on Automated preparation and testing of Quantifiler standards and controls	Feb-07
Project 18	Report on the validation of automate.it STORstar system for automated sequence checking of DNA extracts	Jun-08
Project 19 (A)	Report on the Validation of the Manual 9+1 Method for Capillary Electrophoresis Setup	Feb-08
Project 19 (B)	Close of Post-PCR MultiPROBE® II PLUS ht ex and the Automated Capillary Electrophoresis Setup Method	Not implemented
Project 20	Report on the Verification of the RECAP-96M™ Automated Decapper/Recapper System	Jan-08
Project 21	A Modified DNA IQ™ Method Consisting of Off-Deck Lysis to Allow Supernatant Retention for Presumptive Identification of α-Amylase	Mar-08
Project 22	A Modified DNA IQ™ Method for Off-Deck Lysis Prior to Performing Automated DNA Extraction	Feb-08
Project 23	Hair extractions	Not implemented
Project 24	Sperm Extraction	Not implemented
Project 25	Investigation and evaluation of tapelift materials and procedures	Not implemented
Project 26	Report on Whatman® FTA Concentrator PS™ Parasite Purification	Not implemented
Project 27	DNA IQ Recovery	Jun-09
Project 28	Semen DNA IQ Validation	Not implemented
Project 29	DNA IQ Clean-Up Protocol	Not implemented
Project 30	Rcovery of DNA from IQ Store Plates	Not implemented
Project 31	Suitability of Lovell cuticle pushers as a substrate for fingernail scrapings for DNA Analysis	Not implemented
Project 32	Identifiler	Not implemented

Procedure for Change Management in Forensic DNA Analysis

1 Purpose and Scope

This document describes the change management procedure that is to be used within Forensic DNA Analysis, to ensure that all process changes and projects occur in a controlled and timely manner. This procedure applies to all process changes or projects that:

- involve the validation/verification of equipment
- involve the validation/verification of technical procedures
- are projects with external funding
- are internal projects (minor or major) which impact on sample reporting/processing
- involve major LIMS function/configuration changes
- impact on multiple stakeholders
- require staff training to be implemented
- significantly alter workflow procedures

This procedure does not apply to:

- routine document updates/alterations
- minor technical changes which do not impact on sample reporting/processing (e.g. changes in specimen type, storage configuration changes)

As an appendix to this document - is a checklist that can be used to guide staff on how they might approach a new idea/observation. It will assist in establishing if it should be recorded as an emerging/novel practice, as a minor change, or as a full project/change management.

2 Definitions and Abbreviations

For a comprehensive list of abbreviations refer to QIS 23849 Common Forensic DNA Analysis Terms and Acronyms.

e-sign	Electronic signature
FR:	Forensic Register
FSS:	Forensic Scientific Services
IT:	Information Technology
LIMS:	Laboratory Information Management System used to record information and track exhibits/case files.
NATA:	National Association of Testing Authorities

3 Principle

Changes within Forensic DNA Analysis have the potential to impact on our clients, on stakeholders (internal/external to FSS) and may impact on compliance with NATA. As such changes which occur with Forensic DNA Analysis must be carefully considered and

documented. There are a number of types of changes that may occur within Forensic DNA Analysis; for the purpose of documentation - these are classified into five types: administrative change, IT/LIMS change, minor project, major project, and external projects.

Administrative changes: are restricted to changes in processes/workflows that impact on documentation or administration processes only. These changes will most likely occur within the Administrative team within Forensic DNA Analysis. It does not include any changes of a technical nature.

IT/LIMS change: An IT change would apply to the introduction of new software into Forensic DNA Analysis, in some instances for upgrades in software versions or the introduction of new hardware. This type of change would require collaboration with IT services. A LIMS project would include any alteration that required a change in the LIMS function, or major configuration changes. It would not include minor changes such as storage configurations, or minor changes to specimen types etc.

Minor Project: are generally defined as projects that have a duration of <6 weeks and a budget of <$5,000. These projects have a minor impact on sample processing/reporting. Any project which major impact on workflow or sample reporting should be considered under major projects.

Major Project: are generally defined as projects that have a duration of >6 weeks and/or a budget of >$5,000. Major projects require significant planning and detailed consideration of project impacts and implementation procedures.

External Projects: is to be used for all projects which have been externally funded. Where there are no documentation requirements for an externally funded project – standard change management document as described in this document apply. For RDAC projects, RDAC documentation requirements apply (QIS 33017) with the additional requirements of:

- A change management number will be assigned within Forensic DNA Analysis
- Management Team are to indicate that they have reviewed all RDAC proposals by adding their name to the Excel sheet included within the project folder

The change management procedure utilises a three step process:

- the initial request (Step 1)
- minor change (Step 2a) OR project plan (Step 2b)
- final report, approval/implementation (Step 3)

The utilisation of these steps is dependent on the type of change (administrative, IT/LIMS, minor, major and external) and on the progression of the change management process. Refer to Section 4 for details.

4 Actions

Prior to the preparation of any change management documentation it is recommended that ideas are discussed at the work unit level to determine the merit of each idea or proposal. If the process of change management is initiated it will need to follow the documentation requirements as listed in sections 4.1 to 4.8 and the workflow as shown in Appendix 1.

*An exception is made for projects that are a mandatory requirement for the laboratory e.g. validation/verification of a new process or equipment item. In these cases it is possible to proceed directly to a full project plan (section 4.3).

For large projects an overarching project number is allocated (by quality) to the work, and sub-projects may then be allocated “a letter” such that sections/parts of a project can be signed off separately. For example in validating an amplification kit a project number 1234

may be applied (for the overarching project), with sub-projects 1234a – referring the sensitivity testing, 1234b referring to concordance, 1234c referring to thresholds etc.

In cases where supplementary testing for a project is required (post-sign off), if the data is an extension of previous work - it may also be appropriate to allocate the supplementary work "a letter" ie. part b of the same work. If the supplementary work is substantially different in topic or content a new project number should be allocated.

All project documents are to e-signed and locked at completion. Refer to Appendix 5 for e-sign procedure.

Technical Review:
For major projects and for validations it is a requirement for the project to have a technical reviewer. The role of the technical reviewer is to 'peer view' critical technical aspects of the project (e.g. new instrument programs/settings, new analytical procedures) and/or to review data analysis with the project (e.g. Excel data transformations, formula's and calculations etc.). The technical reviewer/s are nominated by the team leader and/or management team at project proposal stage (section 4.3). The technical review is completed either during the project or at the completion of the laboratory work and data analysis - but prior to final report being presented to the management team. The technical review should provide to the Management Team as a written document that outlines the aspects of the project reviewed and general findings (Refer to Appendix 2 for template)

Communication:

- For large projects regular project updates should be given by the project leader (or delegate) to the management team. This will allow the management team to ensure that the project is meeting all requirements (NATA, internal needs etc), and that they have a full understanding of the project prior to final report preparation and sign off.
- When projects are complete - presentations should be made at team meetings so that all staff have an awareness of new processes and technology as it is released.
- Appropriate communications should be made at time of implementation (emails to applicable staff, additions to minor change registers, records to quality etc).

4.1 Initial Request (Stage 1)

Change requests can be initiated by any staff member within Forensic DNA Analysis, and are to be recorded on an **Initial Request Form** (QIS 31543). Submission of an initial request requires the following actions:

- Complete the **Initial Request Form** (QIS 31543). The initiator is required to complete the blue sections of the form only.
- Initiator is to email the Quality Team and Line manager (of the person initiating the request) with the network location of the document so it can progress.
- Quality will allocate the request a proposal number
- The Line Manager is to complete the red sections of the form, create a PDF of the request form and e-sign the document. Store/save the document to the appropriate project folder in I:\Change Management

The Line Manager will assess the initial request recommending either:

- **Abandon process at Initial Request** (Refer to section 4.7)
- **Proceed to Step 2:**
  - **Minor Change** (Refer to section 4.2)

    or

- **Project Proposal** (Refer to section 4.3)

If the line manager wants to recommend proceeding to a full project proposal – they will need to seek Management Team approval.

If the initial request is abandon - no further action or documentation will be required.

On completion of the initial request form (e-signed and locked), the line manager is to advise quality team

4.2 Minor Change (Stage 2a)

The minor change form is used to document the purpose, method and date of change. If the Line Manager recommends that the change management is to proceed as a minor change, the project initiator must complete the blue sections of the **Minor Change Form** (QIS 31548) and submit it to their line manager. In some circumstances a small amount of experimental data may be included within a minor change – where the data is used for decision making purposes.

The Line Manager must then complete the following actions:

- **E-sign** the minor process change document (QIS 31548). Store/save the document to the appropriate project folder in I:\Change Management\Minor Change Forms - completed
- Add the change to the **Minor Change and emerging or novel practices** register located in: I:\Change Management\Change Register - Minor Changes and emerging or novel practices.xls
- **Inform the quality team and all stakeholders of the change** e.g. team meetings or email
- Update SOPs etc. if required
- Inform the Quality & Projects Senior Scientist to complete the process

The Quality & Projects Senior Scientist must:

- **E-sign and lock** the minor process change document (QIS 31548)
- Ensure all above actions have been completed by the line manager.

4.3 Project Plan (Stage 2b)

If the Management Team recommends that a change management should proceed as a full proposal (administrative, IT/LIMS, major change or external project) the project leader is required to complete the following project documents:

1. **Project Risk Assessment Document** (QIS 22872): A risk assessment must be completed documenting the risks of the project for each team.

2. **Change Management Project Proposal (experimental design) Document**: This document should cover all aspects of what the project is proposing to do: It should include an introduction to the project (including literature review), purpose/background, methodology and experimental design (either laboratory experiments or data analysis as applicable) and a detailed materials and methods section.

   Refer to QIS 23402 for writing guidelines and template for the project proposal. These project proposals will essentially constitute the introduction and materials and methods section of the projects final project report

This document must be prepared and submitted to the Forensic DNA Analysis Management Team along with the Project Risk Assessment Form (QIS 22872).

3. **Consider ethics requirements**: QIS 33268 Police Services – Human Ethics Review Checklist, it may impact on the projects methodology, and ethics approval maybe required before the project can start.
4. **(Optional) Project Budget** (QIS 31052): A budget can be prepared and submitted to the Forensic DNA Analysis Management Team - with the project proposal. A budget template is provided in QIS 31052.

For a new piece of equipment, new chemical or new process a formal risk assessment (QIS 29106) will be needed in addition to the project risks that are outlined in QIS 22872 The formal risk assessment addresses workplace health and safety risks and the project risk assessment is in relation to business risks.

After all project documents have been prepared (as listed above); risk assessments (if applicable) and LIMS documentation completed (if applicable) email your Line Manager and Quality Team (FSS_BiologyQuality@health.qld.gov.au) and advise them of the location of the documents in I:\Change Management. The Line Manager/Project leader will submit the documentation to the Forensic DNA Analysis Management Team for consideration (Refer the section 4.4), with a due date for feedback.

4.4 Forensic DNA Analysis Management Team – Consideration of Project Proposal

The Forensic DNA Analysis Management team will consider the change management project proposal documents as outlined in section 4.3. It is not necessary for all Management Team members to read and approve every proposal; however a quorum of the Management team must approve the proposal. The quorum must include the Managing Scientist, Team Leaders, Quality and Projects Senior Scientist, Senior Scientist that has Line Management of the staff/project and Senior Scientist/s of areas significantly affected by the project. For major projects and validations a technical reviewer suggestion should also be provided to the management team for consideration (Refer to section 4).

Consideration of the proposal should include:

1. *A determination of the impact of the proposed change on all stakeholders*
2. *Cost/Benefit Analysis of the project*
3. *Risk Assessment (Workplace Health & Safety and Business Risks)*
4. *A communication plan for all project participants and stakeholders*

The Forensic DNA Analysis Management Team will then make a recommendation as follows:

- **Implement proposal.** If the proposal is approved, the project documentation will be e-signed by the Management Team. The project leader/appointed staff can initiate the project.
  - Project work must be conducted by a technically experienced and competent person (Refer QIS 10662)
  - For projects that are >3 months, the Senior Scientist Quality and Projects will meet with each project team ~ every 2 weeks to ensure project progression, and to provide advice and resources as required.
- **Implement proposal after change.** If the Management Team requires additions/edits to the project proposal, the Management team will return the document to the project leader/appointed staff with feedback. The project

documents will need to be edited and resubmitted (as per section 4.3.) before further consideration by the Management Team.
- **Abandon process.** Refer to Section 4.7 for details.

After the due date for feedback project leader/line manager should:
- Make edits (if required).
- Create a PDF of the project proposal and project risk assessment documents
- Store/save the document to relevant project folder in I:\Change Management

Management team must:
- Provide feedback on the proposal
- Complete the risk assessment

4.5 Implementation and Final Report (Step 3)

On completion of the change management project - a final report is required, this is usually written by the project leader (Refer to QIS 23402 for report preparation details). A Technical Review - if it is required (Appendix 2), and an Implementation Plan (Refer to Appendix 3) must also be prepared. The implementation plan will be a list of the steps required to be completed either before the change is implemented, or shortly after implementation. Although a proposal may not be implemented on completion, a basic implementation plan that can be refined closer to implementation should still be completed and submitted. On completion of the report, technical review and implementation plan, they are to be forwarded by email to your Line Manager. The Line Manager/project leader will submit the final report, technical review and implementation plan to the Forensic DNA Analysis Management Team for consideration/acceptance.

If the final report is accepted by the Forensic DNA Analysis Management Team it will be e-signed and the project/change management process closed. If the Management Team requires additions/edits to the final report, it will be returned to the project leader/appointed staff with feedback. The final report will need to be edited and resubmitted for consideration by the Management Team.

After the due date for feedback project leader/line manager should:
- Make edits (if required).
- Create a PDF of the project proposal and project risk assessment documents
- Ask the management team to e-sign the document.
- Store/save the document to relevant project folder in I:\Change Management

Management team must:
- Provide feedback on the final report
- E-sign the documents as/when requested by the project leader/line manager.

After acceptance of the final report the Forensic DNA Analysis Management team will recommend that the:

- **Change is implemented** into routine use (Refer to Appendix 3 for implementation plan for project leaders).
- **Change is accepted but will be implemented at a later date** (Refer to Appendix 3 for implementation plan for project leaders).
- **Change is abandoned** (Refer to Section 4.7 for details).

After completion of the project, all stakeholders must receive communications about the findings and outcomes of the project. This may include presentations at meetings, or the provision of final reports to stakeholders. For significant projects, a summary of the project is to be presented at team meetings.

On completion of the final e-signature by the Managing Scientist a communication is to be sent to the Quality team so that they can ensure all documents have been finalised. Quality team can then lock and store data files by loading them to the Forensic Register.

**Please note: in the event the work is to be published, please consider if the publication needs to reviewed by the FSS Ethics committee. Refer to QIS 32177 FSS Publication checklist.*

4.6 Responsibilities in Signing Documentation

When a project proposal or report is submitted for review, it is the responsibility of the reviewer to ensure that all feedback is provided by the due date. Any feedback provided after the due date may not be considered (based on the merit of the feedback).

It is acceptable for a reviewer from the Forensic DNA Analysis Management Team to seek advice from other members of staff where it is deemed appropriate (e.g. where another person may have more experience in the subject of the report). In this instance, it is the responsibility of the person seeking the advice to provide the feedback to the project officer and to do so by the due date.

4.7 Abandoned/Cancellation

Should a change proposal not be approved, or if at any time the change is no longer required, the change management process may be abandoned/cancelled. This shall be recorded on the change management documents (to be forwarded to the Quality Team). If the project is abandon mid-way through a process an electronic file note can be created to detail the date and reason for project cessation.

It is possible to re-start abandon change management processes at a later date, and there are relevant sections in the change management forms to record a restarted process.

4.8 Recording Feedback

Project feedback, including feedback on project proposal and reports, is to be tabulated and stored in the relevant change management folder (under the appropriate project number folder).

All email communications regarding the project are also to be stored in the relevant change management folder.

5 Records

- All change management documentation (plans, reports, data etc.) are to be stored electronically in a network drive (e.g. I:Drive)
- On completion of projects all records (plans, reports, excel files etc) are to be stored in Forensic Register. To store records in FR:
    - Create new FR case Job Type=Research
    - Subject/Complainant=Project number and short title
    - Offence Class=Miscellaneous
    - Location=Forensic DNA Analysis Quality
    - Project documents loaded as an examination summary

6 Associated Documentation

QIS: 10662 FSS Guidelines for Method Validation
QIS: 22872 Project Risk Assessment for Change Management in Forensic DNA Analysis
QIS: 23401 Forensic DNA Analysis Validation and Verification Guidelines
QIS: 23402 Writing Guidelines for Validation and Change Management Reports
QIS: 29100 Health & Safety Risk Assessment Form
QIS: 29106 Risk Management Guideline – conducting and evaluating Health and Safety risk assessments
QIS: 31052 Forensic DNA Analysis - Change Management Budget
QIS: 31543 Initial Request Form for Change Management in Forensic DNA Analysis
QIS: 31548 Minor Process Change Form for Change Management in Forensic DNA Analysis
QIS: 32177 Human Ethics Review Checklist - FSS Publications
QIS: 33017 FSS Research and Development short form
QIS: 33268 Human Ethics Review Checklist - Police Services
QIS: 33333 Participant Information and Consent Form (PICF) - Common Biological Samples
QIS: 33334 Participant Information and Consent Form (PICF) - Semen Samples
QIS: 33335 Participant Information and Consent Form (PICF) - Vaginal Samples

7 Amendment History

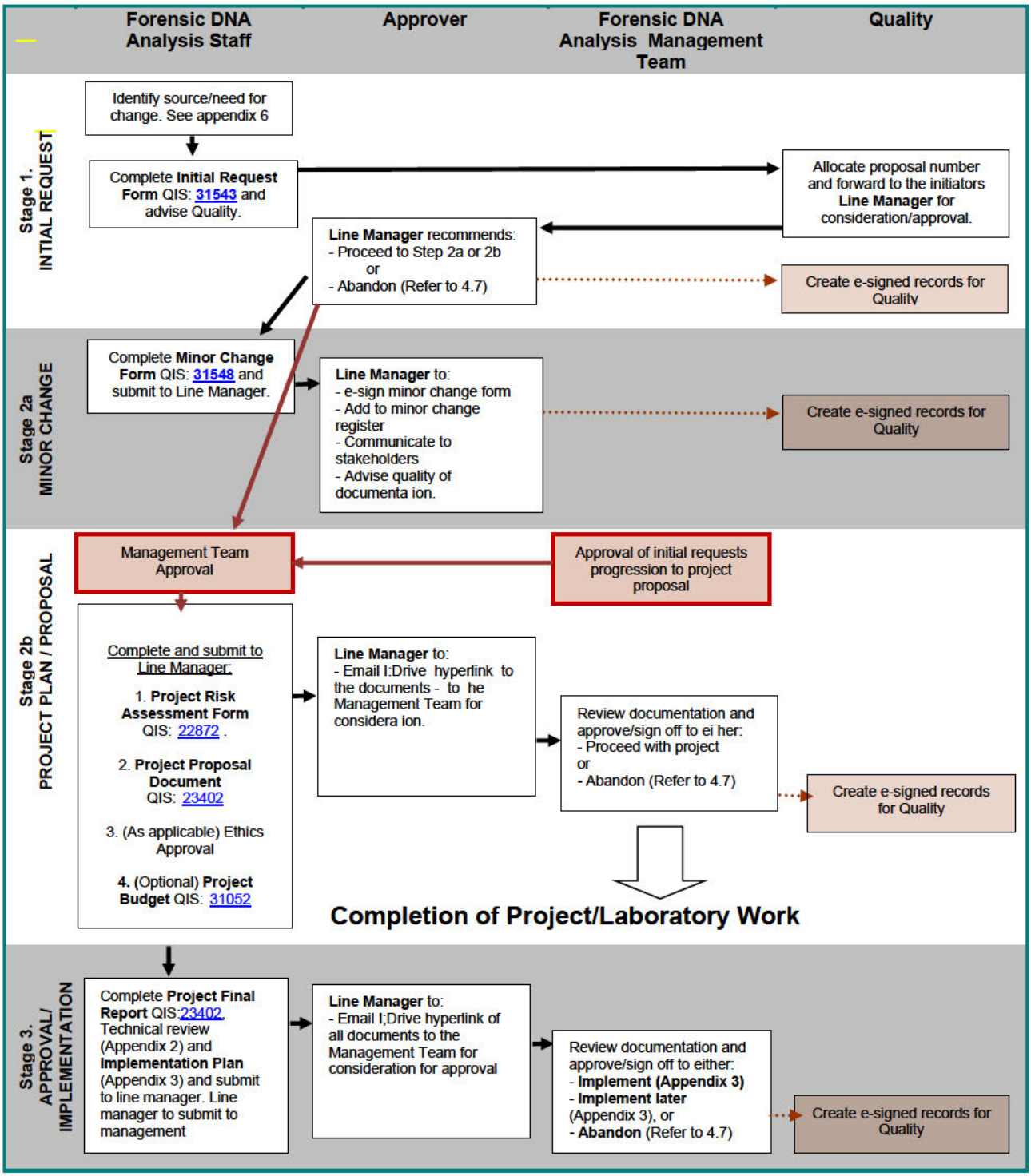
Version	Date	Author/s	Amendments
1	25 Aug 2005	Mary Gardam	First Issue
2	27 Feb 2007	J Olsson, M Gardam V Ientile	Format Changed to include Project Management.
2	April 2008	QIS2 Migration Project	Headers and Footers changed to new CaSS format. Amended Business references
3	25 Sept 2008	Robyn Smith Crystal Revera	Formatting, Changes made to reflect new Laboratory name & Contacts
4	14 May 2012	Shannon Thompson Kirsten Scott	Major revision/re-write as the change management process changed.
5	21 Jan 2013	Kirsten Scott	Update QIS numbers and headers. Add records, associated documents and minor edits.
6	26 Mar 2013	Kirsten Scott	Clarify point 3 in section 4.4. Update hyperlinks
7	6 June 2014	Kirsten Scott	Remove Assessment Phase. Change in actions required by line managers for approving initial plan and minor change documents.
8	19 June 2015	Kerry-Anne Lancaster	New template. Added milestone register and implementation plan. changed AUSLAB to LIMS, defined project proposal and responsibilities of the reviewer. Add QIS 33017
9	21 Oct 2015	Kirsten Scott	Inclusion of consent forms in associated documents. Option for mandatory projects to proceed directly to project plan. Inclusion of RDAC processes & Quality Checklist
10	25 Nov 2015	Kirsten Scott	Inclusion of a technical review for major projects and validations, and minor text update in other section as a result of technical review
11	20 Sept 2016	Kirsten Scott	Specify implementation plan as mandatory, Section 4.5 and 5 add a note on locking of data by quality. Section 3 clarify RDAC requirements

12	1 June 2018	Kirsten Scott	Remove milestone register (section 4.3, 4.8). Add comms and project numbering to section 4. Addition of FR instructions section 5. Add technical review template as appendix 2.
13	19 Nov 2019	Kirsten Scott	Add Human ethics checklist section 6. Additions to section 4.4: meetings with Quality Sen/Sci., and staff competency requirements. Header added to appendix 8.4
14	2 Oct 2020	Kirsten Scott	Edit document to reflect change from hardcopy records to electronic sign-off processes. Additions to appendix 4
15	14 July 2021	Abbie Ryan	Addition of Appendix 5 – e-sign procedure. 4.2 Addition of extra signature step to minor change procedure for Quality Senior Scientist. 4.3.1 Changed title of document 22872 to Project Risk assessment.
16	10 Dec 2021	Kirsten Scott	New header, remove optional Gantt chart for projects, add ethics QIS links and requirements and emerging/novel practices (Appendix 6)
17	30 Mar 2022	Abbie Ryan	Updated Appendix 3 – implementation plan tasks.

8 Appendices

- APPENDIX 1: Change Management Process
- APPENDIX 2: Technical Review Template
- APPENDIX 3: Implementation Plan for project leaders
- APPENDIX 4: Checklist of documents required for a Change Management Project
- APPENDIX 5: Procedure for e-signing documents
- APPENDIX 6: New and emerging novel practices checklist

8.1 APPENDIX 1: Change Management Process



8.2 APPENDIX 2: Technical Review Template

Technical review of Proposal #Project number
Project title

General project observations:

Experiment 1:

Program settings checked: Yes / No / Not Applicable. Comments:_____

Formulas checked: Yes / No / Not Applicable Comments:_____

Data transformations checked: Yes / No / Not Applicable Comments:_____

Calculations checked: Yes / No / Not Applicable Comments:_____

Experimental observations (design/results etc):

Experiment 2: (add additional experiments as required)

Program settings checked: Yes / No / Not Applicable. Comments:_____

Formulas checked: Yes / No / Not Applicable Comments:_____

Data transformations checked: Yes / No / Not Applicable Comments:_____

Calculations checked: Yes / No / Not Applicable Comments:_____

Experimental observations (design/results etc):

Technical Reviewer

Name	Position	Signature	Date

Project Manager

Name	Position	Signature	Date

8.3 APPENDIX 3: Implementation Plan for project leaders

Successful project implementation may require numerous tasks to be completed either prior to implementation, or shortly after the implementation date. Some of the considerations/tasks that may be required are listed below; however, this is not intended to be a comprehensive list of tasks as each project will have different implementation requirements. Project leaders must devise and submit a comprehensive implementation plan for management review prior to the final report being signed off. Ideally, this implementation plan should be provided at the same time as the draft final report.

Once complete, the checklist should be submitted to the quality team for filing with the signed project documents.

Project Title:_____ Project Number:_____

Task	Details	Responsible Line Manager/ Allocated to:	Date Completed
e.g. Create new procedures	New SOPs and training modules to be written and approved		
e.g. Update procedure/s	Existing SOPs and training modules to be revised and approved		
e.g. Staff training	Project members and relevant to staff to be issued with CTT statements as required		
	CTT staff to train relevant staff		
e.g. Software setup	Final version of software to be setup and reviewed on instrument		
	Check if Macro updates are required		
e.g. Equipment tasks	Add equipment to QIS		
	Add equipment to LIMS		
e.g. Consumable tasks	Add consumables to LIMS.		
	Addition of products to FAMMIS		
	Order new consumables		
e.g. Forensic Register development/requirements			
e.g. DNA interpretation/reporting	May include: Model Maker requirements and assessment, Statement of Witness appendix update		
e.g. Impacts/risks assessed	Any risks identified in risk assessment are addressed.		
e.g. Add to minor change register	Ensure that implementation has been added to the minor changes register		
e.g. Communication	Communicate to staff and other stakeholders – by meetings and emails.		

8.4 APPENDIX 4: Checklist of documents required for a Change Management Project

Project Number: _____

Minor Change:

☐ Initial Request Form (31543) (May not be required for mandatory projects)

☐ Minor Change Form (31548)

☐ Added to Minor Change Register and emerging or novel practices register

☐ Implementation (Comments added to SOPs (if required) and communication to staff)

Major Project:

☐ Initial Request Form (31543) (May not be required for mandatory projects)

☐ Project Risk Assessment Form (22872)

☐ Project Proposal Document

☐ (Optional) Project Budget (31052)

☐ Ethics checklist and/or approval - if applicable (33268)

☐ Risk Assessment (As applicable for new equipment and laboratory procedures 29100)

☐ Project Final Report

☐ Technical Review (for validations and major projects only)

☐ Implementation Plan

** Consent forms for staff collections should have been previously provided to quality if applicable.

RDAC project:

☐ RDAC Application Form (Copy only, original stored with Research Office)

☐ RDAC Final Report – if the project is funded (Copy only)

☐ Excel Sheet – with Names of Management Team for acknowledgment of project.

☐ **Quality team have loaded all key project documents to FR for storage**

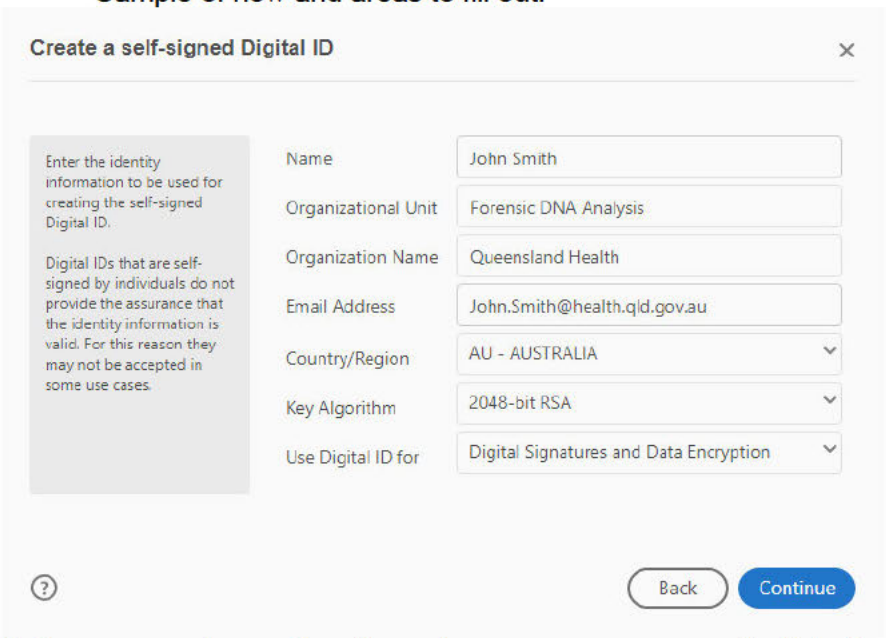
Checklist completed by:_____ Date: _____

8.5 APPENDIX 5: Procedure for e-signing documents in Adobe

First time process to set up digital signature:

1. Scan an image of your personal signature and save to your desktop.
2. Open up a PDF document in Adobe
3. Click tools and Open - Certificate
4. Choose “Digitally Sign”
5. Drag the box to point in PDF document where you want to apply your digital signature.
6. Select Configure Digital ID
7. Select Create a new Digital ID – then continue
8. Select “Save to File” then continue
9. Ensure that you place all your credentials in the name section. (Do not use symbols)

Sample of how and areas to fill out:



Note: you can change the place where you save your credentials, the default saving file location is generally where the adobe program files are kept.

10. Enter a password of your choice. You will use this password every time that you apply it
11. Last step in the process is to attach a copy of your ‘signature’. Click continue
12. Click on the create button
13. Select image then select “Browse” to import in your signature from the file location
14. Click save.
15. To now digitally sign the PDF document, enter password and click sign.

Note: – if you are the final approver, e.g. expenditure delegate, line manager approving the document, you must check the ‘Lock document after signing’ checkbox. This will lock the entire document down and cannot be edited once this has been done.

16. You will be asked to save the PDF file.
17. If the PDF document requires further electronic approvals, it can be forwarded to the next approval for their Digital ID. If the check box is checked ‘lock document after signing’, then the document can no longer be edited or signed.

For all future PDF documents, when you click Digitally sign, you will be asked to select the area to sign and then can select the Digital ID, enter your password and sign the document.

8.6 APPENDIX 6: New and emerging novel practices checklist

This checklist is provided as a template/processes by which staff can consider what to do - when they have seen something new, wish to do something new or are unsure how to proceed with a decide or idea. The emphasis is on the documentation and communication of decisions and thought processes - in line with best quality practices.

Step 1: Gather the facts and define the issue/problem.

Step 2: Make an assessment of your idea or what you have seen: taking into account:
- the case implications
- possible expenditure of resources (time and money)
- impact on clients
- health and safety etc. (refer to Section 4 above).

This will allow you to determine who is accountable for the decision, and how big the required and appropriate process will need to be.

Step 3: Action and documentation: For any issue that have a cost implication (resources or significant staff time), or implications for clients - the full change management process would apply (refer to this document above). For new observations and/or emerging novel practices that are smaller in nature - it maybe more appropriate to use following document to detail the issue, your thinking and the decision:

I:\Change Management\Change Register - Minor Changes and emerging or novel practices.xls

Step 4: Communicate to appropriate audience

Example: **Raised by:** John Smith **Date started:** 20/01/2022

Define your issue:
Apparent artefact at D18S51. Artefact shifts between labelling as a 17.1 or 17.2 variant allele. No stutter is observed for this artefact. Only observed in samples from peri-anal, rectal or penile areas.

Has it been seen before? Yes
Where? Case XXXXXXXxXX
Who can make the decision? Myself

Assessment
Adds contributor to otherwise single source assumed known contributor, height of artefact not consistent with another contribution dropping out. No expenditure of money, time or resources required.

Actions
- Removed artefact from FR GeneMapper table.
- Annotated epgs and re-loaded to Forensic Register
- Notations added to case in Forensic Register.
- Added to Change Register - Minor Changes and emerging or novel practices document

Communication

Who	When	How
All reporters via Microsoft Teams	02/02/2021	Posted
Line manager	01/02/2021	Email

PB140

HealthSupport
Queensland
Forensic and Scientific Services

Miscellaneous Analytical Procedures and Tasks

1 Purpose and Scope

To describe the various miscellaneous tasks and processes not covered by routine DNA extraction, quantification, amplification or capillary electrophoresis within the Analytical Section of Forensic DNA Analysis.

2 Definitions

FR	Forensic Register
FRIT	Forensic DNA Analysis Reporting and Intelligence Team
PP21	PowerPlex® 21 system PCR amplification kit

3 Principle

Samples with high DNA quantification results (>5 ng/µL for CW PP21 and >5 ng/µL for reference PP21) need to be diluted for further processing. Samples are diluted with amplification grade water (PP21) to obtain a target DNA concentration at quantification is approximately 0.5 ng/µL.

Old samples (extracts or substrates) needing to be processed in FR will be registered by the case manager and then transferred by the Analytical staff. DNA extracts and substrates will be transferred into suitable tubes for further processing.

A pooling process is ordered to combine the extracts of two or more samples from the same case. Pooling will be ordered by the case scientist or evidence recovery scientist.

4 Reagents, Equipment and Consumables

4.1 Reagents

Table 1 outlines all the reagents and their storage locations required for the dilution and pooling procedures.

Table 1: Reagents with storage room and location

Reagent	Room	Location
Amplification Grade Water	3188	In-use tray or shelf

4.2 Equipment

Table 2 outlines the equipment and their locations required for the dilution, pooling and transfer procedures.

Table 2: Location of required equipment

Equipment	Location
Fridge	3189
Freezers	3194
Pipettes 100 – 1000 µL	3189
Pipettes 20 – 200 µL	3189
Pipettes 1 - 10 µL	3189

4.3 Consumables

Table 3 outlines the consumables and their locations required for the dilution, pooling and transfer procedures.

Table 3: Location of required consumables

Consumables	Location
1.5 mL and/or 2 mL tubes	3189
Nunc™ tubes	3189

Additional consumables can be found in the store room (3184).

4.4 Entering Reagents, Equipment, Consumables and Locations into FR

1. Access the batch according to the Forensic DNA Analysis Workflow Procedure (34034).
2. Click the **Edit/Update Batch** icon.
3. Using the relevant dropdown menu select the correct consumables and reagents.
4. Scan the equipment and location barcodes into the required fields (Figure 1).
5. Click the **Save Batch** icon.

Note: Fields should be filled out contemporaneously while processing the batch. These steps can be performed at any stage prior to batch completion and entries can be modified after saving.

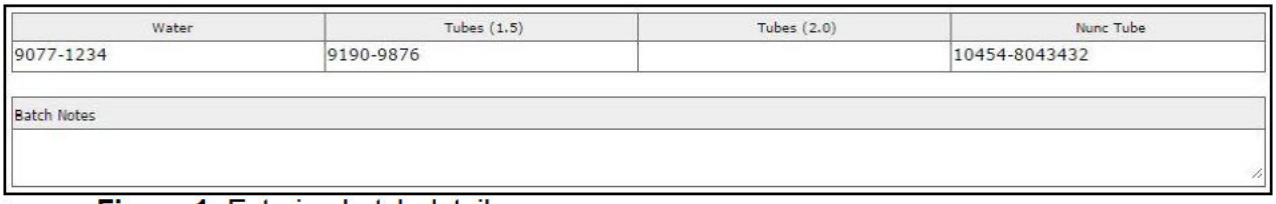


Figure 1: Entering batch details

Note: A notation is added on the pooled or transfer sample for any reagents, equipment, consumables or location used in the procedure. Refer to Section 8.1.2 for adding a notation.

5 Safety

As per the Anti-contamination Procedure (22857), PPE is to be worn by all staff when performing this procedure.

6 Sample Location and Batch Preparation

6.1 Forensic Register Workflow Diary

The FR Workflow diary displays a Worklist Summary (number of samples pending processing under each batch type) and a Batch Schedule (list of batches that are either scheduled, created (in progress) and completed for each day). Batches (e.g. Extraction and Pre-PCR batches) are scheduled in the FR Workflow diary to specify to the Operation staff, which batches need to be created and the samples located. It also specifies to the Analytical staff as to what tasks are required to be processed that day. The FR Workflow diary also allows the analytical staff to communicate and monitor the daily work for each laboratory area in Analytical.

To access the FR Workflow diary, refer to Forensic DNA Analysis Workflow Procedure (34034), Section 5.

6.2 Batch Creation

Dilution batches are created as per the Forensic DNA Analysis Workflow Procedure (34034).

Pooling and transfer procedures do not have a specific batch type, these are scheduled in the Forensic Register Workflow Diary as individual samples. See below for procedure.

6.2.1 Pooling

1. Access the **Sample Management Tab** as per the Forensic DNA Analysis Workflow Procedure (34034).

2. Click the **Worklist** tab and move the cursor down to the **Awaiting Review** sub-menu, then click **Pooling**.

3. The Analytical HP4 will schedule a **Pooling** in the **Analytical Workflow Diary** and will manually enter in the **Assigned To** column a list of **Child** barcodes that require pooling from the **Pooling Review Worklist**.

6.2.2 Transfers

1. Access the **Sample Management Tab** as per the Forensic DNA Analysis Workflow Procedure (34034).

2. Click the **Worklist** tab and move the cursor down to the **Awaiting Review** sub-menu, then click **Transfer**.

3. The Analytical HP4 will schedule a **Transfer** in the **Analytical Workflow Diary** and will manually enter in the **Assigned To** column a list of barcodes (or DNA#) that require transfer and the 'type' of sample that the transfer is coming from (e.g. extract, spin).

6.3 QC Samples

One negative control will be registered automatically by FR when creating a dilution batch according to the Forensic DNA Analysis Workflow Procedure (34034).

6.4 Sample Location and Locating Samples

Samples that are awaiting dilution, pooling or transfers are stored in the freezer in room 3194 and once the batch or samples are pulled (located), it is stored in the fridge room 3189 awaiting processing.

Locate samples according to Forensic DNA Analysis Workflow Procedure (34034).

6.5 Analytical Notes

For Dilution batches:

1. Access the dilution batch as per the Forensic DNA Analysis Workflow Procedure (34034).

2. On the batch **Exhibit Analysis** page, if any samples are coloured half orange, hover the cursor over it to check for analytical notes that request for specific processing comments (e.g. DILN factor) (Figure 2).

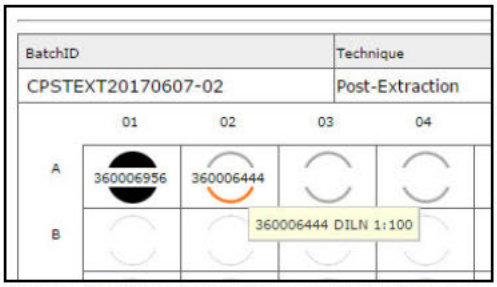


Figure 2: Checking for analytical notes

For Pooling and Transfer samples:

1. Analytical notes for pooling or transfer samples can be viewed in the **Exhibit Testing** table in FR (Figure 3). If required, click on the **Date/Time** of the **Pooling** line to view the full notes.

Exhibit Testing

Date / Time	Technique	Testing	Linked No	Employee	Reviewer
07/06/2017 09:51	Pooling	Pooled from 360005230,360005257		[redacted]	
07/06/2017 09:52	Analytical Note	Please pool and microcon			

Figure 3: Exhibit Testing Table

6.6 Creating and Printing Sample Labels

1. Access the batch according to the Forensic DNA Analysis Workflow Procedure (34034).
2. Click the **Subsamples** icon to create additional barcodes refer to Table 4.

Table 4: Subsample labels required for Dilution batches

Method	SPIN	SUPNAT	EFRAC	SLIDE	DILN
Dilution	No	No	No	No	Yes

3. Ensure the negative control **DILN** box is unchecked.
4. Click **Create Subsamples** icon.

Note: Once subsamples have been created and saved, this option is no longer available for the batch.

5. Click the **Batch Labels** icon to display the sample labels. Print the labels to the designated label printer.

Note: Print the barcode for pooling and transfer samples by clicking its Date/Time hyperlink in the **Exhibit Testing Table** and click the **3 Part Tube Barcode** icon and print to the appropriate label printer.

6.7 Sequence Checking

1. Perform a sequence check of all tubes for the dilution batch by clicking the **Sequence Check & Lock** icon.

2. Scan all tubes in the order they are positioned in the rack corresponding with the FR platemap. The parent barcode must be the first barcode scanned.

Note: If a barcode is scanned incorrectly during the sequence check an error message will appear above the virtual rack. Check the position the error message indicates and rescan with the correct barcode.

3. To complete the sequence check, check the **Confirm Sequence Check** box. (Figure 4). The **Confirm Sequence Check** box should not be checked if there are any errors showing on the sequence check screen.

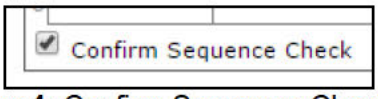


Figure 4: Confirm Sequence Check box

4. Click the **Lock Batch** icon.

Note: For **Reference Dilutions** the parent barcode (visible above the barcode on the extract Nunc™) will need to be physically typed into the first row for each of the samples on the batch.

Note: For Pooling and Transfers a second operator is to check that all labelled tubes are correct and edit the pooling or transfer exhibit test by clicking its Date/Time hyperlink and adding to the **Notes** field "Sequence checked by …" then click the **Save** icon

7 Procedure

7.1 Dilution Procedure

1. All dilutions are performed within a biological safety cabinet in Room 3189.

2. Print labels required as per Section 6.6.

3. For each sample label:
   - 1.0 mL Nunc™ tube (**DILN** barcode)
   - 1.5 mL or 2.0 mL tube as required (**parent** barcode)

4. Vortex mix and pulse spin the original DNA extract tubes (perform prior to sequence check).

5. Ensure a sequence check is performed as per Section 6.7 prior to continuing.

6. Click the **Sample Transition** icon and print the page to the appropriate printer. **Do not** click the **Save Batch** icon, as this will complete the batch, use the **Back** button.

7. Add to the negative control Nunc™ tube 100 µL of amplification grade water.

8. Add to the **DILN** Nunc™ tubes the required amount of amplification grade water as stated in the dilution factor Analytical Note (e.g. 1:25 = 4:100 = 4 of extract to 96 µL of amplification grade water).

Note: The minimum total volume in the Nunc™ tube should be 100uL.

9. Add to the **DILN** Nunc™ tubes the required amount of DNA extract from the original DNA extract tube, according to the dilution factor on the Analytical Note.

10. Vortex mix the diluted DNA extract mix and pulse spin.

11. Ensure all reagents, equipment, consumables and locations are selected against the batch as per Section 4.4.

12. Click the **Sample Transition** icon. Ensure that the Negative control and the DILN subsamples have "DNA Quantification" **Technique** and "Quantifiler Trio" **Method** selected and that all parent barcode **Technique** and **Methods** are blank.

13. Click the **Complete Batch** icon.

14. Click the **Edit/Update Batch** icon.

15. Complete the **Run Date & Run Time** fields and click the **Complete Batch** icon.

16. Store original DNA extract tubes in permanent storage and DILN Nunc™ tubes in temporary storage within the Pre-PCR sorting room (3194 A) in the freezers as per Storage Guidelines for Forensic DNA Analysis (23959).

7.2 Pooling Procedure

1. Pooling is performed within a biological safety cabinet in Room 3189.

2. Print labels required as per Section 6.6.

3. For each sample label:
   - 1.0 mL Nunc™ tube (new **parent** barcode)

4. Ensure a manual sequence check is performed by another operator. The sequence check operator must add a note (eg. BM 06/06/2020: Sequence check performed.) to each sample under the 'Pooling' technique prior to continuing.

5. Vortex mix and pulse spin the original DNA extract Nunc™ tubes.

6. Transfer the original DNA extracts to the new **parent** DNA extract Nunc™ tube.

7. Vortex mix and pulse spin the newly pooled DNA extracts.

8. In the **Exhibit Testing** table click the Date/Time hyperlink of the **Pooling** technique.

9. Click the **[CLICK TO VALIDATE]** text (Figure 5).

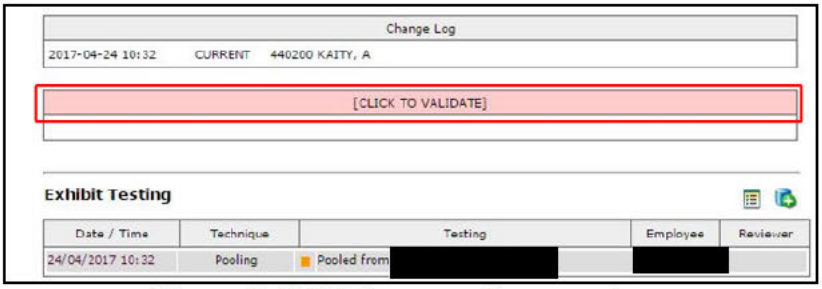


Figure 5: Validating a pooling sample

10. Order the appropriate procedure (eg. microcon or quantification) as per the notations of the case scientist as per Section 8.1.8.

11. Store original DNA extract tubes in permanent storage and newly pooled DNA extract Nunc™ tube in temporary storage within the Pre-PCR sorting room (3194 A) in the freezers as per Storage Guidelines for Forensic DNA Analysis (23959).

7.3 Transfer Procedure

1. Transfers are performed within a biological safety cabinet in Room 3189.

2. Print labels required as per Section 6.6.

3. Assess each sample tube and label as follows:
   - If tube is suitable to carry through next procedure cover barcode with new **parent** barcode leaving the original number visible.
   - If tube is unsuitable (older tubes), select appropriate 1.0 mL Nunc™ or 1.5 mL / 2.0 mL tube and label with **parent** barcode.

4. Ensure a manual sequence check is performed by another operator. The sequence check operator must add a note (eg. BM 06/06/2020: Sequence check performed.) to each sample under the 'Transfer' technique prior to continuing.

5. If required, carefully transfer the extract or substrate from the original tube to the new labelled parent tube.

6. In the **Exhibit Testing** table click the Date/Time hyperlink of the **Transfer** technique.

7. Click the **[CLICK TO VALIDATE]** text (Figure 5).

8. Order the appropriate procedure (microcon, nucleospin cleanup, quantification or amplification) as per the notations of the case scientist as per Section 8.1.8.

9. Store original DNA extract tubes in permanent storage and newly pooled DNA extract Nunc™ tube in temporary storage within the Pre-PCR sorting room (3194 A) in the freezers as per Storage Guidelines for Forensic DNA Analysis (23959).

8 Forensic Register Tasks / Functions

8.1 How to add an Exhibit Test

To add a Process or Technique to a sample, access the **Exhibit Detail** page (Figure 6) by entering the sample barcode into the FR search field. Click the **Add Exhibit Test** icon.

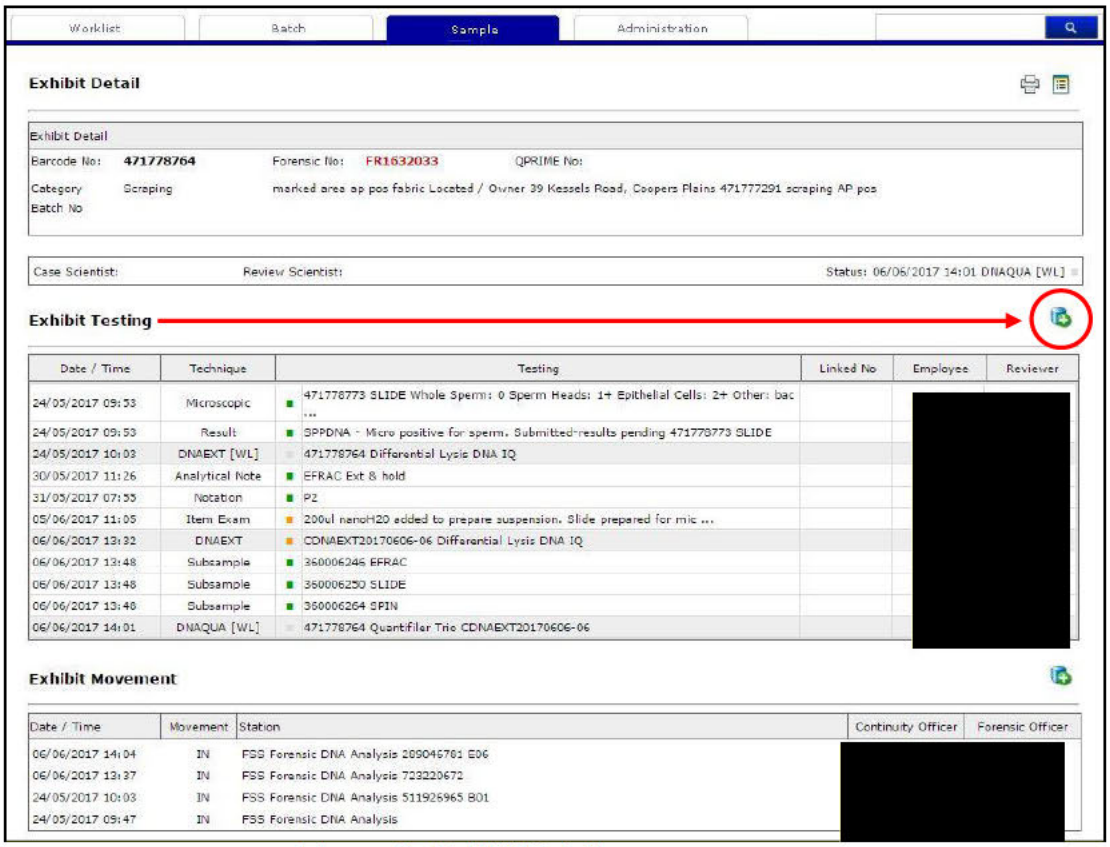


Figure 6: Exhibit Detail page

To add a Process or Technique to a *Subsample*, access the **Exhibit Detail** page of the *Parent* sample (Figure 6) by entering the subsample barcode into the FR search field. Click the **Add Exhibit Test** icon. Ensure that the Subsample barcode is entered into the **SubID** field of Testing / Analysis (Figure 7) before adding a Process / Technique.

8.1.1 Analytical Note Process

The **Analytical Note** process adds a comment to the sample that will be visible as a half orange circle on platemaps and in the Priority / Analytical Note columns on the Sample Transition and Quant Results pages.

1. On the Exhibit Testing page choose **Analytical Note** from the **Process*** drop down box (Figure 7).

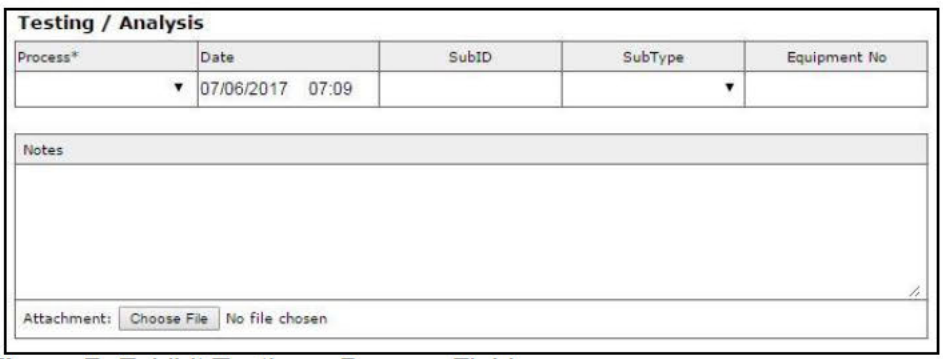


Figure 7: Exhibit Testing – Process Fields

2. Add the required comment in the **Notes** field.

3. Click the **Save** icon.

Note: Any previously entered Analytical Note can be accessed and changed by clicking its Date/Time hyperlink in the **Exhibit Testing Table** for the sample.

8.1.2 Notation Process

A **Notation** process adds a sample notation which can be used to describe adverse events and other anomalies which may require a lengthy account of events for the sample. A notation **is not** visible on platemaps, or on the Sample Transition or Quant Results pages.

1. On the Exhibit Testing page choose **Notation** from the **Process*** drop down box (Figure 7).

2. Add the details of the notation in the **Notes** field.

3. Click the **Save** icon.

Note: Any previously entered Notation can be accessed by clicking its Date/Time hyperlink in the **Exhibit Testing Table** for the sample.

8.1.3 Pooling Process

For adding a **Pooling** process, refer to Procedure for Profile Data Analysis using the Forensic Register (33773). For performing a Pooling procedure refer to Section 7.2.

8.1.4 Reallocate Process

The **Reallocate** process removes a sample from **ALL** its current worklists (including the PDA worklist if present). If a sample is only required to be removed from one worklist when it is on multiple, add the corresponding Technique/s and Method/s as per Section 8.1.8 to add it back on the required worklists.

1. On the Exhibit Testing page choose **Reallocate** from the **Process*** drop down box (Figure 6).

2. Add the reason for the reallocation process in the **Notes** field.

3. Click the **Save** icon.

Note: Hovering over the reallocation exhibit test in the Exhibit Testing table will display all the worklists the reallocate process has removed the sample from.

8.1.5 Subsample Process

The **Subsample** process is used to add a new child subsample to the sample.

1. On the Exhibit Testing page choose **Subsample** from the **Process*** drop down box (Figure 7).

2. Scan an unused barcode number into the **SubID** field.

3. Choose the type of subsample from the **SubType** drop down box.

4. Add a note if required in the **Notes** field.

5. Click the **Save** icon.

6. Print the barcode of the created Subsample by clicking its Date/Time hyperlink in the **Exhibit Testing Table** of the *Parent* sample.

7. Click the **3 Part Tube Barcode** icon and print to the appropriate label printer.

8.1.6 Transfer Process

For adding a **Transfer** process, refer to Procedure for Profile Data Analysis using the Forensic Register (33773). For performing a Transfer procedure refer to Section 7.3.

8.1.7 Changing Priority

The priority of a sample is automatically allocated according to the QPS registration. A change of priority for a sample is to be assessed and performed by the Analytical HP4/HP5.

1. On the Exhibit Testing page choose **Notation** from the **Process*** drop down box (Figure 9).

2. Add the reason for the change of priority in the **Notes** field.

3. Change the priority in the **Priority** field (Figure 8).

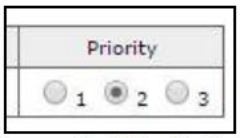


Figure 8: Priority field

4. Click the **Save** icon.

8.1.8 Adding to Worklists / Ordering a Procedure

Adding a sample to a worklist can mean that either a new procedure is required for that sample (i.e. Extraction / Quantification / Amplification / CE / Supernatant Testing procedure) or that the sample needs to be added to a worklist for review (i.e. On Hold / Profile Data Analysis / STRMix).

Note: If adding a **STR Amplification** Technique, ensure there are suitable volumes in the volume fields (SV1, TV1, SV2 & TV2) for the Method selected.

1. On the Exhibit Testing page choose a **Technique*** and its corresponding **Method** from the drop-down boxes (Figure 9).

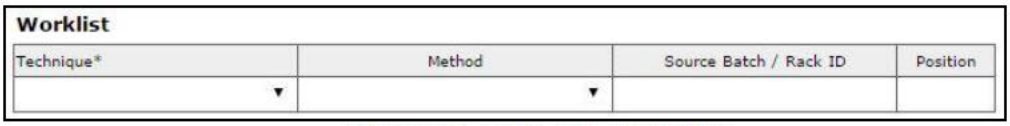


Figure 9: Adding to a worklist

2. Click the **Save** icon.

Note: If adding a **Profile Data Analysis** Technique, ensure the **Source Batch / Rack ID** field has been automatically filled with the samples *most recent* amplification batch. This field will need to be added if not automatically filled.

8.1.9 Ordering a Reference Dilution

A dilution cannot be ordered on an EREF extract as subsamples cannot be created from another subsample. For reference dilutions a dilution is ordered on the *parent* barcode (i.e. FTA or Blood cloth barcode) and an **Analytical Note** is added to specify the barcode of the extract.

1. Access the **Exhibit Detail** page by entering the *extract* barcode into the FR search field. Click the **Add Exhibit Test** icon (Figure 6).
2. On the Exhibit Testing page choose **Analytical Note** from the **Process*** drop down box (Figure 7).
3. Type the dilution factor and the comment "**Dilution of EREF subsample…**" followed by the subsample extract barcode in the **Notes** field.
4. Choose the "Post-extraction" **Technique*** and "Dilution" **Method** from the drop-down boxes (Figure 9).
5. Click the **Save** icon.

8.2 Creating an Exhibit from a Subsample

If a subsample is required to go through a procedure (e.g. an EFRAC that was previously extract & hold or SPIN that requires a re-extract) it must be changed into an exhibit in the FR to continue processing.

To register a subsample as an exhibit, refer to Procedure for Profile Data Analysis using the Forensic Register, Appendix 10- Registering a Sub-Sample as an Exhibit (33773).

8.3 Registering Positive and Negative Controls

Positive and negative controls are required to be registered when creating validation batches for testing of reagents (e.g. DTT, TNE, PowerPlex® 21) or for contamination batches on instruments (e.g. Soccerball for QIAsymphony® and STARlet or Zebra for Maxwell®).

1. Enter into a recent extraction batch in the FR as per the Forensic DNA Analysis Workflow Procedure (34034).
2. Copy and paste the positive control barcode into the FR search field and **Enter**.
3. Copy the FR number for the positive control (numbers only).
4. On the **Case Management Tab** in FR, click **Case Files**.
5. Paste the FR number into the **Forensic No** field and **Enter**.
6. Click **Exhibit Register** (Figure 10).

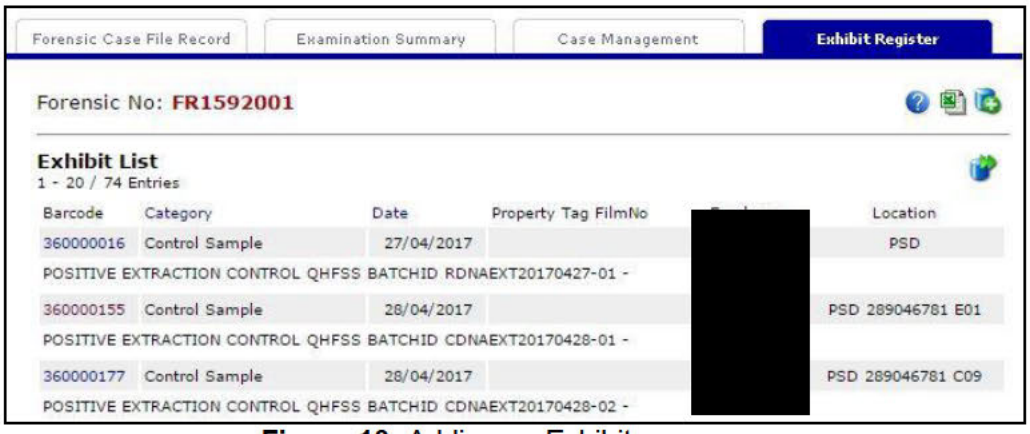


Figure 10: Adding an Exhibit

7. Click the **Add Exhibit** icon.

8. Enter in an unused barcode number into the **Exhibit Barcode** field and choose *Control Sample* from the **Category** drop down box.

9. Type "Positive Extraction Control" in **Description** field and "FSS" in **Located / Owner** field.

10. Type a brief description (e.g. TNE testing, Maxwell A contamination check or QIA A contamination check) into the **Exhibit Notes & FSS Advice** field.

11. Check the *Admission / Intel* box in the **Relationship / Prioritisation** field and ensure the *FSS DNA Analysis* box is checked in the **Examination Section**.

12. Check the *Sample has been collected in strict compliance with CSE101 Biological Evidence [Required]* box in the **Forensic Biology Analytical Advice** field.

13. Enter FR user number into **Delivery Officer Rego** field.

14. Click the **Save** icon.

15. Repeat steps 2 – 13 for the negative control on the extraction batch (ensure "Negative Extraction Control" is typed as the description).

8.4 Registering FSS Environmental Samples

Environmental controls are required to be registered for the monthly environmental monitoring performed in the Analytical laboratories. For registration of FSS environmental sample refer to Environmental Monitoring (34280).

8.5 Locked Batches that Require Changes

Note: To correct a batch that has been created incorrectly and is not at lock status, refer to Forensic DNA Analysis Workflow Procedure (34034).

1. Re-create batch (new batch) with the correct Template, Technique, Method and Type as per Forensic DNA Analysis Workflow Procedure (34034) using original batch ID as the source batch.

2. Ensure reagent, consumable and equipment details from the original batch are entered into the new batch as per Section 4.4.

3. Add a batch note stating, "Batch created from *original batch ID*".

4. Ensure sequence check is performed as per Section 6.7 and process as per relevant procedure.

Note: For CE batches ensure correct statuses have been chosen and if processed run date/time needs to be the same as the original batch.

5. On the original batch add batch note stating reason for batch not progressing.

6. On the original batch change status to N/R.

9 Analytical Tasks

9.1 No DNA Detected (NDNAD) / DNA Insufficient for Further Processing (DIFP) List

The NDNAD / DIFP List is to be checked and actioned by the scientists rostered in Pre-PCR (or if required, other areas also). The scientist who has uploaded the quantification results file cannot validate the NDNAD / DIFP page for samples from that quantification.

1. On the **Sample Management Tab** in FR, click **Worklist**.

2. From the drop-down list choose *Awaiting Review* → *Result.*

3. Click the **[NDNAD/DIFP]** filter.

4. Click the **ExhibitNo** of the sample.

5. In the **Exhibit Testing** table, review the most recent Result. Ensure that the quantification value is within the correct range – See Table 9 Defaults for Quant Results Page (Section 8) in Quantification of Extracted DNA using the Quantifiler Trio DNA Quantification Kit (34045).

6. When reviewing Diff samples, ensure that the Diff microscopy line has been validated prior to validating the DNAD/DIFP line.

7. Click the Date/Time hyperlink for the Result.

8. Click the **[CLICK TO VALIDATE]** text (Figure 5).

Note: Ensure all NDNAD & DIFP DNA extract Nunc™ tubes have a final storage location in permanent storage within the Pre-PCR sorting room (3194 A) in the freezers as per Storage Guidelines for Forensic DNA Analysis (23959).

9.2 No Work Required QPS (NWQPS)

QPS will dictate whether a sample is to cease processing by checking *No Testing Required* on the registration page of an exhibit. Once this has been selected, an Analytical Note of **NWQPS** will be added to the sample which will be visible on platemaps and in the Priority / Analytical Note column of the Sample Transition and Quant Results page.

A NWQPS sample can be halted before or after extraction and quantification batches but after amplification it will be continued on through to capillary electrophoresis. Once a

NWQPS sample has been identified and the process halted, the sample is to be validated and stored in its appropriate final storage location.

1. If the sample is on a batch but has not been processed yet, remove the sample from the batch and replace with another sample.

2. Access the **Exhibit Detail** page (Figure 6) by entering the sample barcode into the FR search field.

3. To remove the sample from the worklist, refer to Section 8.1.4.

4. Return to the **Exhibit Detail** page (Figure 6).

5. In the **Exhibit Testing** table, click the Date/Time hyperlink for the NWQPS.

6. Click the **[CLICK TO VALIDATE]** text (Figure 5).

7. Store any unextracted substrate tubes in the "No Further Work" box and any DNA extract Nunc™ tube in permanent storage within the Pre-PCR sorting room (3194 A) in the freezers as per Storage Guidelines for Forensic DNA Analysis (23959).

Note: If a sample was removed from the batch by the operational staff the sample still needs to be validated. This can be done by checking the contents of the "No Further Work", any sample with an orange square in the right hand side of the exhibit column needs to be validated.

9.3 Discarding Substrates, PCR and CE plates

9.3.1 Substrates

Discard storage boxes in the freezer within the Pre-PCR sorting room (3194 A) can be emptied one month from the date of the last sampled stored. Substrates stored in discard boxes should be limited to positive controls, environmental swabs (QPS or FSS), CTS and substrates that are unlikely to produce a DNA profile upon re-extraction (e.g. cigarette butts, paper, straws).

1. On the **Case Management Tab** in FR, click **Equipment**.

2. Enter the storage box barcode in the **Storage Boxes** field and press **ENTER**.

3. Click the **Contents** tab.

4. Enter into each exhibit number that shows a 'QP' number in the 'Case' column and ensure that the substrate is ok to be discarded.

Note: 'QP' numbers that has the year and a string of zeros for example 'QP1900000000' are police environmental samples and are therefore ok to be discarded.

5. Click the **Empty Storage Box** icon.

6. A warning will appear confirming the destruction of the substrates (Figure 11) click the **Empty Storage Box** icon to confirm.

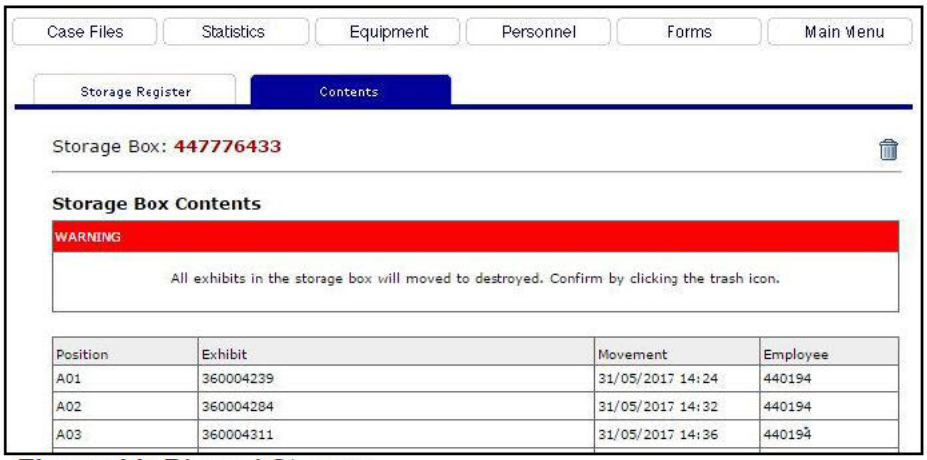


Figure 11: Discard Storage

7. Substrate tubes are discarded in a biohazard bin.

Note: If a substrate has been "destroyed" in error, store the substrate tube in a spin storage box within the Pre-PCR sorting room (3194 A) in the freezer as per Storage Guidelines for Forensic DNA Analysis (23959). Add a notation to the sample / subsample indicating that the substrate was not destroyed as per Section 8.1.2.

9.3.2 PCR Amplification Plates

The different types (e.g. CSTRAMP, RFTAAMP (FTA/RPT/OSD/RUN), RSTRAMP) of PCR amplification plates each have a minimum length of time that they are required to be kept. After this time has lapsed, the PCR plates can be discarded.

Storage requirements for each plat type are as follows:
- Direct amp reference (RFTAAMP): 3 months
- Extracted reference (RSTRAMP): ≈12 months
- Casework (CSTRAMP): minimum 18 months, longer if storage space is available
- Validation plates: ≈12 months after sign off (ensuring the plates are **not** routine plates)
- TestAmp an routine Soccerball check: ≈1 month

1. For each plate being discarded, access the batch according to the Forensic DNA Analysis Workflow Procedure (34034).
2. Click the **Edit/Update Batch** icon.
3. Enter in Batch notes **"Amp plate discarded"** with initials and date.
4. Click the **Save Batch** icon.
5. Discarded the PCR plate into a biohazard bin.

9.3.3 CE plates

CE plates can be discarded from the freezer after approximately 1 week and are discarded in a biohazard bin.

9.4 Weekly Stocktake

The weekly stocktakes of the manual extraction, auto and Pre-PCR laboratories is the responsibility of the rostered HP2 staff member or an Analytical staff member if required.

This task is to be completed at the start of the week preferably before Thursday. The CE stocktake is to be performed by the rostered CE scientists.

Note: If the rostered staff member has a valid reason for not being able to complete this task, the line manger should be notified so that an alternative arrangement can be made so that it gets done.

1. Access the stocktake in I:\AAA Analytical\Analytical Spreadsheets

Note: Each time the stocktake list is revised the document must be saved as a new version. This is expected to occur semi-regularly as usage levels change according to operational needs.

2. Complete the stocktake for each laboratory area and initial and date the spreadsheet tab corresponding to the laboratory.

3. The operational staff supervisor will periodically access the stocktake spreadsheet and place orders as required.

4. Re-stock all rooms with the necessary consumables from either Block 3 or 6. If there is insufficient stock, this must be indicated on the stocktake list so it can be ordered.

5. Any reagents that are running low in the clean room that require the operational staff to make in-house can be noted on the whiteboard within the ante-chamber (room 3187).

10 Analytical Diaries, Logs and Smart Roster

10.1 Overview of the Electronic Diaries

The Electronic workflow diary is no longer in use (replaced by FR Workflow Diary), however there are 3 electronic (instrument) diaries still in use. These are the CE, QIAsymphony and Pre-PCR diaries. All electronic diaries (in use and older/archived versions) are located in I:\AAA Electronic Workflow Diary. The diaries allow instrument maintenance and schedules (e.g. consumable or part changes, PM/regular maintenance), to be recorded for each area, along with a log of batches/processes performed. As the diaries are used to log tasks in a chronological order, they are also useful for troubleshooting requirements.

10.2 Overview of Analytical Logs

Electronic logs are maintained by the Analytical staff located in I:\AAA Analytical\Analytical Logs. The Logs maintained include:

- **QC Swab and Reagent results Log** – monitors the preparation and results of the Blood and Diff. controls

10.3 Contamination and Adverse Events in Analytical

Contamination events may have occurred when the presence of DNA is suspected within a negative control, when a positive control contains an unexpected profile, or when a sample yields an unexpected DNA profile when viewed in context (e.g. a suspected mixture present in a person sample).

Adverse events are any occurrences that happen during the analytical procedures that differ from the standard order of procedure. Adverse events are recorded by the analytical staff in I:\Adverse Events DNA Analysis and the log is maintained and monitored by the Analytical HP4\HP5.

For investigating of adverse events refer to Investigating Adverse Events in Forensic DNA Analysis (30800).

10.4 Smart Roster Instructions

The Smart Roster program is used to roster the "late" (4.30pm) finishers within the Analytical team and the plate reading roster. Refer to Appendix 14.1 for instructions on how to use the software.

11 Quality Assurance/Acceptance Criteria

A negative control is included in a dilution batch and is processed as normal samples through to completion. If a result is obtained from the negative control, either at quantification or during the CEQ check, then the possible source of the contamination is investigated. The samples extracted with this control are thoroughly checked and repeated if required. This is covered in Capillary Electrophoresis (CEQ) Quality Check (34131).

12 Associated Documents

22857 Anti-Contamination Procedure
23959 Storage Guidelines for Forensic DNA Analysis
30800 Investigating Adverse Events in Forensic DNA Analysis
33773 Procedure for Profile Data Analysis using the Forensic Register
34034 Forensic DNA Analysis Workflow Procedure
34044 DNA IQ Method of Extraction Using Maxwell 16
34045 Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit
34052 Amplification of Extracted DNA Using the Powerplex21 System
34062 Capillary Electrophoresis Setup
34131 Capillary Electrophoresis Quality (CEQ) Check
34132 DNA Extraction and Quantitation of Samples using the QIAsymphony® SP and AS - FR
34280 Environmental Monitoring
34514 Preparation & Testing of Quantification Standards, In-house Controls, Quantification Kits and Amplification Kits

13 Amendment History

Version	Date	Author/s	Amendments
1	June 2017	A Kaity, M Mathieson, L Farrelly	Changeover of LIMS from AUSLAB to Forensic Register. Update relevant sections.
2	June 2019	P Acedo	Removed sections related to P+, added a section for the STARlet contamination checks, updated hyperlinks and minor formatting.
3	Sept 2020	B Micic	Removed Contamination Batch sections (7.4,7.5,7.6) as moved to QIS 34280, updated 6.2. Split Section 8 into 3 sections. Reworded Sections regarding electronic workflow diary. Minor changes.

14 Appendices

14.1 Smart Roster Instructions

1. Open the shortcut to SmartRoster on desktop 

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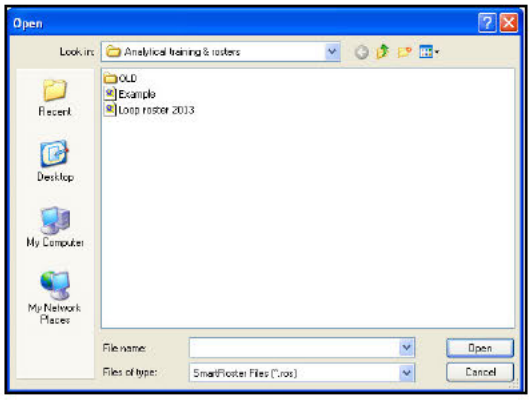
2. The "Welcome to SmartRoster" prompt box will appear.

3. Choose "Open – An existing SmartRoster file"

4. Open box will appear. Navigate to location of file e.g. G:\ForBiol\AAA Analytical\Analytical Training & rosters

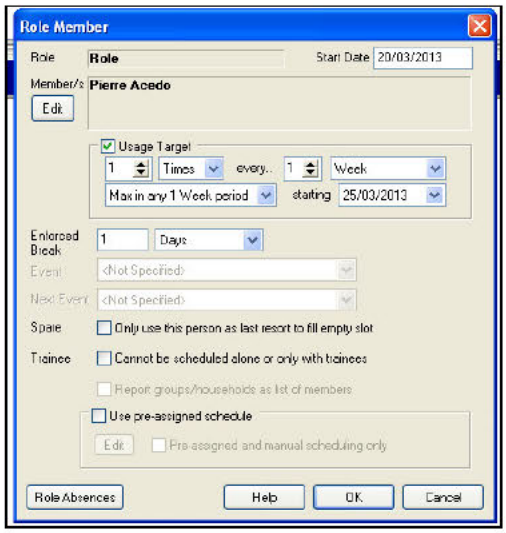


5. Select the file for the roster by either double clicking or click on the file then click open. File type stays as smart roster (.ros)

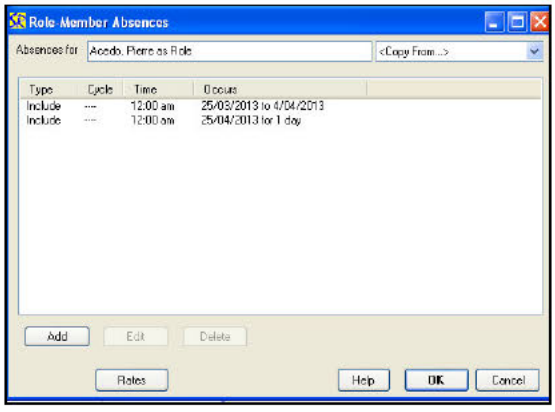
6. In the "Schedules section", change the scheduling period by choosing the dates for the next six weeks (top left corner).

7. Open the DNA Leave calendar to check for staff leave (G:\ForBiol\AAA Administration\Timesheets and HR forms\DNA Analysis Leave Calendars\DNA Team)

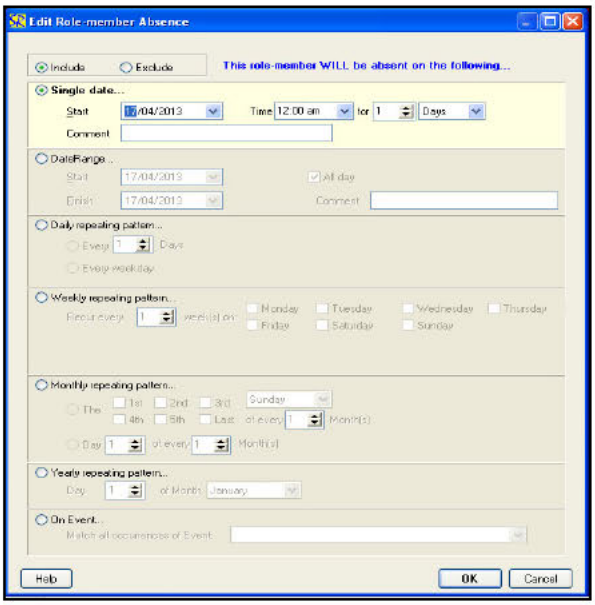
8. To add absences for each staff member, in the "Roles section" double click on the staff member and a Role member box will appear.



9. In the bottom left hand corner click "Role absences" and another box appears "Role-Member absences" then select "Add".



10. "Edit Role-Member Absence" box will appear, select either "Single Date" if staff member is having 1 day off or "Date Range" if a block of dates is being taken off. Once dates are selected, click "OK".



11. Repeat steps 8-10 for each staff member taking leave in the six week period.

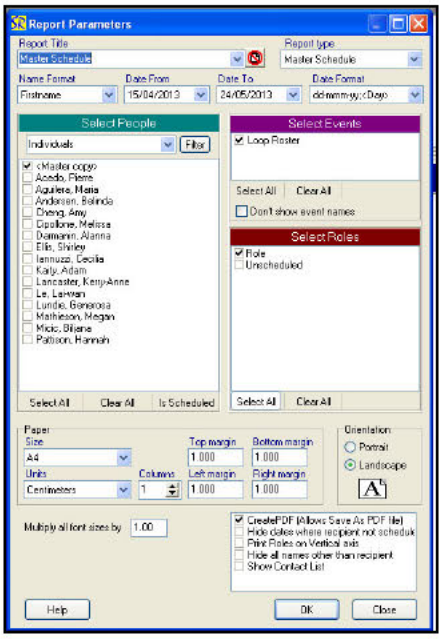
12. Press Auto-Fill button on the tool bar, this will fill in the roles in the schedule.

13. Check all the spaces are filled.

14. To copy the schedule to a word document, press the report button on the tool bar.

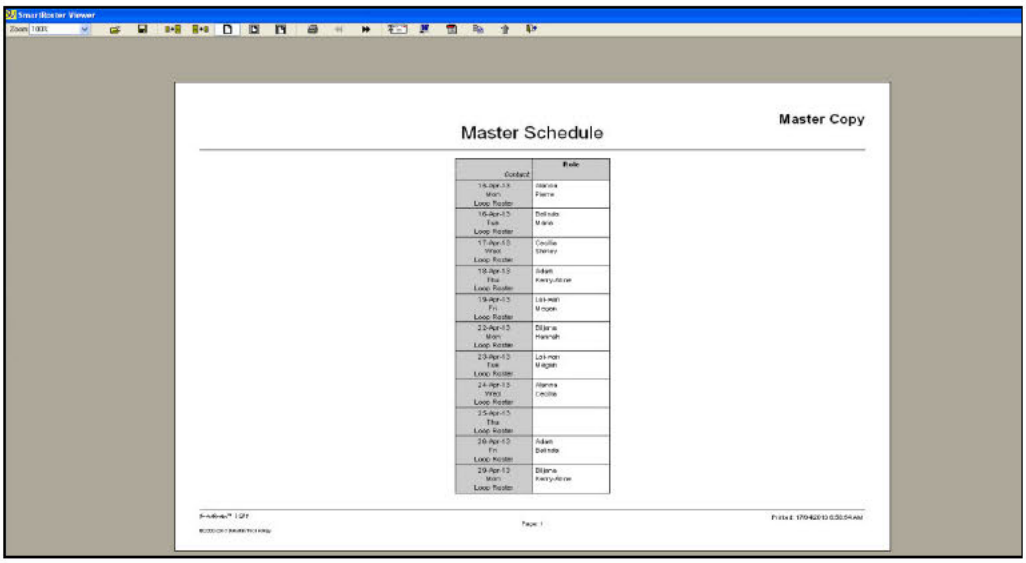
15. Then choose schedule from drop down menu

16. Report parameters box appears, make sure the date format is in the format required, master copy is selected, the event is selected and the role is selected then click “OK”.



17. Smart roster viewer appears, on top tool bar click on the Microsoft Word icon, this will convert the schedule to a word document.

18. Modify document in Word into the required format then “Save As” e. g. Loop roster 4th April to 5th May.



19. Close smart roster, prompt appears. Save changes to. Eg G:\ForBiol\AAA Analytical training & rosters\Loop roster 2013.ros? Click “Yes”

20. Back up reminder box pops up click “Yes”

PB141

Queensland Health

Forensic and Scientific Services

Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit

1 Purpose and Scope

This document describes the routine automated and manual methods for the quantification of extracted DNA from casework and reference samples in Forensic DNA Analysis, using the Quantifiler® Trio DNA quantification kit (Life Technologies/Thermo Fisher Scientific, Carlsbad, CA, USA). The procedure for manual methods will be utilised during extended downtimes of the liquid handler platforms. This document applies to all DNA analysis staff performing this procedure.

2 Definitions

AB / ABI	Applied Biosystems
BP	Base pairs
C_T	Cycle threshold
Decapper	LabElite® Integrated I.D. Capper™
DNA	Deoxyribonucleic acid
DI	Degradation index
FR	Forensic Register
IPC	Internal PCR control
LAT	Long autosomal target
MGB	Minor groove binding
PCR	Polymerase chain reaction
QS5	QuantStudio™ 5
RT-PCR	Real time polymerase chain reaction
SAT	Short autosomal target
STARlet	Microlab® STARlet
STR	Short tandem repeats
Y-Target	Y-chromosome target

3 Principle

The Quantifiler® real-time PCR assay measures the relative amount of a DNA target during each amplification cycle of the PCR in real-time. The Quantifiler® Trio DNA quantification kit is designed to simultaneously quantify the total amount of amplifiable human DNA and human male DNA in a sample. It uses multi-copy target loci for improved detection sensitivity. The kit provides DNA quantification results for the following targets:

- SAT (80 bp) – is the primary quantification target for total human genomic DNA, its smaller amplicon size makes it better able to detect degraded DNA samples.
- LAT (214 bp) – is used mainly as an indicator of DNA degradation, by comparing the ratio of its quantification result with that of the SAT.
- Y-Target (75 bp) – allows the quantification of the human male genomic DNA component of samples and can be useful in assessing mixture samples of male and female genomic DNAs.
- IPC (130 bp) – is a synthetic DNA template present in each sample and provides positive confirmation that all assay components are functioning as expected. It confirms the validity of negative results and is useful to identify samples that contain PCR inhibitors.

The results obtained using the Quantifiler® Trio kit can determine the following:

- If the sample contains sufficient human DNA and/or human male DNA to proceed with STR analysis.
- The amount of sample to use in STR analysis.
- The relative quantities of human male and female DNA in a sample that can assist in the selection of an applicable STR kit.
- The DNA quality, with respect to both the levels of DNA degradation and inhibition, which is useful for determining if the STR loci with larger amplicon sizes will likely be recovered in the STR profile.

Two TaqMan® MGB probes labelled with VIC® and FAM™ dye are used to detect amplified SAT, and Y-Target respectively. Also, two TaqMan® QSY® probes labelled with ABY® and JUN® are used to detect amplified LAT and the IPC amplicon respectively.

A set of five prepared DNA standards (in duplicate), reagent blank and samples are added to a 96-well reaction plate and amplified using the AB QS5 real time PCR system (Table 1). At the end of each amplification cycle, each well in the reaction plate is flooded with light from an LED lamp, which excites the fluorescent dyes in each well of the plate. A Complementary Metal Oxide Semiconductor (CMOS) camera collects the differing wavelengths of light emitted. Data analysis algorithms are then applied to the raw data collected using the AB QS5 sequence detection system software.

Table 1 Thermalcycling parameters for the Quantifiler® Trio DNA quantification kit.

STEP	**PARAMETERS**	
Taq Activation	95ºC 2 mins	
Denaturation	95ºC 9 sec	40 cycles
Primer annealing & template extension	60ºC 30 sec	
Reaction volume 20 µL	**9600 Emulation mode**	

Amplification of a sample on the AB QS5 instrument is displayed on the amplification plot. The curves observed in the amplification plot represent the increasing fluorescent signal as the amount of specific amplified product increases. The curve consists of geometric, linear and plateau phases. During the geometric phase, amplification is characterised by a high and constant efficiency. In the linear phase, the slope of the amplification plot decreases steadily as amplification efficiency begins to lower because one or more of the PCR reaction components is below critical concentration. Amplification reaches the plateau phase when the reaction is saturated by product and PCR amplification stops. The Blue (SAT, LAT & Y-Target) and Red (IPC) threshold line should be positioned at approximately the middle of the curve at geometric phase.

On the standard curve of the amplification results, a slope close to -3.3 (SAT), -3.4 (LAT) and -3.3 (Y-Target) indicates optimal, 100 % PCR amplification efficiency. The R^2 value indicates the closeness of fit between the standard curve regression line and the individual cycle threshold (C_T) points.

The Quantifiler® Trio DNA Quantification Kit also uses the ratio of quantification results for the SAT and LAT to give an estimate of degradation in a sample expressed as the DI. According to the manufacturer a DI of 1-10 is considered slightly to moderately degraded and a DI above 10 is considered significantly degraded. DI results may be able to be used to guide sample workflow which may streamline processing. It is calculated by the software using the formula:
[DI = Small autosomal target DNA conc. (ng/µL) / Large autosomal target DNA conc. (ng/µL)]

The Quantifiler® Trio DNA Quantification Kit also includes a Y-Target which provides a quantification concentration for male DNA in a sample including in mixtures of male and female DNA. This will enable the identification of samples suitable for testing with Y-STR analysis. It is calculated by the software using the formula:
[Male DNA:Female DNA Ratio = Quantity of Male DNA/Quantity of Male DNA : (Quantity of Human DNA – Quantity of Male DNA)/Quantity Male DNA]
(All quantities in the equation are ng/µL)

4 Reagents and Equipment

4.1 Reagents

All reaction components are stored at -15 to -25°C and must be stored after initial use at 2 to 8°C. Table 2 outlines all reagents and the storage locations required for quantification.

Table 2 Reagents with storage room and location.

Reaction Component	Room	Storage Location (Initial)	Storage Location (after initial use)
Quantifiler® THP PCR Reaction mix	3188	Freezer B	Two-way Fridge
Quantifiler® Trio Primer Mix	3188	Freezer B	Two-way Fridge
Quantifiler® prepared standards	3194	Fridge	Fridge

Prepare fresh Quantifiler® Trio master mix in the biosafety cabinet in room 3188 just prior to commencing quantification.

1. Determine the required volume of reagents by referring to Table 3.

Table 3 Quantifiler® Trio master mix volumes

Reaction Component	Equation
Quantifiler® THP PCR Reaction mix	*n* x 10
Quantifiler® Trio Primer Mix	*n* x 8

Note: Where *n* is indicative of the number of samples on a batch.
Note: For each batch prepare *n* + 4 samples.

2. Remove the Quantifiler® THP PCR reaction mix and Quantifiler® Trio primer mix from the fridge / freezer and thaw if required. Vortex and centrifuge before use.
3. Pipette the required amount of Quantifiler® THP PCR reaction mix into a single 2 mL or 2 x 2 mL tubes.

Note: Small quant batches may only require 1 x 2 mL tube for the PCR master mix. For larger batches, please note the STARlet will run faster with 2 master mix tubes.

4. Pipette the required amount of Quantifiler® Trio primer mix to the master mix tube/s containing the Quantifiler® THP PCR reaction mix. Gently vortex and centrifuge.
5. Label with "**QUA**" CW or REF, initial and date.

4.2 Equipment

Table 4 outlines the equipment and the locations required for quantification.

Table 4 Location of required equipment.

Equipment	Location
STORstar (B)	3194
Labogene Scanspeed 1248	3191
AB QS5 A RT-PCR	3196
AB QS5 B RT-PCR	3196
Microlab® STARlet with LabElite® Integrated I.D. Capper™ A	3194
Microlab® STARlet with LabElite® Integrated I.D. Capper™ B	3194
Eppendorf Mixmate	3194

4.3 Consumables

Table 5 outlines the consumables and the locations required for quantification.

Table 5 Location of required consumables.

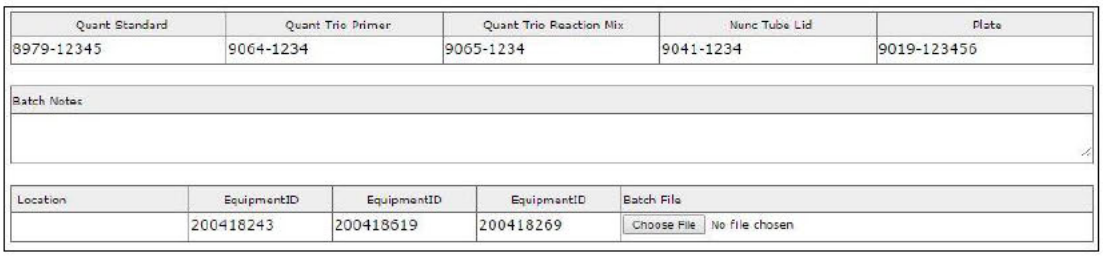
Consumables	Location
50 µL CO-RE tips with filters	3194
96-well optical plate	3194
Nunc™ caps	3194
Optical seal	3194
2 mL QIAGEN tubes	3191

Note: Additional consumables can be found in the Store Room (3184).

4.4 Entering Reagents, Equipment, Consumables and Locations into FR

Fields should be filled out contemporaneously while processing the batch. These steps can be performed at any stage prior to batch completion and entries can be modified after saving. Fields cannot be entered or edited once the batch is completed.

1. Access the batch according to the Forensic DNA Analysis Workflow Procedure (34034).
2. Click the **Edit/Update Batch** icon.
3. Using the relevant dropdown menus, select the correct consumables and reagents.
4. Scan the equipment and location barcodes into the required fields (Figure 1).



Quant Standard	Quant Trio Primer	Quant Trio Reaction Mix	Nunc Tube Lid	Plate
8979-12345	9064-1234	9065-1234	9041-1234	9019-123456

Batch Notes

Location	EquipmentID	EquipmentID	EquipmentID	Batch File
	200418243	200418619	200418269	Choose File No file chosen

Figure 1 An example of quantification batch details.

5. Click the **Save** Batch icon.

5 Safety

As per the Anti-Contamination Procedure (22857), PPE is to be worn by all staff when performing this procedure. No part of the body should be placed inside the STARlet while the instrument is performing any procedure.

6 Sample Location and Sample Preparation

6.1 FR Workflow Diary and Electronic Workflow Diary

Batches that require processing can be found in the FR Workflow diary tab (refer to the Forensic DNA Analysis Workflow Procedure (34034).

The electronic Pre-PCR instrument diary (I:\AAA Electronic Workflow Diary\AAA PrePCR Diary) is used to record daily use of the instruments. Maintenance, processed batches and any issues/errors are recorded in the diary.

6.2 Batch Creation

Create or schedule quantification batches according to the Forensic DNA Analysis Workflow Procedure (34034).

6.3 QC Samples

Duplicates of the Quantifiler® standards and a reagent blank will be automatically allocated by the FR when creating the quantification batch according to the Forensic DNA Analysis Workflow Procedure (34034).

6.4 Sample Location and Locating Samples

Samples awaiting quantification are stored in the fridge as described in Table 6.

Table 6 Sample storage location.

Sample type	Storage Device	Storage Location
DNA Extracts	Fridge or Freezer	3194

Locate samples according to Forensic DNA Analysis Workflow Procedure (34034).

6.5 Analytical Notes

1. Access the batch according to the Forensic DNA Analysis Workflow Procedure (34034).
2. On the batch **Exhibit Analysis** page, if any samples are coloured half orange, hover the cursor over it to check for analytical notes that request for specific processing comments (e.g. Quant and hold) (Figure 2).

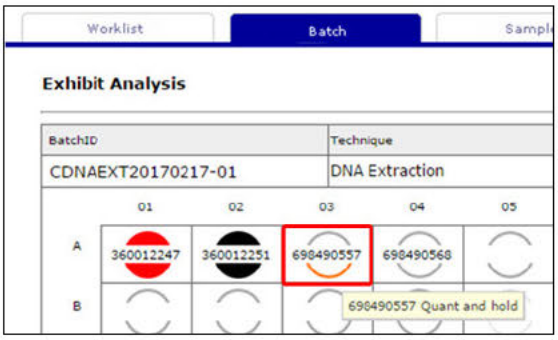


Figure 2 An example of an Analytical Note displayed with an orange semi-circle.

6.6 Uploading Files

1. Access the batch in FR as per the Forensic DNA Analysis Workflow Procedure (34034).
2. Click **Edit/Update Batch** icon.
3. Click the **Choose File** button in the **Batch File** field (Figure 1).
4. Browse the I:\ drive folders for the required file and click **Open.**

Note: If the file cannot be seen (e.g. .trc files) change the drop down box "All files".

5. Click the **Save Batch** icon

7 Procedure

7.1 Quantification Set up

1. In the Clean Reagent Room (3188) prepare Quantifiler® Trio master mix. Refer to Section 4.1 for preparation of reagents.
2. Set up of quantification is performed using the dedicated Microlab® STARlets A and B located in Room 3194.
3. Access the batch in FR as per the Forensic DNA Analysis Workflow Procedure (34034).
4. Check for Analytical notes as per Section 6.5 and action as required.
5. In the **Batch File** field, click the **BatchID_Trio_QS5_Map.txt** file and save to I:\ABI Quantifiler.
6. In the **Batch File** field click the **BatchID_QUANT_MAP.xls** file and save file to I:\Pre PCR STARlet\All Plate Maps. Check for duplicate samples as the STARlet is **NOT** programmed to have the same sample on a batch twice.

Note: If duplicate samples are required, please refer to Section 14.2.4 Duplicate Sample/s on Batch.

7. Ensure STARlet Daily Start-up has been performed as described in Operation and Maintenance of the Microlab® STARlet and LabElite® Integrated I.D. Capper™ (34050).
8. Launch the Method Manager software via the desktop icon (Figure 3).

Figure 3 The Method Manager desktop icon.

9. Select the '**QUANTS & AMPS**' button on the home page (Figure 4).

Figure 4 Method Manager home page.

10. Click on the **Layout** button to open the layout for the Quantifiler Trio method.
11. Centrifuge the Nunc™ rack of samples for 1 min at 2000 rpm (657 *xg*).
12. Using the Mixmate, vortex the Nunc™ rack of samples for 1 min at 1000 rpm.
13. Check the first and last barcodes of the extracts against the FR plate map and place the Nunc™ rack of samples onto the platform of the Decapper.
14. Decontaminate and place all the required labware onto the autoload tray in the designated track positions as outlined in the layout (Figure 5).

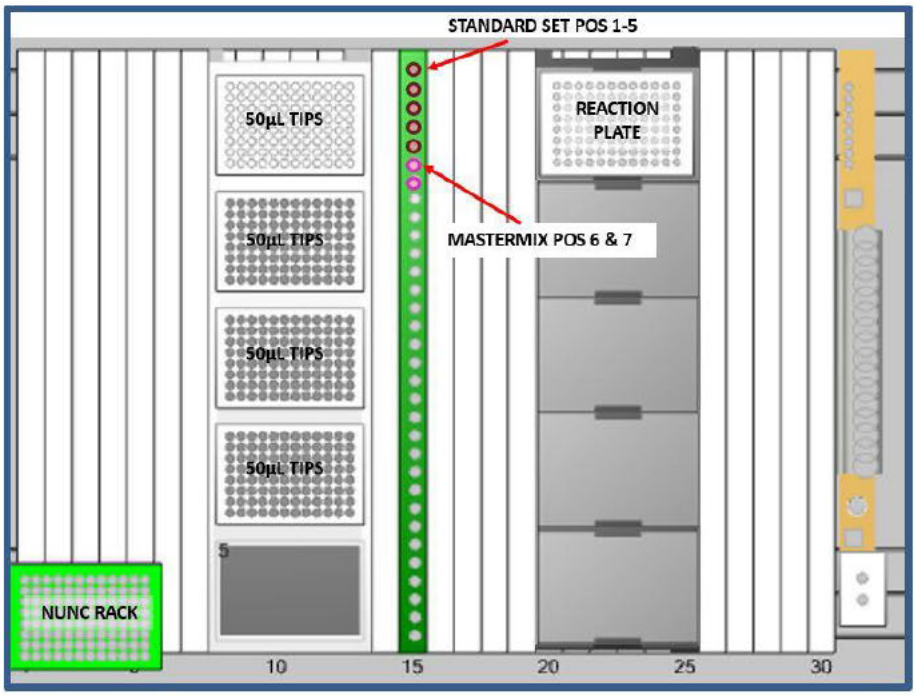


Figure 5 Quantifiler Trio deck layout. Note that the Nunc rack of samples is placed on the decapper platform at the beginning of the method.

15. Ensure there are at least one and a half full racks of 50 µL CO-RE filter tips in the tip carrier (TIP-CAR) in **Tracks 8-13** of the autoload tray.
16. Briefly vortex and centrifuge the Quantifiler® prepared standards and place in positions **1-5** of the sample carrier (SMP-CAR) in **Track 15** (Figure 5).

Note: Ensure the Quantifiler® prepared standards are within the expiry date and there is sufficient volume remaining (>20 µL).

17. Briefly vortex and centrifuge the master mix tube/s and place in **position 6** (& **7** if using two tubes) of the sample carrier in **Track 15** (Figure 5).
18. Label a skirted 96-well optical plate with the Batch ID on the front and the Batch ID barcode on the right side and place into position **1** of the multiflex carrier (APE-CAR) in **Tracks 20-25**.
19. After ensuring all the necessary labware has been positioned on the autoload tray, close the deck layout and click **Run** on the Quantifiler Trio screen of the Method Manager.
20. A prompt will appear to check the deck layout. Select **Continue** once the carriers on the autoload tray match the deck layout.
21. A prompt will appear to load the plate and sample carriers onto the deck. Select **OK** to load the carriers.
22. A prompt will appear **Edit Tip Count** asking for the first and last position of the tips in the tip carrier (Figure 6).

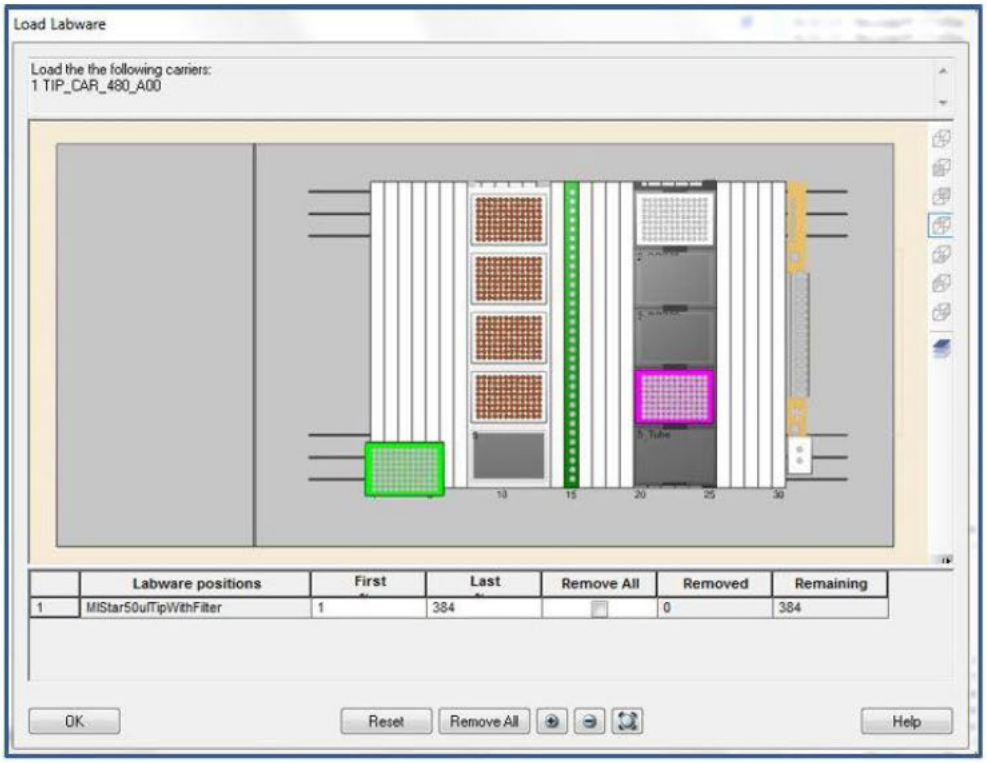


Figure 6 Edit tip count dialogue box.

23. To edit the tip counter, highlight individual tips are present by clicking on the individual positions or click and drag to highlight multiple positions. These methods will also remove already highlighted tips. If it is necessary to remove all highlighted tips, check the **Remove All** box and then highlight the positions of present tips. When the tip counter matches the tips in the carrier (TIP-CAR), click **OK**.

24. A prompt will appear to enter the quantification Batch ID, link the platemap and select the number of MasterMix tubes to be loaded (Figure 7).

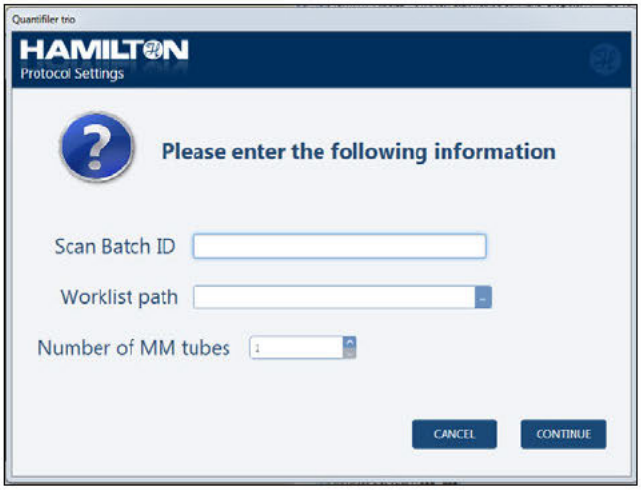


Figure 7 Dialogue box prompt.

25. Scan the barcode to enter the quantification Batch ID. Select the correct platemap by browsing to **I:\Pre PCR STARlet\All Plate Maps\BatchID_QUANT_MAP.xls** and select the number of master mix tubes (1 or 2).
26. Select **Continue** to begin the method.
27. A prompt will appear to load samples and an empty Nunc™ rack onto the Decapper (Figure 8).

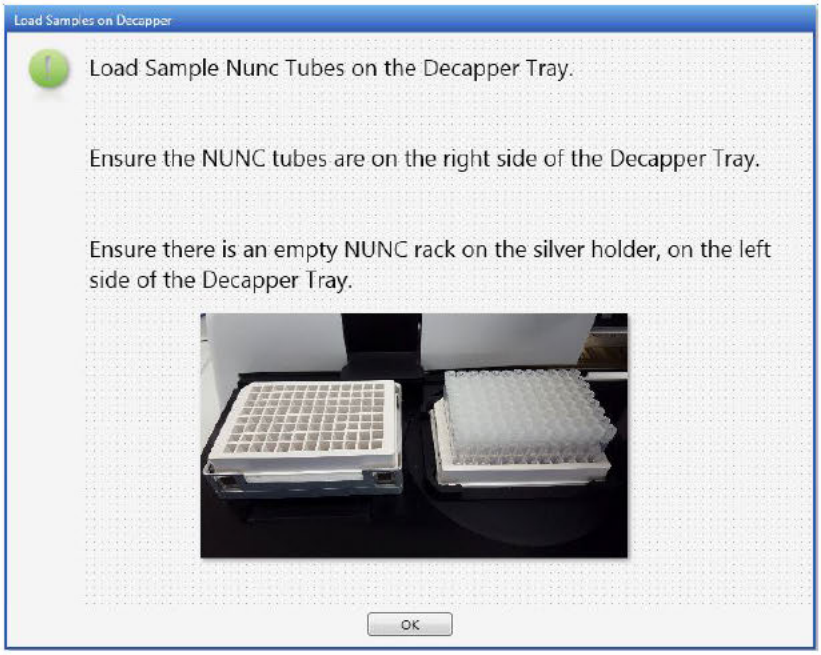


Figure 8 Prompt to load samples and empty Nunc™ rack to Decapper.

28. Ensure that the samples and empty Nunc™ rack have been loaded. Select **OK** to continue the method.
29. A prompt will appear to unload the plate carrier and check volumes and bubbles of master mix within the reaction plate (Figure 9).

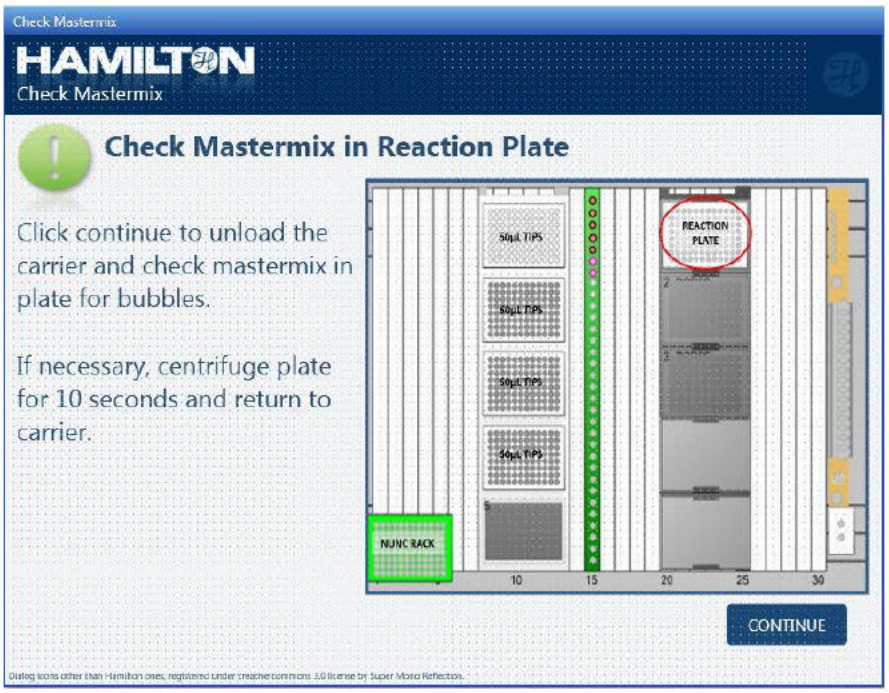


Figure 9 Prompt to check master mix in the reaction plate.

30. Select continue to unload the multiflex carrier (APE-CAR) in **Track 20-25**.
31. Check that the liquid within the plate is sitting at the bottom of the wells. If the master mix liquid is not at the bottom of the well, seal the plate and centrifuge for 10 sec at 2000 rpm (657 g).
32. A prompt will appear to load the reaction plate on the multiflex carrier (APE-CAR) (Figure 10).

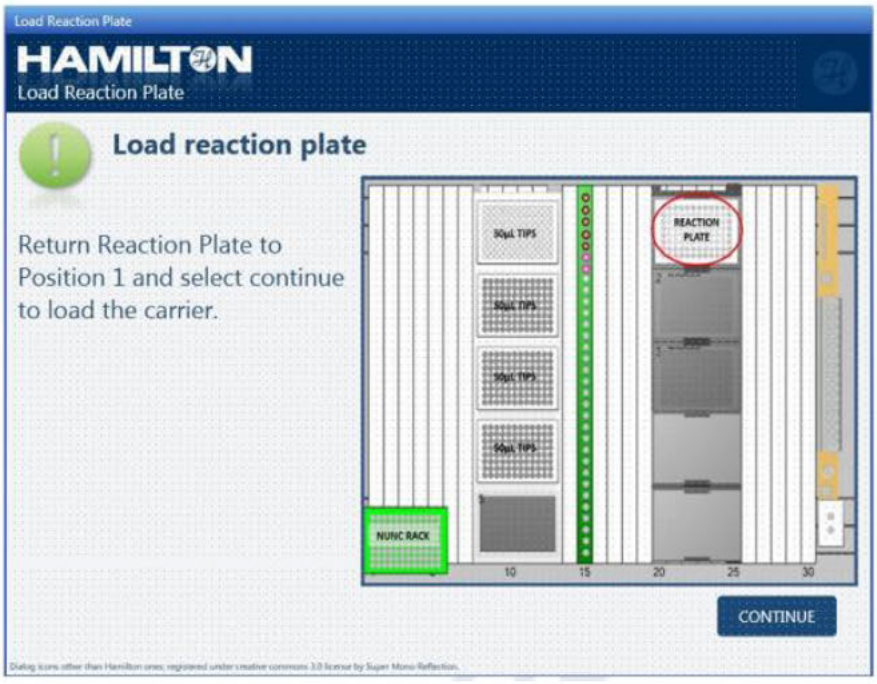


Figure 10 Prompt to reload the reaction plate.

33. Remove the seal and replace plate in position **1** of the multiflex carrier (APE-CAR).
34. Select "**Continue**" to load the multiflex carrier (APE-CAR) and continue the method.
35. A prompt will appear to discard old Nunc™ caps and load new Nunc™ caps onto the Decapper (Figure 11).

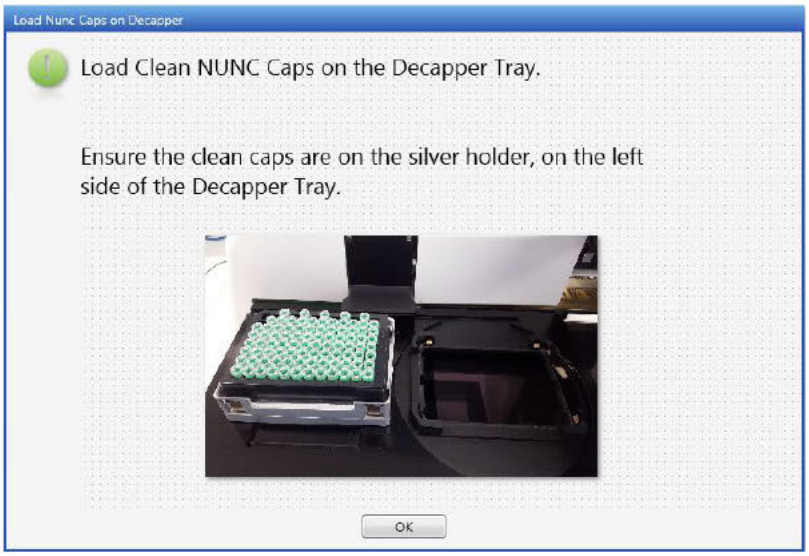


Figure 11 Prompt to load new Nunc™ caps on the Decapper.

36. Discard the old Nunc™ caps and clean the tray with 5% TriGene™, followed by 70% Ethanol. Load new caps onto the Decapper. Click "**OK**".
37. A prompt will appear to unload the carriers (Figure 12). Click OK and the carriers will automatically unload and the DNA extracts will be recapped.

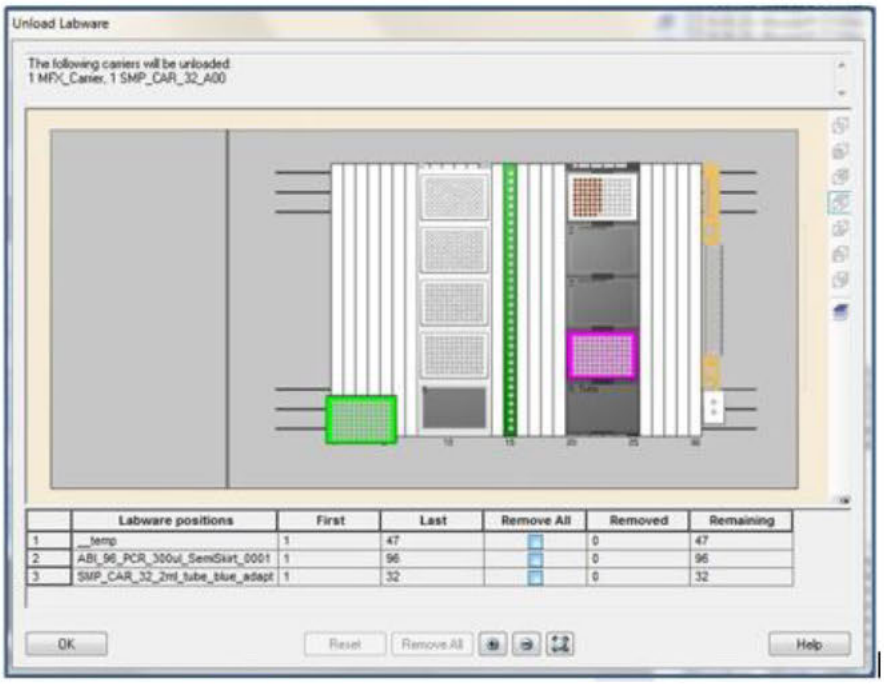


Figure 12 Prompt to unload carriers at the end of the run.

38. Check the plate to ensure all wells contain the required volume and that no bubbles are present. Seal the 96-well plate with an optical adhesive seal.

Note: Excessive bubbles in the wells are critical and may affect the accuracy of the quantification results.

39. Centrifuge the optical plate for 1 minute at 2000 rpm. Place the plate in the pass-through hatch to the PCR/CE Room (3194) and notify the CE Operators.

40. Check the plate again to ensure all wells contain the required volume and that no bubbles are present.

41. Refer to Section 7.2 for performing the quantification on the QS5 instrument and Section 7.3 for Quantifiler® results analysis.

42. The STARlet should have begun re-capping the open tubes. Re-cap and return Quantifiler® prepared standards to the fridge. Discard empty master mix tubes into the biohazard waste bin.

43. Store the re-capped Nunc™ tube extracts in the upright freezer in the Pre-PCR sorting room (Room 3194 A).

44. On the PC, navigate to **C:\Program Files (x86)\HAMILTON\Log Files**, sort by date modified and locate the most recent Quantilifer Trio trace file, e.g. "Quantifiler_Trio_Setup_v2_4c08086aa87e4a829cccb8438c349283_Trace.trc".

45. Open the trace file, check that it belongs to the batch and then close. Rename the file as the Batch ID, e.g. "CDNAQUA20160525-04.trc".

46. Copy the renamed trace file into **I:\Pre PCR STARlet\Trc Files**

47. Upload the trace file to FR as outlined in Section 6.6.

48. Ensure all reagents, equipment, consumables and locations are selected against the batch as per Section 4.4.

49. Once uploaded, electronically archive the trace file within the appropriate month folder within **I:\Pre PCR STARlet\Trc Files\01 – Jan**

50. If not performing another run remove all labware from the deck and clean with 5% TriGene™ followed by 70% Ethanol.

Note: When changing between casework and reference batch preparations on the STARlet, the sample carriers and modules are required to be cleaned with 5% Trigene and 70% Ethanol (not the deck).

7.2 Performing Quantification on the QS5 Instrument

1. In the PCR/CE room (3196) retrieve the optical plate from pass through hatch.
2. Check the plate to ensure all wells contain the required volume and that no bubbles are present. If required centrifuge the plate for 1 minute at 2000 rpm.
3. Turn on the AB QS5 and login to the PC using the "**INSTR-ADMIN**" username.

4. Once the desktop has loaded, log into Novell using the "**biology**" username and the current password.
5. Launch the QS5 system software (Figure 13).

Figure 13 HID Real-Time PCR analysis software v1.3 desktop icon.

6. Login using the "**Robotics**" username and click **OK** (Figure 14).

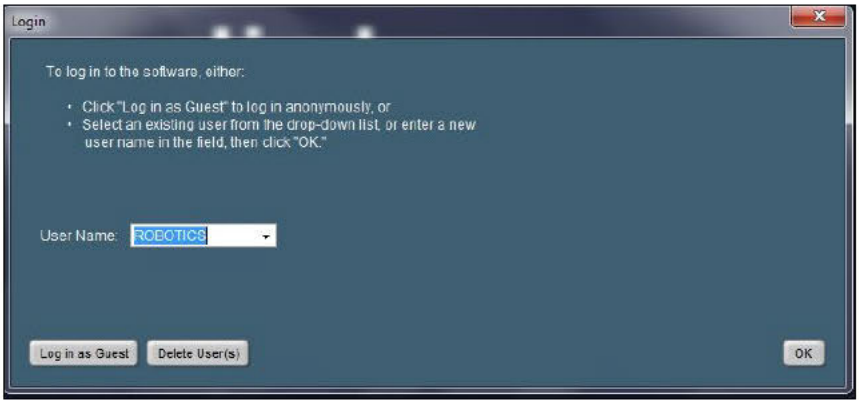


Figure 14 HID Real-Time PCR analysis software login screen.

7. From the home screen click on **Quantifiler® Trio** button.
8. From the main screen, click **File → Import**.
9. Select **Browse** and navigate to I:\ABI Quantifiler and select the relevant platemap file.
10. Click **Start Import**. When prompted click **Yes**.
11. Enter the Batch ID and user initials on the **Experiment Properties** page.
12. View the plate map by selecting **Setup → Plate Setup → Assign Targets and Samples** tab **→ View Plate Layout** tab.
13. Access the batch in FR as per the Forensic DNA Analysis Workflow Procedure (34034).
14. Check the last barcode of the FR platemap against the imported platemap on the QS5. (Use cursor to hover over well position on the plate layout screen for barcode to appear).
15. Using the QS5 touchscreen, click on the eject button on the top righthand corner to eject the loading tray. Place the 96-well plate with the Batch ID label on the front facing out onto the loading tray. Click on the eject button again to close the loading tray.
16. Click **START RUN**.
17. A prompt will appear to save the file. Ensure the filename is the Batch ID and click **Save**.

18. Ensure the progress bar appears indicating the run has started.

19. Ensure all equipment is selected against the batch in FR as per Section 4.4.

7.3 Quantifiler® Trio Results Analysis

Upon completion of the Quantification the QuantStudio®5 HID Software analyses the data and prepares the report.

Note: In the CT Settings tab of the Analysis settings, the CT Settings for the LAT, SAT and Y standard curves must be set to Automatic. This is a hard setting for all runs that may only need to be checked during troubleshooting.

1. On the left navigational panel of the screen click on the **Analysis** tab → **Amplification Plot** to observe the morphology of the amplification (Figure 15).

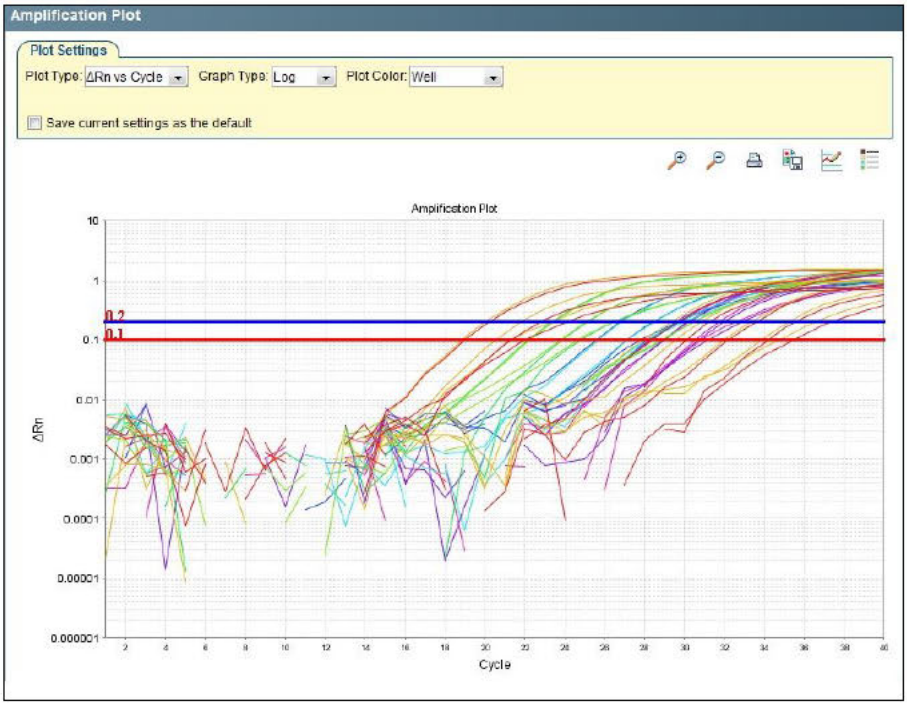


Figure 15 Quantifiler® Trio amplification plot.

Note: If jagged peaks, blips or spikes are observed (Figure 16), this is an indication of an electrical interruption. See the Analytical HP5 to determine if the quantification plate is to be repeated.

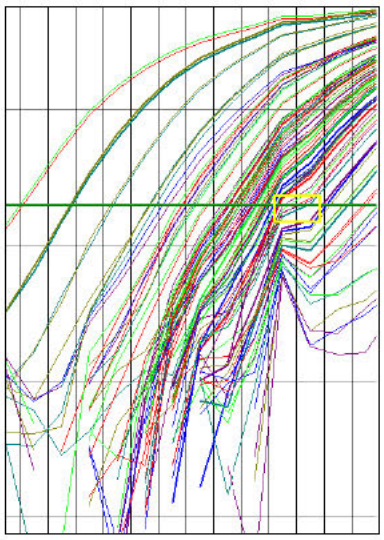


Figure 16 Amplification plot displaying abnormal amplification morphology.

2. Click on **Multicomponent Plot** to observe the amount of fluorescence of all targets. Take note of any spike or blips in the plot between cycles 3-15 (Figure 17).

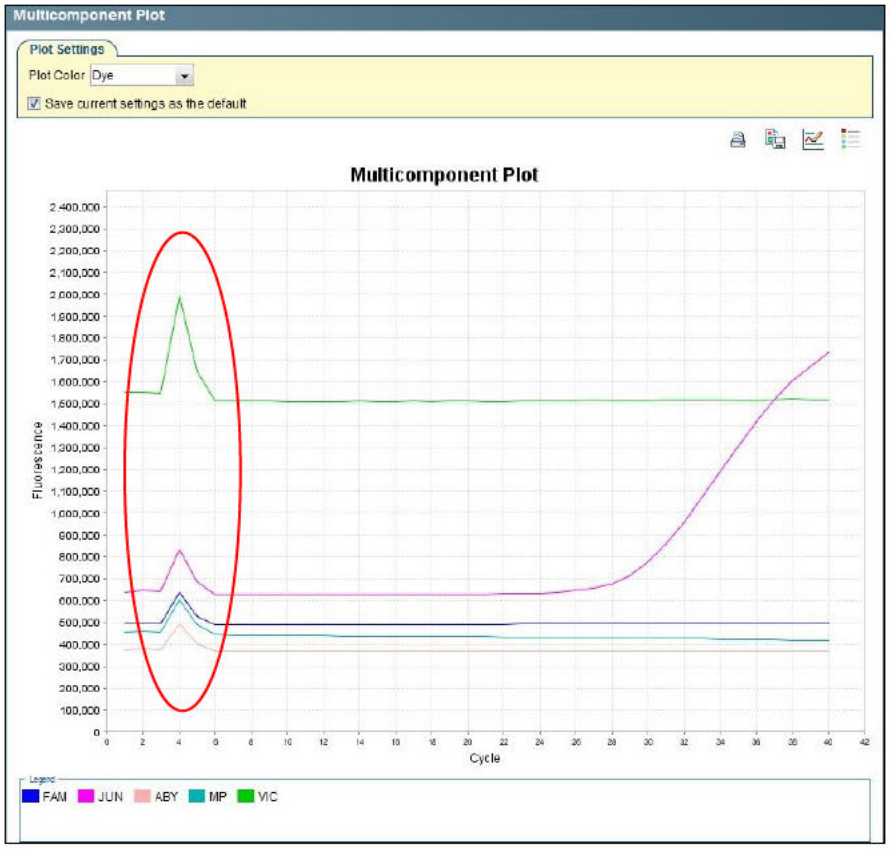


Figure 17 Quantifiler® Trio multicomponent plot displaying the fluorescence of one well.

Note: An abnormal plot that displays any spikes or short / long blips in the fluorescence readings between cycles 3-15 may indicate signs of bubbles in the wells or well evaporation. This in turn can affect the C_T value calculated for the DNA targets and sample/s should be re-quantified.

3. If a sample has excessive noise in the baseline between cycles 3-15 on the Multicomponent Plot, flag the sample/s so it appears red on the Results PDF by performing the following:

   a) Change the well colour on the platemap for that sample by selecting **Setup → Plate Setup → Define Samples and Targets**.

   b) On the **Define Samples** window on the right, find the sample that requires the flag and change the colour to Red by clicking on the drop-down option under the **Color** column.

4. Click on **Standard Curve** in the Analysis tab to observe the standard curve of all targets (Figure 18).

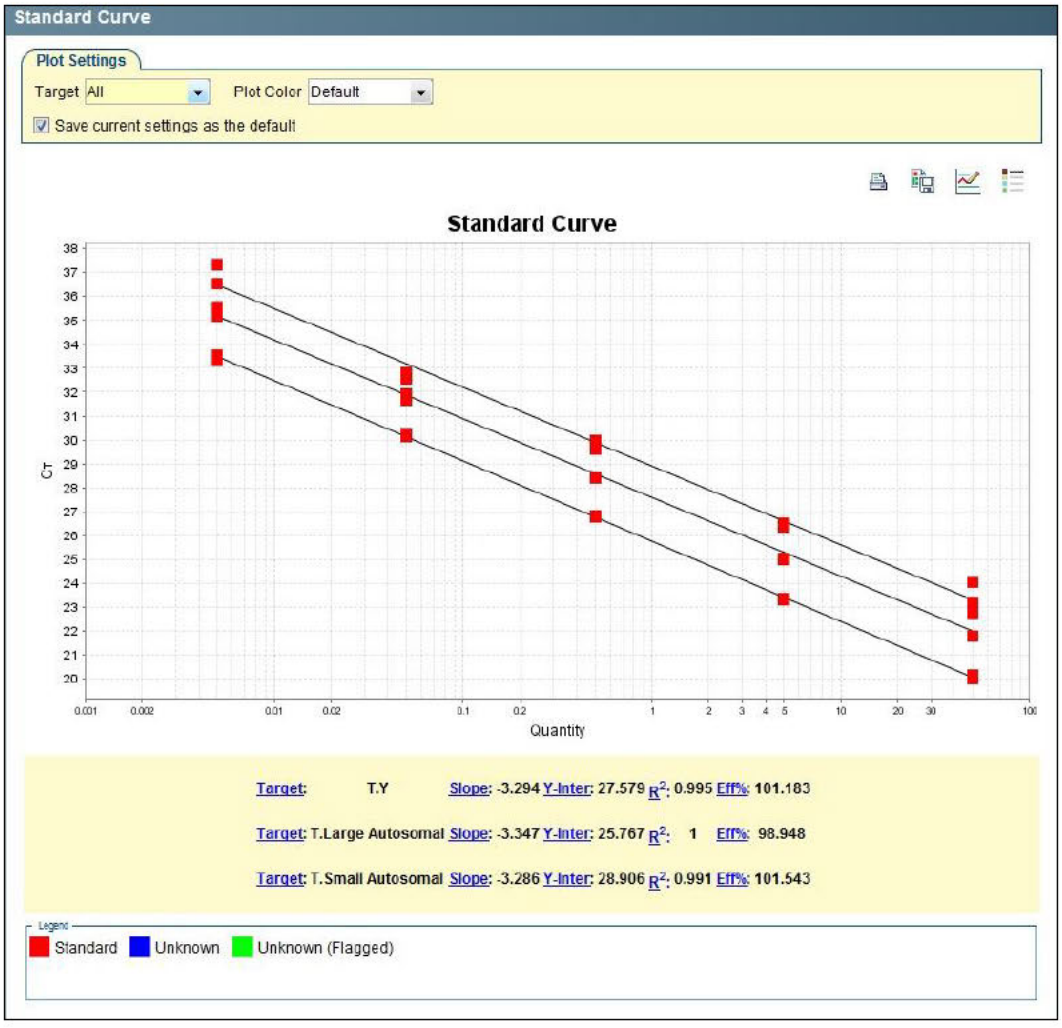


Figure 18 Standard Curve Results in the Analysis Tab, the standard at the top left is STD#5.

5. Assess the standard curve results and ensure that the slope, Y-intercept and R^2 values fall within the allowable ranges for all targets (Table 7).

Table 7 Criteria thresholds for the Quantifiler® Trio standard curve.

Target-Y Criteria	**Allowable Thresholds**
Slope	-3.0 to -3.6
Y-Intercept	25.64 – 26.58 (3SD)
R^2	≥0.98000
SAT Criteria	**Allowable Thresholds**
Slope	-3.0 to -3.6
Y-Intercept	25.35 – 28.94 (3SD)
R^2	≥0.98000
LAT Criteria	**Allowable Thresholds**
Slope	-3.1 to -3.7
Y-Intercept	19.71 – 30.47 (3SD)
R^2	≥0.98000

6. To improve the Slope, Y-Intercept and R^2 thresholds, up to 2 standard curve data points (not from the same standard) can be omitted by performing the following:
   a) Select the **View Plate Layout** tab in the right navigational panel.
   b) Highlight the relevant well/s, right click and select **Omit → Well**.
   c) Click **Analyze** on the main screen, this will re-analyse the data without the selected standard replicate.
   d) If the well needs to be added back for analysis, right click and select **Include.**
   e) Note in the FR batch notes which standards have been omitted (e.g. single replicate of Std#1 omitted from the standard curve).
   f) If more than two individual data points from the standard curve are to be omitted, notify Analytical Senior Scientist (HP5).
7. If the SAT Y-Intercept values fall outside the range, notify the Pre-PCR scientists and proceed to step 10.
8. If either of the Target-Y or LAT values for Y-Intercept, Slope or R^2 are outside the ranges, notify the Analytical Senior Scientist (HP5).
9. Check the reagent blank by moving the cursor over the reagent blank well position on the **View Plate Layout** tab and verify that no DNA quantity is present. If amplification did result, take note of the quantification value and notify the Pre-PCR scientists.
10. Using the QS5 touchscreen, click on the eject button on the top righthand corner to eject the loading tray.
11. Remove the optical plate from the QS5 and check each well for evaporation before discarding the plate into a biohazard bin. Click on the eject button again to close the loading tray.
12. Evaporated wells should be noted by adding a comment in the quantification batch in FR and by changing the colour of the sample well to red in the Result PDF (as per step 3 of Section 7.3). These samples are also repeated by ordering another quantification as per step 10 of Section 8.

13. **Ensure all wells** are selected and click **Export…** from the top toolbar (Figure 19).

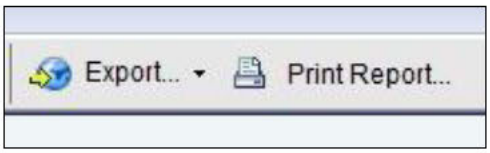


Figure 19 Export and Print Report buttons.

14. On the export data window remove the "_data" from the Batch ID in the **Export File Name** field (Figure 20). Ensure that the **File Type** extension is (*.xls) and the **Export File Location** is I:\Results\Qres.

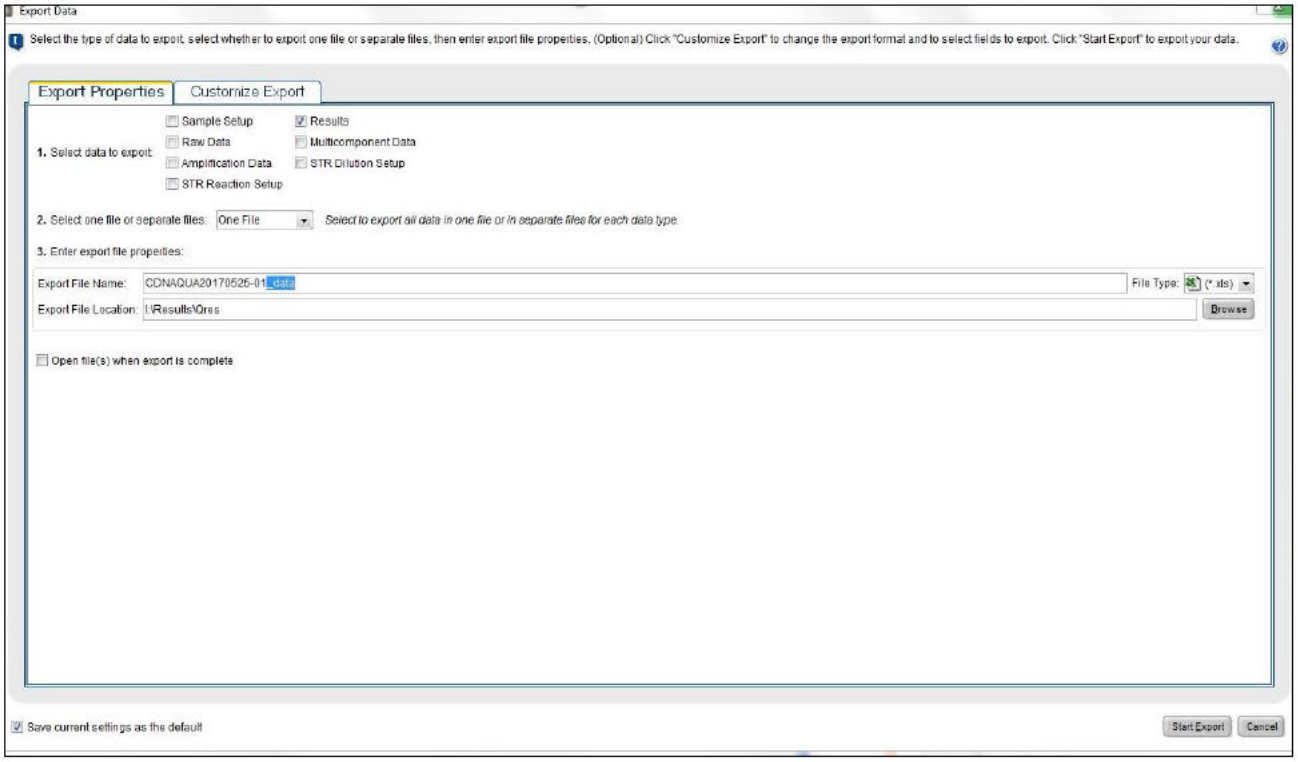


Figure 20 Export data window.

15. Click **Start Export** and close Export Tool when complete.

Note: The DI and the Male:Female ratio results can both be accessed in the exported file.

16. **Ensure all wells** are selected and click **Print Report…** on the top tool bar (Figure 21).

17. Ensure that the boxes shown in Figure 21 are checked and click **Print Report**.

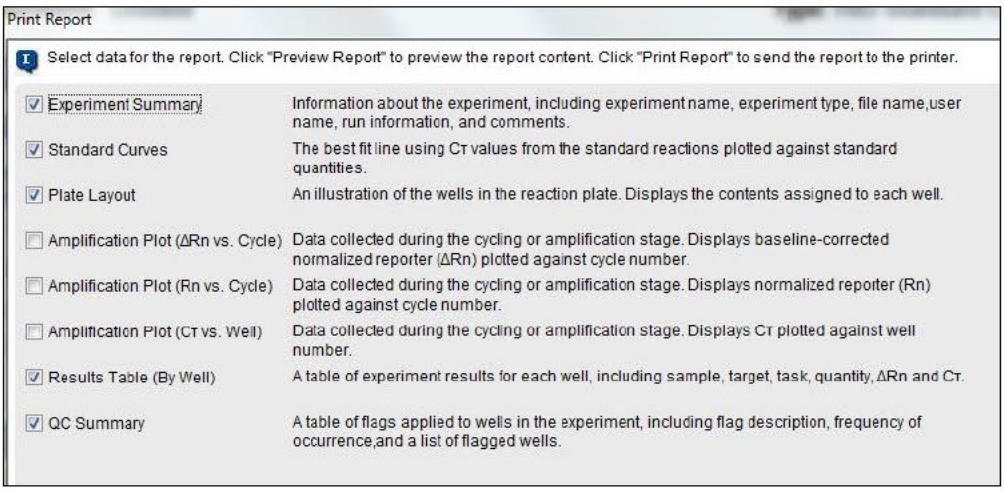


Figure 21 Print Report selection window to export.

18. Select **CutePDF Writer** in the print window and click **OK**.
19. Save the report to **I:\Results\Qres** using the Batch ID (eg.CDNAQUA20161118-01.pdf).
20. Close the experiment tab and click **Yes** when prompt appears to save changes.

8 Batch finalisation

1. Open the **Results PDF** for the batch saved in **I:\Results\Qres**
2. Ensure the standard curve slopes, Y-intercept and R^2 values are within the allowable ranges for all targets.
3. If the SAT Y-Intercept values are outside the range, the batch is to be repeated. Fail and re-create the quantification batch as follows and upload **Results PDF** only (step 5).
   a) In FR, click the **Edit/Update Batch** icon, change the batch status to FAIL (Figure 22), add a suitable batch note, select the PDF result file only and click the **Save** Batch icon.

Run Date	Run Time	Batch Controls / Status
09/03/2017	10:56	○ PASS ◉ FAIL ○ INV ○ N/R ○ CEQ

Figure 22 Batch status.

   b) Create a quantification batch in FR using the failed batch as a template as shown in the Forensic DNA Analysis Workflow Procedure (34034).
   c) Use the **Batch / SBox / File** sample source option.
   d) Use the Batch ID of the quantification batch that is to be repeated as the **Batch Source / Storage Box**.
   e) Click the **Save** icon.

4. Ensure the Reagent Blank (located in well C2) has an undetermined quantification value for the LAT, SAT and Target-Y. If the Reagent Blank has a value, refer to Table 8 for actions required.

Table 8 Criteria threshold for the Quantifiler® reagent blank control.

Reagent Blank (ng/µL)	Action Required
>0 to ≤ 0.001	Note the quantification value in the FR as a batch note (e.g. reagent blank 0.00081 ng/µL < acceptable threshold).
> 0.001	Contamination may have occurred. Note the quantification value in the FR as a batch note entry (e.g. reagent blank 0.0052 ng/µL > acceptable threshold). Notify analytical senior scientist before further action. Actions required will either be to monitor the reagent blank on the following quantification batch for contamination issues or clean the block on the QS5 instrument as per Operation and Maintenance of the AB QuantStudio 5 RT-PCR Instrument (35028) then fail and repeat quantification batch as per step 3 of Section 8.

5. Upload the **Results PDF** to FR as outlined in Section 6.6.

6. Upload the **Results Excel** file to FR as outlined in Section 6.6. This will take some time to upload and once finished the Quant Results page will open (Figure 23).

BatchID	Technique	Method	Plate / Rack ID
CDNAQUA20170519-01	DNA Quantification	Quantifiler Trio	

Well	SampleID	T.SA (Qty)	Priority / Analytical Note	µL	Technique	Method	Dil'n
A1	STD 1 (50ng/uL)	50					
A2	STD 5 (0.005ng/uL)	0.005					
A3	360002469	0.000000	P1		STR Amplification	PowerPlex21 3130xl	
B1	STD 1 (50ng/uL)	50					
B2	STD 5 (0.005ng/uL)	0.005					
B3	360002378	0.000000	P2		STR Amplification	PowerPlex21 3130xl	
C1	STD 2 (5ng/uL)	5					
C2	Reagent Blank	0.000000					
C3	360002367	0.225500	P2		STR Amplification	PowerPlex21 3130xl	
D1	STD 2 (5ng/uL)	5					
D2	360000702	0.001000	P1 Micron 32.0 uL	32.0	STR Amplification	PowerPlex21 3130xl	
D3	360000276	0.000000	P2		No DNA Detected		
E1	STD 3 (0.5ng/uL)	0.5					
E2	360002431	0.000000	P1		STR Amplification	PowerPlex21 3130xl	
E3	690149580	0.555000	P2		STR Amplification	Profiler Plus 3130xl	
F1	STD 3 (0.5ng/uL)	0.5					
F2	360002420	0.248700	P1 EXTPB 360001639 used as positi ...		STR Amplification	PowerPlex21 3130xl	
F3	360000947	0.012000	P2		STR Amplification	Profiler Plus 3130xl	
G1	STD 4 (0.05ng/uL)	0.05					
G2	360001031	0.457000	P1		STR Amplification	PowerPlex21 3130xl	
H1	STD 4 (0.05ng/uL)	0.05					
H2	360002475	0.412000	P1		STR Amplification	PowerPlex21 3130xl	

Figure 23 Quant results page.

7. Ensure samples have correct **Technique** and **Method** selected as per sample type and quantification value as shown in Table 9.

Table 9 Default values for Quant Results page.

Type	Quantification Value	Priority	Technique	Method
CW	<0.001 ng/µL	P1	No DNA Detected	Blank
	≥0.001 – ≤0.0088 ng/µL	P1	Post-Extraction	Microcon PowerPlex21
	>0.0088 ng/µL	P1	STR Amplification	PowerPlex21 3500xl
	>5 ng/µL	P1	Post-Extraction	Dilution
	<0.001 ng/µL	P2 or P3	No DNA Detected	Blank
	≥ 0.001 – 0.0088 ng/µL	P2 or P3	DNA Insufficient	Blank
	>0.0088 ng/µL	P2 or P3	STR Amplification	PowerPlex21 3500xl
	>5 ng/µL	P2 or P3	Post-Extraction	Dilution
	<0.001 ng/µL	QPS ENVM*	No DNA Detected	Blank
	≥0.001 – ≤0.0088 ng/µL	QPS ENVM*	DNA Insufficient	Blank
	>0.0088 ng/µL	QPS ENVM*	STR Amplification	PowerPlex21 3500xl
	>5 ng/µL	QPS ENVM*	Post-Extraction	Dilution
Ref	<0.0088 ng/µL	All	On Hold	Reference Sample Review
	≥0.0088 – ≤0.0176 ng/µL	All	Post-Extraction	Microcon PowerPlex21
	>0.0176 ng/µL	All	STR Amplification	PowerPlex21 3500xl
	>5 ng/µL	All	Post-Extraction	Dilution

Note: QPS ENVM samples will have the well designation highlighted blue.

8. Check for any samples with a **Quant & hold** or **NWQPS** analytical note in the Priority / Analytical Note column and:
   - if the **Technique** is no DNA detected or DNA Insufficient, no action is required.
   - If the **Technique** is anything else (e.g. STR Ampflication or Post-Extraction) change the **Technique** and **Method** fields to blank on the Quant Results page in FR.

Note: Ensure truncated analytical comments ending in "…" are expanded and checked as Quant and Hold samples must not progress to further processing.

9. Check for any Microcon® samples which will have a volume in the **µL** column. Ensure that the sample has defaulted to the correct STR Amplification Method, which is PowerPlex21 3500xL for volumes ≥ 23 µL and PowerPlex21 3500xL Manual for volumes < 23 µL.

10. Check the plate layout page of the **Results PDF** for any samples flagged red. For these samples, change the **Technique** and **Method** fields to blank on the Quant Results page in FR. If required, add an exhibit testing for another quantification as per the Miscellaneous Analytical Procedures and Tasks (34064), include an appropriate sample notation (e.g. "Excessive noise in the Multi-component plot which may have affected quant results – sample to be re-quanted" or "well evaporation").

11. For any concentrated samples (> 5 ng/µL), the default **Technique** will be a "Post Extraction" and **Method** a "Dilution" on the Quant Results page in FR. Calculate the dilution factor required (aiming for a C_T value of approximately 0.5 ng/µL) and enter into the **Diln** column. **Do not** press **Enter** after entering dilution factors.

12. For any samples that are displayed on the QC summary page of the **Results PDF** (Figure 24), open the exhibit detail page for each of the samples in a new tab in FR.

- If a sample has come from either a Microcon® batch or a Nucleospin® batch, contact the case scientist before changing the **Technique** and **Method**.
- If a sample has come from any other batch type change to "Post-Extraction" **Technique** and "Nucleospin" **Method** on the Quant Results page in FR.

Note: ***Inhibited samples*** are those that have an IPC C_T value of undetermined or values 2 units above the average IPC C_T from the standards.

Experiment:Untitled | Experiment Results Report | Applied Biosystems 7500 Instrument

QC Summary

Total Wells	96	Processed Wells	64	Targets Used	4
Well Setup	64	Flagged Wells	18	Samples Used	35

Flag	Name	Frequency	Locations
AMPNC	Amplification in negative control	0	
BADROX	Bad passive reference signal	1	E3
BLFAIL	Baseline algorithm failed	0	
CTFAIL	CT algorithm failed	0	
EXPFAIL	Exponential algorithm failed	0	
HIGHQT	High Quantity of DNA	0	
HIGHSD	High standard deviation in replicate group	0	
IPCCT	Internal PCR Control CT value	16	A3, A7, B3, B7, C3, C7, D3, D7, E3, F2, F3, G2, G6, H1, H2, H6
LOWQT	Low Quantity of DNA	2	E6, F6
MTFR	Ratio of Male to Female DNA quantities	0	
NOAMP	No amplification	0	
NOISE	Noise higher than others in plate	5	A3, C3, D3, E3, F3
NOSIGNAL	No signal in well	0	
NTCCT	Non-Template Control sample	0	

Figure 24 QC report summary page.

13. Click the **Save** icon. This will automatically fill the **Run date / time** and select **PASS** as the batch status for the quantification batch.

Note: Once uploaded to FR the Results PDF can be deleted from I:\Results\Qres after one week.

14. Review the Quant Results page after saving to check the **negative extraction controls** quantification values. Refer to Table 10 for appropriate actions.

Table 10 Actions for extraction negative controls.

Extraction Negative Controls Quantification Value	**Action Required**
0.000000 ng/µL	Nil
≤ 0.001 ng/µL	Note the quantification value in the **extraction batch notes** (e.g. extraction negative control 0.00081ng/µL < acceptable threshold) and allow sample to be amplified.
> 0.001 ng/µL	Contamination may have occurred. Note the quantification value in the **extraction batch notes** (e.g. extraction negative control 0.0052 ng/µL > acceptable threshold). Order another quantification as per the Miscellaneous Analytical Procedures and Tasks (34064), allow the sample to progress to amp and notify the Analytical Senior Scientist (HP5).

15. Review the Quant Results page after saving to check the **positive extraction controls** quantification values. If the value is undetermined or lower than expected, order another quantification as per the Miscellaneous Analytical Procedures and Tasks (34064) and allow the sample to progress to amp. Enter a batch note in the **extraction** batch.

9 Validation

- Acedo, P., Mathieson, M., Ryan, L., Allen, C. 2015 Validation of Quantifiler® Trio.
- Dwyer, T., Darmanin, A., Ryan, L. and Allen, C. (2016). Project Proposal #173: Validation of Hamilton STARlet A for Quantification and Amplification Assay Setup.
- Kaity, A., Ryan, L., Mathieson, M., Allen, C. 2019 Validation of two QuantStudio™ 5 Real-Time PCR Systems.

10 Quality assurance/acceptance criteria

Refer to Section 7.3 for the quality assurance/acceptance criteria for quantification.

11 References

- Applied Biosystems (2015). QuantStudio 3 and 5 Real-Time PCR Systems. Installation, use and maintenance. [PN MAN0010407, Revision C.0].
- Applied Biosystems (2017). HID Real-Time PCR Analysis Software. Version 1.3. [PN MAN0009819, Revision D.0].
- Applied Biosystems (2017). QuantStudio 5 Real-Time PCR System for Human Identification. Site preparation guide. [PN MAN0016701, Revision A.0].
- Thermo Fisher Scientific, Quantifiler® HP and Trio DNA Quantification Kits User Guide, Publication Number 4485354, Revision A. Publication Number 4485354, Revision A ed2014.
- J.Y. Liu, Direct qPCR quantification using the Quantifiler® Trio DNA quantification kit. Forensic Science International: Genetics 13 (2014) 10-19

- [D.T. Chung, J. Drabek, K.L. Opel, J.M. Butler, B.R. McCord, A study of the effects of degradation and template concentration on the amplification efficiency of the miniplex primer sets. J. Forensic Sci. 49 (2004) 733–740
- S. Vernarecci, E. Ottaviani, A. Agnostino, E. Mei, L. Calandro, P. Montagna, Quantifiler®Trio Kit and forensic samples management: A matter of degradation. Forensic Science International: Genetics 16 (2015) 77-85.
- J.M. Roberston, S.M. Dineen, K.A. Scott, J. Lucyshyn, M. Saeed, D.L. Murphy, A.J. Schweighardt, K.A. Meiklejohn, Assessing PreCRTM repair enzymes for restoration of STR profiles from artificially degraded DNA for human identification. J Forensic Science International: Genetics 12 (2014) 168-180.
- Thermo Fisher Scientific, Introduction of Improvements to the Quantifiler™ HP and Quantifiler® Trio, Customer Notification Letter (May 2015).
- Bright, J.-A., Cockerton, S., Harbison, S., Russell, A., Samson, O. and Stevenson, K. (2011), The Effect of Cleaning Agents on the Ability to Obtain DNA Profiles Using the Identifiler™ and PowerPlex® Y Multiplex Kits. Journal of Forensic Sciences, 56: 181–185.

12 Associated documents

QIS: 17195 Spill Control
QIS: 22857 Anti-Contamination Procedure
QIS: 35028 Operation and Maintenance of the AB QS5 RT-PCR Instrument
QIS: 34034 Forensic DNA Analysis Workflow Procedure
QIS: 34042 Procedure for the use of the STORstar unit for automated sequence checking
QIS: 34050 Operation and Maintenance of the Microlab® STARlet and LabElite® Integrated I.D. Capper™
QIS: 34063 Preparation of DNA Quantification Standards and In-house Quality Controls
QIS: 34064 Miscellaneous Analytical Procedures and Tasks
QIS: 34103 Receipt, Storage and Preparation of Chemicals, Reagents & Kits
QIS: 34132 DNA Extraction and Quantification of Samples using the QIAsymphony SP and AS - FR

13 Amendment history

Version	Date	Author/s	Amendments
1	18 April 2017	T Dwyer, A Kaity, L Farrelly, M Mathieson	FR changeover from AUSLAB to Forensic Register. Liquid handler changeover from MPII to STARlet. Minor editing.
2	08 Nov 2017	T Dwyer	Update Y-Intercepts for all Standard Curve thresholds. Remove re-quant of negative controls with a quant value <acceptable threshold. Change negative control and reagent blank threshold to 0.001ng/uL to align with CW samples and as per the recommendation in Validation. Update hyperlinks to link to FR SOPs. Enter actions for QPS ENVM samples.

Version	Date	Author/s	Amendments
3	06 June 2019	A Kaity	Replacement of 7500 RT-PCR instruments with QS5 RT-PCR. Update transition table. Remove references to Profiler Plus.
4	July 2020	B Micic T Prowse	Minor formatting. Removed figures 16-18 not required, updated Fig 14, Section 6.1, Tables 7 & 9, moved checking evaporated wells to prior to result export, removed ordering Ref dilutions manually, added check for DILN samples. Update Section 7.3 to specify Automatic Baseline used following Minor Change July 2020.
5	January 2021	A Darmanin	Updated 3130xl to 3500xL.
6	April 2021	A Darmanin	Changed STARlet MasterMix position from the reagent module to the sample carrier. Updated images. Removed the 2nd centrifugation of the Nunc tube rack prior to quant setup. Corrected the referencing of all Figures and Tables.
7	Mar 2022	B Micic	Minor formatting. Removed Fig5. Updated Table 7 with new Y-intercept values. Table 9- corrected Ref On hold value. Re-arranged Appendix section, added note to (now) 14.4 section, added sections 14.2 STARlet Troubleshooting programs & 14.5 Locked batch troubleshooting. Updated to new template, amended figures, removed dNTO and NTC from Definition section.

14 Appendices

14.1 Appendix 1 - Manual Quantifiler® Trio Quantification Setup Procedure

The following procedure describes the method for the manual quantification of extracted DNA when the liquid handler is expected to be unavailable for ≥48 hours or if the DNA extract volume is <20 µL.

1. In the Clean Reagent Room (3188) prepare Quantifiler® Trio master mix. Refer to Section 4.1 for preparation of reagents.
2. Manual DNA quantification is to be performed in room 3194 in a biosafety cabinet.
3. Access the batch in FR and check for Analytical notes as per Section 6.5 and action as required.
4. Print the platemap from the **BatchID_QUANT_MAP.xls** file ensuring lab numbers and volumes are visible.
5. Centrifuge the Nunc™ rack of samples for 1 min at 2000 rpm (657 g).
6. Using the Mixmate, vortex the Nunc™ rack of samples for 1 min at 1000 rpm.
7. Centrifuge the Nunc™ rack of samples again for 1 min at 2000 rpm (657 g).
8. Check the first and last barcodes of the extracts against the FR platemap.
9. Briefly vortex and centrifuge the Quantifiler® prepared standards.

Note: Ensure the Quantifiler® prepared standards are within the expiry date and there is sufficient volume remaining (> 20 µL).

10. Briefly vortex and centrifuge the master mix tube/s.
11. Label a skirted 96-well plate with the Batch ID on the front and the Batch ID barcode on the right side.
12. Dispense 18µL of master mix to each required well of the optical reaction plate (as per the platemap).
13. Apply a breathable sealing film to surface of labelled 96-well plate.

Note: Well designations may be written on the surface of the breathable sealing film for ease of reference.

14. For the following steps, check that the well position being pipetted into matches the standard or barcode according to the FR platemap.
15. Pipette 2µL of Quantifiler® prepared standard or DNA extract into each of the wells of the labelled 96-well plate as per the platemap.
16. ***CAREFULLY*** remove the breathable sealing film and seal the 96-well plate with an optical adhesive seal.
17. Centrifuge the optical plate for 1 minute at 2000 rpm. Place the plate in the pass-through hatch to the PCR/CE Room (3194).

18. Check the plate to ensure all wells contain the required volume and that no bubbles are present.

Note: Excessive bubbles in the wells are critical and may affect the accuracy of the quantification results.

19. Ensure all reagents, equipment, consumables and locations are selected against the batch as per Section 4.4.

20. Proceed to Section 7.2.

14.2 Appendix 2 - Troubleshooting: STARlet Troubleshooting Programs

If a method is aborted part-way through a run, there are troubleshooting methods to allow the operator to restart the method from the last completed step. Which troubleshooting program used is dependent on what stage of the set up the run was aborted. The operator should be able to identify where the method has aborted by reading the trace file. (**Note:** Mastermix is added to the plate first, followed by Standards and then DNA extracts). Once this has been determined, the operator can then choose the appropriate troubleshooting method to continue the run.

The Quantifiler Trio program cannot process batches with duplicate samples. If duplicate samples are required there is a troubleshooting program that can prepare batches with duplicate samples.

The four troubleshooting programs are:

1. Q1. Quant Start from Add Master Mix
2. Q2. Quant Start from Add Standards
3. Q3. Quant Start from Add Samples
4. Quantifiler Trio DUPLICATE SAMPLES

14.2.1 Quant Start from Add Master Mix

This troubleshooting program is used when:

- samples have been decapped
- some or no mastermix has been added to the plate
- no Standards have been added to plate
- no samples have been added to plate

1. If some mastermix has been added to the plate, edit the platemap to change the mastermix volume (MMVOL column) to 0 for the samples that already have mastermix.
2. Upload the edited platemap to FR.
3. If required, launch the Method Manager software via the desktop icon.
4. Select the '**Troubleshooting**' button on the home page (Figure 4).
5. Select '**Q1. Quant Start from Add Master Mix**'.
6. A dialogue box will appear (Figure 25). Read and follow the prompts:
    a. check that you have selected the correct program
    b. check that all required carriers are loaded onto the STARlet deck
    c. NUNC rack must be on the decapper stage, inside the STARlet

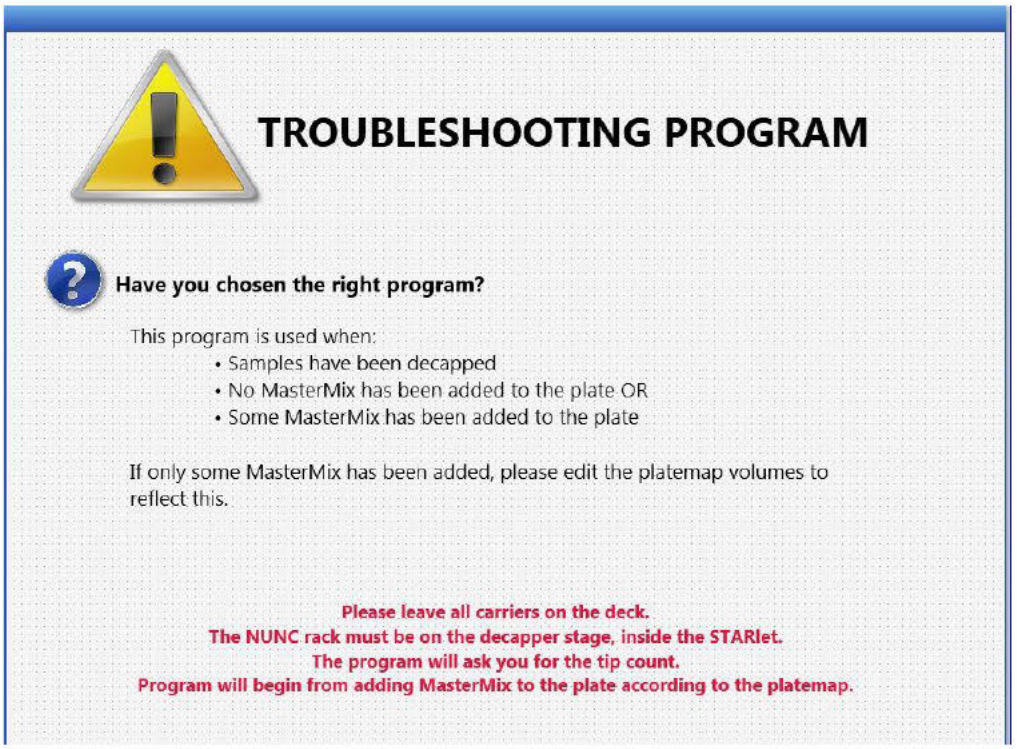


Figure 25 Troubleshooting Program Dialog box- starting at the addition of MasterMix.

7. Continue the program, the program will start from adding mastermix to the plate as per the platemap.

14.2.2 Quant Start from Add Standards

This troubleshooting program is used when:

- samples have been decapped
- mastermix has been added to the plate
- some or no Standards have been added to plate
- no samples have been added to plate

1. If some Standards have been added to the plate, edit the platemap to change the sample volume (SVOL column) to 0 for the Standards that have already been added to the plate.
2. Upload the edited platemap to FR.
3. If required, launch the Method Manager software via the desktop icon.
4. Select the '**Troubleshooting**' button on the home page (Figure 4).
5. Select '**Q2. Quant Start from Add Standards**'.
6. A dialogue box will appear (Figure 26). Read and follow the prompts:
    a. check that you have selected the correct program
    b. check that all required carriers are loaded onto the STARlet deck
    c. NUNC rack must be in position 4 of the Multifelx carrier in Tracks 20-25

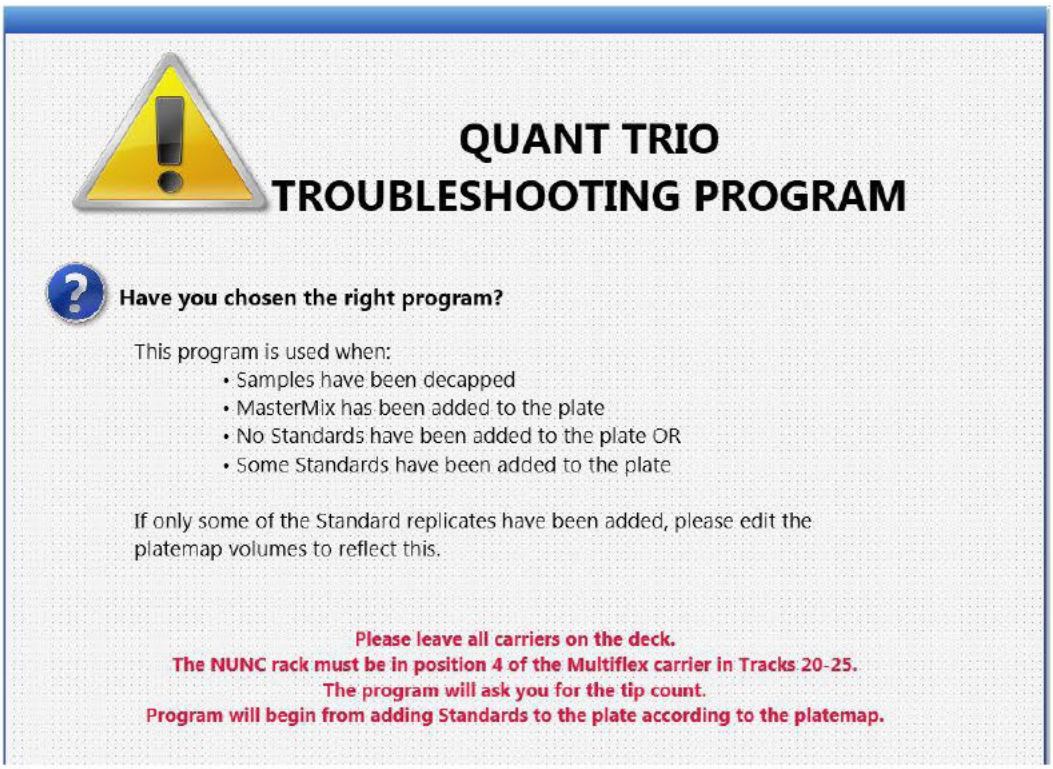


Figure 26 Troubleshooting Program- starting at the addition of Standards.

7. Continue the program, the program will start from adding the Standards to the plate as per the platemap.

14.2.3 Quant Start from Add Samples

This troubleshooting program is used when:

- samples have been decapped
- mastermix has been added to the plate
- Standards have been added to plate
- Some or no samples have been added to plate

1. If some samples have been added to the plate, edit the platemap to change the sample volume (SVOL column) to 0 for the samples that have already been added to the plate.
2. Upload the edited platemap to FR.
3. If required, launch the Method Manager software via the desktop icon.
4. Select the '**Troubleshooting**' button on the home page (Figure 4).
5. Select '**Q3. Quant Start from Add Samples**'.
6. A dialogue box will appear (Figure 27). Read and follow the prompts:
    a. check that you have selected the correct program
    b. check that all required carriers are loaded onto the STARlet deck
    c. NUNC rack must be in position 4 of the Multifelx carrier in Tracks 20-25

Figure 27 Troubleshooting Program- starting at the addition of samples.

7. Continue the program, the program will start from adding the samples to the plate as per the platemap.

14.2.4 Duplicate Sample/s on Batch

1. Complete steps 1-8 of Section 7.1 Quantification Set up.
2. Open the STARlet quant platemap and the duplicate quant template (I:\Pre PCR STARlet\All Plate Maps\Duplicate Quant Template) (alternatively any amp platemap can also be used). Copy the '**Source Pos**' column and paste it into the quant platemap into **column G** (to the right of the 'SVOL' column).
3. Locate the duplicate sample/s in the platemap and edit the '**Source Pos**' column to reflect the correct position for the physical extract. Save the platemap.
4. Upload the edited platemap to FR.
5. If required, launch the Method Manager software via the desktop icon.
6. Select the '**Troubleshooting**' button on the home page (Figure 4).
7. Select '**Quantifiler Trio DUPLICATE SAMPLES**'.
8. Continue as per step 10 of Section 7.1 Quantification Set up.

14.3 Appendix 3 - Troubleshooting: Batch Completed prior to Results Upload

If the quantification batch is completed in FR prior to the upload if the result file (.xls), the samples do not transition to the next required step of processing. The result file (.xls) cannot then be uploaded to a completed batch to transition the samples. The following procedure describes the method used to progress samples when the quantification batch has been completed prior to the import of the result file (.xls).

1. For the original batch add “See Batch” to the batch comments field.
2. In the Batch Notes field add: “Batch completed prior to results being uploaded. Results have been uploaded under (New Batch ID)”.
3. Change the batch status to “Pass”.
4. Upload the PDF results file to this original batch.
5. Create a new quantification batch of the same type using the original batch as the source batch.
6. Sequence check and lock the batch.

Note: Check if the samples from the quantification batch have been stored. If the samples have been stored, locking the newly created quantification batch will remove all the samples from the storage location in FR. If this occurs, you must re-store all the samples back into FR storage.

7. Add a “See Batch” comment to the new batch.
8. In the Batch Notes field add: “Samples processed on (Original Batch ID). This batch was created solely to upload the results for processing to continue.”
9. Copy and rename the .xls results file to match the new Batch ID in the results folder.
10. Open the new .xls file and edit the Batch ID inside the file to match the new Batch ID. Save and close the file.
11. Upload the .xls results file to the new batch and complete as per routine procedure.

14.4 Appendix 4 - Troubleshooting: Locked Batch that needs samples to be removed

Once the quantification batch is locked in FR, samples cannot be removed from the batch. If there are samples that are required to removed (duplicate samples, on hold, etc), ideally the quantification batch would be re-created in FR (using the original batch). The samples removed (that require removal) and the (new) batch re-STORstarred. Sometimes due to time constraints this is not possible. The following procedure describes the method used for processing a quantification batch that contains samples that do not require quantification.

1. For samples that do not require quantification, edit the quantification batch platemap for the STARlet and change the sample volume (in SVOL column) to zero. Upload the edited platemap into FR.
2. In the Batch Notes field add a comment to note each sample that required to be removed (but could not be removed in FR as the batch was locked) and that no sample was physically used/added to the quantification reaction plate. Also add that these samples/positions are not to be transitioned from the quant result page.
3. Add a notation to each sample noted in the Batch notes to explain that the sample was not physically used/added to the quantification reaction plate.
4. Prepare the quantification batch as per normal procedure.

Note: When running the batch on the QS5, the instrument platemap must match FR. **Do not remove/omit any samples from the QS5 platemap**. If the samples that were not added to the quantification plate are removed, then the QS5 result file cannot be uploaded into FR.

5. Upload the result file and blank out the Technique and Method for the samples / positions that did not require quantification and then complete the normal result finalisation steps.

PB142

HealthSupport Queensland

Forensic and Scientific Services

Procedure for Case Management

1 Purpose

The purpose of this procedure is to describe the components of a case record, processes involved in compiling and completing a case record and tracking of case records.

2 Scope

This procedure shall apply to all Forensic DNA Analysis staff that case manage any component of a case record.

3 Definitions

AUSLAB	Laboratory Information System (routinely used prior to the FR)
Case managing scientist	The scientist(s) that has (or have) been involved in the assessment of results and compilation of the case file in preparation for statement writing or peer review.
Case record	All information relating to a particular case. This can include all case histories, receipts, communication with clients, examination notes, Analytical data, internal communications, results and reports.
CE	Capillary Electrophoresis
DAD	DNA Analysis Database
DNA Master	Repository of DNA profiling information prior to FR
DNA Mgt	DNA Management Unit – A QPS Unit that transfers the exhibit results and link results from the Forensic Register to QPRIME. They also perform quality checks on the validity of the information/results received.
EPG	Electropherogram
Examining scientist	The scientist/s who has/have examined exhibits for a case.
FR	Forensic Register – Laboratory Information Management System since July 2017.
GMIDX	GeneMapper ID-X, software used for allele designation after capillary electrophoresis
In tube	An item that has been sub-sampled by the QPS and submitted to the laboratory in a tube ready for analysis.
LR	Likelihood Ratio
NCIDD	National Criminal Investigation DNA Database
OLA	Off ladder allele
PDA	Profile Data Analysis – page in the FR to record the DNA profile interpretation and actions
Profiler Plus	AmpF*l*STR® Profiler Plus®: The amplification kit made by Life Technologies
PP21	PowerPlex® 21 system kit
Paperless	A type of case that does not involve a traditional paper case file.
PowerPlex® 21 system kit	The amplification kit made by Promega that is currently used for all samples.
QFLAG	Quality checking procedure to investigate potential staff and elimination database matches

QPRIME	Queensland Police Records and Information Management Exchange (Post 2008)
Reporting Scientist	The scientist who is responsible for writing a Statement of Witness outlining the results of a case and for presenting evidence in a court of law.
RFU	Relative Flourescent unit (a measure of peak heights in electropherograms)
SCI	QPS Scientific Officer
SOCO	QPS Scenes of Crimes Officer
SSLU	Scientific Services Liaison Unit
StatsPWG	Statistics Project Working Group
STRmix™	A statistical program used during case management to interpret certain types of DNA profiles.
UKN	Unknown DNA profile
ULP	Unlabelled allele
VAR	Variant allele
XOVER	Cross over allele, allele migrates into an adjacent marker bin.

4 Case file overview

Since the 1st of September 2009, low priority Volume Crime cases have been treated as 'paperless' and therefore do not have case files. In April 2010, paperless case management and review was expanded to also include all cases of both high and low priority (Volume and Major Crime) and some Sexual Assault cases except for cases involving excessive numbers of crime scene/reference samples or complex profiles. In April 2015 all cases are initially managed as paperless cases.

Case files are generally created

- At the time of case management (for complex cases) or
- When a statement is requested or
- When a case manager/reporter deems it necessary for efficient case management.

For cases previously managed paperlessly that become reactivated upon receipt of further items, they may be considered for conversion to a paper file. Case and examination notes (when the case was managed paperlessly) are stored in 'Paperless' folders stored in Evidence Recovery, Reporting and Admin areas.

If a case has been converted from paperless to paper, it is not necessary to annotate all of the EPGs with the item description or interpretations unless a statement has been requested. At such a time, the reporting scientist may continue with EPGs not being annotated as long as the casefile also includes a printout of the relevant PDA page from the FR.

4.1 How to create a case file

To request a casefile to be created, email FSS.FDNA.Admin@health.qld.gov.au with instructions. Admin edit the Statement Request/Task that a casefile is being created, assign a barcode for the casefile and create a storage location (see QIS 33773 and 34248).

4.2 Additional Elements of a case file

Upon completion, a case file may also contain:

1. Examination notes

2. Diagrams, photographs and/or photocopies
3. Statistical calculations.
4. Copies of results (GeneMapper ID-X printouts).
    a. As a minimum, reference samples require the final/reported profile. Casework samples should have all EPGs printed.
5. Interpretations of results
6. Copy of statement or intelligence report
7. Records of any internal or external communication relating to the case, e.g. Casefile Notations, Requests/Tasks or emails.
8. STRmix™ output files/report. STRmix™ v2.7 it is not recommended to include the STRmix™ report, rather a printout of the PDA page with the EPG is sufficient.

4.3 Handwritten results and corrections within a case file

As is required by NATA ISO 17025 - as case notes etc. are subject to subpoenas; no pencil is to be used in the case file (unless used in diagrams or pictorial representations).

Any calculations, interpretations or changes to notes or results must be initialled and dated by the person performing the action.

4.4 Case file storage and movement

Case files are required to be kept indefinitely as per accreditation requirements.

Exhibits are not to be stored in the case file. This includes external proficiency samples. Original QPS property tags or reference sample envelopes are also NOT to be stored in the case file.

Case file movements are to be recorded in the FR. If a case previously managed within AUSLAB is reactivated, remove the tracking from AUSLAB, create a casefile in the FR (using the same barcode) and track in the FR.

Active case files are stored with the case analyst or in a designated storage location for the work area.

Upon completion, scientists should transfer cases to Admin via the FR. Administration assistance slips are available to attach to the front of the case file to direct the storage of the file or to outline any further administrative tasks that need to be performed prior to storage. Admin In-Tray – Casefile Finish is the location from which administrative staff will track case files (sequentially) into the compactus or another designated storage location. No further administrative tasks will be carried out on these cases.

If a casefile in the custody of the case scientist is taken out of the laboratory for court, or for court preparation, movement of the casefile should be recorded as a casefile notation in the FR.

5 Workflows

5.1 Priorities

Table 2 details the DNA priorities that are used in Forensic DNA Analysis. These are not to be confused with case priorities eg. one sample may be processed as Priority 1, but the case as a whole is Priority 2 (Major Crime).

Table 1 - DNA Priorities in Forensic DNA Analysis

Priority	Description	CW Use	Ref Use
1	Urgent	Urgent	Priority/investigation
2	High Pri	Major crime	High priority
3	Low Pri	Volume	Normal

Urgent (5-day Turnaround (TAT)) cases are specifically allocated to a case scientist and/or reporting scientist as they arrive into the department. The Managing Scientist and Team Leaders will be notified of the arrival of an urgent case by email and appropriate notes will be entered. A supervising scientist will allocate to an appropriate case manager. This does not mean that the case managing scientist will necessarily become the reporting scientist should a statement be required, however this is preferred to maintain consistency in reporting.

P1 samples must be managed as soon as results become available and reviewed as soon as results are interpreted. To ensure there is no delay in QPS being informed of 5-day TAT results as soon as they are available, a workflow has been created for samples that are expected to be completed on a Friday (see QIS 23968, 33773 and 34006).

5.2 PowerPlex®21 system kit vs AmpFℓSTR® Profiler Plus® case management

Since the end of testing with AmpFℓSTR® Profiler Plus® (Profiler Plus) in January 2018, all samples are received and processed with PowerPlex®21 system kit (PP21).

This does not mean the reporting method for Profiler Plus samples is invalid; therefore, in consultation with a senior scientist, samples may be re-processed with PP21 for case consistency or only newly received items will be processed and reported with PP21 and STRmix™.

5.3 STRmix™ versions

The date of first installation and processing of cases with various versions of STRmix™ are listed in Table 3 below.

Table 2 – STRmix™ version use

Date case received	Decon	LR (at time of receival)	LR (New comparison)
19 Dec 2012	v1.05	v1.05	v2.0.6
1 July 2014	v2.0.1	v2.0.1	v2.7.0
30 Jan 2015	v2.0.6	v2.0.6	v2.7.0
16 Jan 2019	v2.6.0	v2.6.0	v2.7.0
24 June 2019	v2.6.2	v2.6.2	v2.7.0
10 Feb 2020	v2.7.0	v2.7.0	v2.7.0

If new samples are received for cases that had other samples in the case previously analysed with earlier STRmix™ versions, they are to be analysed with the current version of STRmix™. Discussion with a Senior Scientist on whether to migrate previously reported samples to the current version should be held.

5.4 Case management workflows

For the process to allocate samples and/or cases, see QIS 33773.

For worklists and information on how these are populated, refer to QIS 33773.

Allocation of cases to a particular scientist usually only happens if a statement is required, the case is large or has been assigned an Operation by QPS. These cases will otherwise be routinely case managed by the competent case managers. However, to reduce the amount of double handling by case managers, individual samples initially case managed by a particular person will be completed by the same person. This includes reworking and STRmix™ deconvolutions.

Unallocated paper case files may be stored in the filing cabinets stored in the far end of the reporting area in Block 3.

Internal controls, external and internal proficiency (where applicable), internal and external environmental monitoring samples are case managed by the Analytical, Evidence recovery and Quality teams.

Various tools may be employed to assist in meeting timeframes and to cover absence such as scheduling Outlook appointments or tasks.

6 Case management

The purpose of case management is to collate and report any DNA results that have been obtained and to prepare the case file for a statement (if required) or for peer review. To achieve this, the case managing scientist may be required to:

1. Assess DNA results to determine whether reworking is required to improve or confirm results.
2. Enter final Exhibit reports via the Profile Data Analysis (PDA) page in the FR.
3. Compile case file.

6.1 Check quality

Samples should not be progressed or reported until the various quality checks that are in place have been completed. These checks are designed to identify potential issues with samples before they are reported to the QPS.

6.1.1 Batch statuses

Check that the statuses of the processing batches are fully completed (see QIS 33773).

If there has been an issue noted during processing of a sample, the Analytical staff member/delegate will enter a status of 'See batch'. The case managers (PDA operator and reviewer) **MUST** check the batch audit and add a Sample Note to detail that they have deemed the sample OK to report.
It is acceptable that the note is added by the PDA operator or reviewer. If there is a critical element to a Batch that could affect the sample processing or interpretation strategy, and there is no note added by the PDA operator, then a discussion between the PDA operator and reviewer should occur.

Results can be released prior to the batches being formally 'passed'. In these instances, the PDA operator and reviewer will need to check the relevant batches and added a comment or sample notation to describe this.

6.1.2 Casefile Notations

Check Case Management tab in the FR for Casefile Notations and Request/Tasks (and UR notes for cases processed with AUSLAB) for relevant information related to the case. This may include information such as allocation to an individual case manager/reporter, court timeframes, communication with DNA Management etc.

6.1.3 Notations

Check for relevant information in the Exhibit Testing tables for notations and Analytical Notes (see QIS 33773), and Specimen Notes for cases processed with AUSLAB.

6.2 Check case information

Case information may be relevant to only particular samples or the whole case. This information may be used to guide the case manager's choice of processing and reporting.

6.2.1 Check for reference samples associated to the case

The presence or absence of reference samples may affect the workflow path a sample takes. If reference samples have been received for a case, these will be compared against all single source DNA profiles, and all interpretable mixed DNA profiles to generate a LR.

See QIS 33773 and 34006.

6.2.2 Check for case allocation

It is necessary to check if a case has been allocated to a particular case manager or reporter before case managing a sample.
Check the Case Management tab in the FR for details or on the PDA page, it can be viewed in the 'Case Scientist' field. See QIS 33773.
In AUSLAB (if some or all of the case was processed with AUSLAB (pre July 2017), it may be recorded in the UR notes and/or the CS page.

6.2.3 Check for paper file/case notes.

Check the Exhibit Register for a barcode created for a casefile to enable storage and tracking (see QIS 33773).

6.2.4 Check ownership of item

Ownership of an item may be required before interpretation of a DNA profile or an exhibit is sampled. If unknown, send a Request/Task to the SOCO or SCI in the first instance to obtain this information. If a response is not received in a timely manner, send a Request/Task to QPS DNA Management for the information.

6.2.5 Finalising samples no longer required

See QIS 34006.

6.3 Assess results

All samples have alleles designated as per QIS 34112.

When results become available for a sample, an assessment needs to be made as to whether reworks are required or whether sufficient information has already been obtained. This can be performed as each result becomes available. Not all results need to be available at the same time for these assessments to take place.

If viewing a case via AUSLAB and with samples processed with Profiler Plus, the EPGs were saved to AUSLAB as jpegs, or if they were samples from major crime cases, they had their EPGs saved to the P drive.

If the case was processed before implementation of the FR, the EPG PDF will be stored on the network.

To assess the stutter percentages, a worksheet or macro may be used to perform the calculation checks (see QIS 35008 or QIS 35406). The former requires manual addition of the alleles and peak heights to calculate the stutters, and the latter spreadsheet uses a macro to calculate the stutters after importation of the STRmix™ text file generated by the FR.

6.3.1 Assess the number of contributors to the DNA profile

The number of contributors to a DNA profile is required to perform interpretation. Counting the number of alleles at each locus (above and below Limit of Reporting threshold, above Limit of Detection) is the first step in assessing the number of contributors.

However, counting called alleles alone may not be suitable in determining the number of contributors due to the presence of PCR artefacts such as stutter. Allelic imbalance (AI) also known as heterozygote balance (Hb) can also be used as an indication of the number of contributors. Forensic DNA Analysis does not have a threshold for AI for casework DNA profiles because STRmix™ is designed to model the heterozygote balance as a continuous system. Although internal validation studies (Nurthen et al 2013) indicate that the calculated AI threshold varies depending on the DNA input, the values detailed in the study can be used as a guide.

See Appendix 1 for a workflow designed within the internal Change Management project #149 to assist in deciding on a reasonable number of contributors to the DNA profile. Note that the stochastic range in RFU values will be different depending on the CE instrument. The workflow is a guide only.

The validated stutter thresholds (as published in QIS 34112) are used as a guide to aid in the determination of number of contributors to a DNA profile.

6.3.2 Assess the overall quality of the DNA profile

The quality of the DNA profile in conjunction with the number of contributors will determine if a DNA profile is suitable for interpretation.
The following factors should be considered

1. Whether a reasonable assumption of the number of contributors can be made.
2. The degradation slope (the tendency for higher molecular weight loci to have lower peak heights compared with smaller molecular weight loci).
3. The total amount of DNA input used in the amplification
4. Adverse events affecting the sample.

6.3.3 Check VAR/OLA/ULP/XOVER calculations

If a variant and/or off ladder allele or stutter has been observed on a GeneMapper ID-X (GMIDX) profile it is not necessary to re-amplify to confirm its presence.

For mixed DNA profiles with variant and/or off ladder alleles, the repeat of these samples is at the case manager/reporter's discretion. Things to consider include whether the profile with variant and/or off ladder alleles has already had this questioned allele confirmed, matches a deconvoluted contribution, or if the sample description suggests the mixed DNA profile could be conditioned on the reference DNA profile (with variant and/or off ladder alleles).

A case manager must independently perform the calculation for allele designation including if the calculated allele falls in the stutter position. Refer to QIS 33773.

Variant/OLA/ULP/crossover calculations do not require checking if the DNA profile has been assessed as unsuitable for interpretation.

If there are broad peaks observed in the EPG and the sample has not been Re-CE'd, the case manager may order a Re-CE. This is especially important if the DNA profile is to be assessed by STRmix™, or if the case manager determines that the broad peak could be masking other peaks such that it may affect the number of contributors assessment.

6.3.4 NAD samples

If a sample is flagged as No Analysed Data (NAD) at CE quality checking stage, the sample will be re-prepared by Analytical staff.

6.3.5 Edit DNA profiles

See QIS 33773 and 34006.

6.3.6 Rework DNA extract if necessary.

For processes relating to ordering reworks, see 33773.

See Appendix 2 for information on reworking strategies and considerations when assessing sample information and profiles.

If a sample was completed in DNAMaster/DAD and AUSLAB, any subsequent reworks that are required are requested in the FR.

As of 30 June, 2019, any rework on a previously reported Major Crime (Priority 2) result is not to be ordered without Managing Scientist or Executive Director authorisation. A MS Form can be used to provide information to the Managing Scientist of Executive Director to assess the reasons for the rework, and the potential risks associated with proceeding (or not proceeding) with a requested rework. This form can be accessed via Office 365, then selecting MS Forms. The operator fills out the details in the DNA Rework Authorisation form. After submission, the form then goes to the Team Leader for consideration and endorsement prior to the Managing Scientist (or Executive Director) for final consideration.

Internal validation studies (Nurthen et al 2013) have shown that samples with low template DNA (~132 pg) that are amplified with PP21 may exhibit significant stochastic effects such as large allelic imbalance and allele drop-out. These effects can complicate the interpretation of both single source and mixed DNA profiles. Reworking may improve the quality of the DNA profile. It is standard for P2 samples with less than 132pg (Quant of 0.0088ng/µL) to not be processed initially and a result line of 'DNA Insufficient for further processing' be released. DNA Mgt may request these samples to be reactivated for processing by sending a Request/Task in the FR to the Supervising Scientist of the Analytical Section.

In 2008, QPS in conjunction with Forensic DNA Analysis decided that for Low priority Volume Crime (Priority 3) cases, samples are only to be reworked via re-amplification, or Re-CE'ing until 12 alleles are obtained (National Criminal Investigation DNA Database-NCIDD uploading threshold). NucleoSpin cleanups or Microcon concentrations are not to be ordered on low priority samples, unless in exceptional circumstances. Other valid reasons for reworking these samples include investigations of adverse events or if other quality issues are suspected.

If a partial profile or NSD profile is obtained for a sample, an assessment should be made as to whether reworking that sample will be beneficial or if there are other profiles within the case that satisfy reporting requirements.

Amplification products are not kept indefinitely. The availability of a PCR product should be checked prior to ordering a Re-CE. For more recent batches, the Analytical Section enters audit notes against the amplification batch when the PCR product has been discarded.

Rework strategies:
If it is determined that a better profile is required, the following should be considered when determining the best rework strategy:

1. **The type of sample**
   e.g. blood versus cells. Due to the generally high number of nucleated white cells in whole blood, a DNA profile is usually obtained from such samples. If a DNA profile is not obtained, this may be due to insufficient nucleated cells in the sample, or could indicate an issue with the efficacy of the processing, or it could be that the sample is inhibited. Reworks may assist in obtaining an interpretable profile.

2. **The Quantitation value**
   The quantitation value is displayed in the FR. The quantitation value is an estimate and should be assessed in conjunction with other factors. Sample workflows based on the quantitation value are listed below:

1. PP21 samples with a quantitation value <0.001 ng/µL will not be further processed and will be reported post-quant with the result line 'No DNA detected', regardless of priority.
2. PP21 samples with an initial quantitation value between 0.001 ng/µL and 0.0088 ng/µL will be reported post-quant with the result line of 'DNA insufficient for further processing'. Priority 1 samples will proceed to a microcon concentration step prior to re-quant and amplification as per QPS –Forensic DNA Analysis agreement.
3. Samples reported as 'No DNA detected' or 'DNA insufficient for further processing' can be requested by QPS for further processing via the Request/Task system to the senior scientist of the Analytical section.
4. PP21 samples with an initial quantitation value of > 0.0088 ng/µL are amplified.

A partial or NSD profile from a sample with a high quantitation value may indicate inhibition or may be due to degradation. The Degradation Index is available within the Quantification data and provides an indication that degraded DNA may be present. It should be noted that while quantitation values can be used as an indicator for the presence of inhibitory compounds in an extracted sample, lack of inhibition in a quantitation amplification (as indicated by the IPCCT and possibly the CT as well) does not necessarily mean there will be no inhibition in an STR amplification. This is because different primers, target DNA and amplification conditions are used in each reaction and this could result in inhibition to one reaction and not the other. Also, 2 µL of extracted sample is added to a quantitation amplification, whereas in an STR amplification the sample may be diluted before being added (which would decrease the concentration of any inhibitory substances in the amplification reaction). Up to 15 µL of DNA extract can be used for a PP21 amplification (which would change the relative concentration of inhibitory substances in the amplification reaction). Further information on DNA quantification is found in QIS 34045.

3. The number of alleles obtained

A full DNA profile is the aim of any DNA amplification but a partial DNA profile does not necessarily need to be reworked.

The minimum number of alleles required to upload to NCIDD is 12 alleles. Samples below this stringency, but above 6 alleles, may be loaded to NCIDD under special circumstances and searched against the database (refer to QIS 34246 and 33773).

If an assumption of a single contributor has been determined, partial DNA profiles do not have to be reworked to obtain a full DNA profile.

4. Examination notes

Certain substances are known to be inhibitory to the PCR process. This includes a variety of commonly encountered substances, such as dyes used in clothing (particularly denim dyes) and some biological material (in particular, the haem in blood). If managing a case where semen samples were extracted with Chelex – for example, if the case is reactivated for further processing - these samples were sometimes observed to return an NSD profile after initial extraction with no indication of inhibition. Performing a NucleoSpin clean up was noted to improve the chances of obtaining an interpretable DNA profile for these samples.

5. **Offence Details (if available)**
   Information from the QPS entered into the FR, present on item packaging, or from case conferences may assist in determining the evidential value of a particular item.

6. **Results already obtained**
   If multiple samples have been submitted for an item and one or more full profiles or mixtures have already been obtained there may be no need to continue reworking other samples from that same item. A partial 'matching' profile is often sufficient if other better profiles already exist for the same item. This must be considered carefully and in the context of the case. If it is a possibility that there may be a different profile present, such as in the case of multiple offenders, then reworks should be considered.

6.4 Manage samples

The sample management tab in the FR contains the worklists relevant to PDA entry and review (see 33773 and 33744).

Cases are not usually allocated to an individual case manager/reporter. The exception to this rule may be some urgent cases, QPS operations, linked cases or sensitive matters. Samples are case managed by staff from the worklists in the FR.

Cases with paper files may have EPGs annotated with the results and interpretations, although if the PDA page is also printed, this may be not required (see 33773). If annotated, as a minimum, the type of DNA profile. e.g. single source matching UKM1 is required. These annotations need to be signed and dated by the case manager.

6.4.1 Interpret

6.4.1.1 Paired Kinship/Paternity Trios

Any samples for Paternity trios etc. are interpreted as detailed in QIS 25303.

Reporting of the analysis outcomes is detailed in QIS 34006 and QIS 34308.

6.4.1.2 PP21 interpretation

Statistics for PP21 results are generated by the STRmix™ program as outlined in QIS 35007.

If a sample has replicate amplifications they must all be included in the STRmix™ deconvolution unless they have a particular processing issue such as excess peak heights and pull up, a Re-CE has been performed, or the runs are not consistent with each other (at the discretion of the case manager). A Re-CE and the source amplification results cannot be included in the same deconvolution as they come from the same amplification, a choice as to the best or most appropriate run must be made by the case manager and replaces the less informative result. At a minimum, a Sample Note should be added to explain why particular amplifications were not included.

All reference samples received for a particular case are to be compared against all interpretable mixtures (to generate a Likelihood Ratio - LR) and single source samples within a case.

The number of contributors will have been determined as per section 6.3.1 above.

STRmix™ V2.7 uses a stratified approach to reporting the Likelihood Ratio where the relative proportions of the population are factored into the final LR.

Single source DNA profiles

Deconvolution with STRmix™ is required if:

1. The sample is the first single source DNA profile that matches a reference sample and needs to be loaded to NCIDD, or
2. The sample requires loading to NCIDD (e.g. UNK), and/or
3. This DNA profile has less than 32 allelic peaks. The count of peaks is such that homozygous loci are counted as one peak. It is only through STRmix that single-peak loci are determined to be homozygous.

LR generation with STRmix™ is not required for single source DNA profiles:

1. If a reference sample does not match the single source sample.
2. If a matching reference sample has previously had an LR generated (and the new interpretation would not be more probative).
3. If the single source DNA profile has 32 or more allelic peaks, the sample can be reported with the appropriate result line (as per QIS 34229) and doesn't require deconvolution and an LR generated as per the recommendations in the document 'The determination of the threshold number of alleles, above which single source DNA profiles can confidently be ascribed a likelihood ratio in excess of 100 billion.' [Parry et al 2014] and further Risk Assessment after moving to STRmix™ V2.7.0.

If a single source DNA profile has one peak at a locus and another peak is visible sub threshold, STRmix™ may designate the locus as a homozygote (with a ≥99 % weighting), the case manager should consider ordering a rework in an attempt to amplify the second peak.

Homozygote alleles for single source samples that will not be loaded to NCIDD do not require editing in the FR PDA page.

A mixed DNA profile would be reported as a single source profile with sub-threshold peaks using the appropriate exhibit result line in the following circumstances:

1. If the only indication of a mixture is a labelled Y peak at Amelogenin or
2. If the only indication of a mixture is a labelled Y peak at Amelogenin and sub-threshold peaks that do not affect the called alleles.

This is done because STRmix™ cannot 'see' Amelogenin or sub-threshold peaks and the low-level contribution does not affect the interpretation of the 'single source' profile.

Mixed DNA profiles (two, three, four person mixtures)

Deconvolution with STRmix™ is not required if:

1. The case does not have any reference samples and the profile is not likely to be deconvoluted by STRmix™ into contributions for NCIDD, or
2. The case does not have any reference samples and if the DNA profile is likely to be deconvoluted into a contribution that matches an already reported unknown in the case.

If reference samples are later received then the deconvolution will be run and these reference sample profiles will be compared against the mixture and the LRs reported back via exhibit result lines.

Deconvolution with STRmix™ is required for all other two, three and four person mixtures.

Deconvolutions of mixed DNA profiles may run for extended periods of time. Additional support is provided by other staff in Forensic DNA Analysis (mostly Forensic Technicians) to run deconvolutions on dedicated STRmix™ computers. This releases Reporting Scientists' computers for other tasks.

To have another staff member run a deconvolution, see QIS 33773.

Conditioning mixtures

It may be possible to condition mixtures from intimate swabs and items (said to have come from a person). The decision to condition is at the discretion of the case manager (and reviewer). Additional information regarding ownership may be required.

Table 3 – Quick reference when to use STRmix™

Scenario	Decon	LR
SS <32 & matches assumed known contributor	No	No
SS <32 & matches a reference sample	Yes	Yes
SS <32 & new Unknown profile & NCIDD	Yes	N/A
SS <32 & matches an Unknown profile	No	N/A
First SS >32 DNA profile & matches a reference sample & NCIDD	Yes	No*
First SS >32 DNA profile & matches a reference sample no NCIDD	No	No*
SS >32 DNA profile & new Unknown profile & NCIDD	Yes	No
Subsequent SS >32 DNA profile and matches a reference sample/Unknown profile	No	No*
2P to 4P & no reference samples & not likely to resolve for NCIDD	No	N/A
2P to 4P cond & no other reference samples & not likely to resolve for NCIDD	No	N/A
2P to 4P & reference samples	Yes	Yes

*Where matching a reference samples, a Likelihood Ratio is not calculated in these instances, but they are reported in the FR as >100 billion favouring contribution.

STRmix™ results output

After the STRmix™ deconvolution and/or reference comparison has been completed and processed, the following quality checks must be performed on each result produced by STRmix™.

1. STRmix™ version
2. Casework sample number is correct
3. Reference sample number (if any) is correct
4. Number of contributors assumed to be present is correct
5. Casework DNA profile (correct allelic designations entered and correct run(s) have been included)
6. Individual locus LRs appear have an intuitive fit
7. Check all loci had successfully deconvoluted (component interpretation complete)
8. Check that the Diagnostic tools are all performing to expectation
9. Settings values (especially check full vs. half variances)
10. Reference DNA profile (correct allelic designations entered)

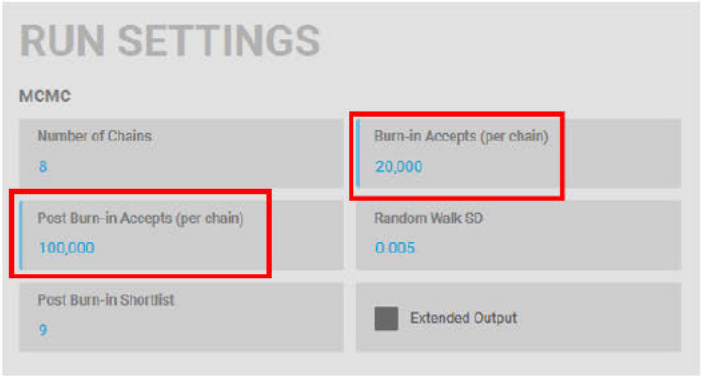
11. The overall LR is reasonable given the reference and casework DNA profiles

It is important when a STRmix™ analysis is carried out, that the results are interpreted by examining the weightings of various genotypes and the DNA profile(s) observed. There are instances when the results obtained do not intuitively seem correct. Sometimes (particularly if the model must account for drop-in) the failure of the Markov chain to properly converge means that some parameters will not have optimised properly. Examples of this are:

1. Large LRs are obtained for each locus, except one where the LR is low or 0
2. The mixture proportions do not reflect what is observed
3. The degradation does not reflect what is observed
4. Genotype combinations do not reflect all likely allele sets (especially likely if sub-threshold peaks are present at a locus)
5. The probability of dropout at a particular locus has been given a low value but sub-threshold peaks are clearly visible in the DNA profile.

Effectively, a zero LR means that the genotype of the POI has not been accepted by the MCMC at any time through the course of the analysis. Common causes for making a genotype an unlikely contributor are large dropouts, drop-ins or imbalances, or when the peak heights at a locus exceed the general degradation slope (and are therefore penalised). If further iterations are chosen, then the MCMC will have more opportunity to accept the less supported genotypes, however a reference sample with a poor fit to the DNA profile will still have a low LR for a particular locus or loci. It is best practice to attempt to resolve the mixture biologically first, that is through rework, prior to resorting to increased iterations.

It is possible that the deconvolution does not fit with the intuitive assessment of the DNA profile, e.g. there is a clear major profile but the deconvolution has not resolved C1 (Contributor 1) to ≥99%. There are a number of reasons why this may occur including there being insufficient accepts to enable STRmix to converge on the best probability space. In this instance, the user can increase the number of burnin accepts and post-burnin accepts by a factor of 2 (to 20,000 and 100,000 respectively) in the run settings when setting up the deconvolution.



If it is noted that the EPG has a plate reading error, such as a stutter peak that has been inappropriately removed or an artefact that has been left in, then the sample can be edited in the FR and EPGs manually edited as per QIS 33773.

It is not necessary for STRmix™ v2.6 (and beyond) cases to have the STRmix™ report printed and included in the casefiles. A printout of the PDA page and EPG is sufficient. All cases have the pdfs imported and retained in the FR (see QIS 33773).

Repeated Analysis

Each time a DNA profile is analysed using STRmix™ the results will vary slightly. This is a natural consequence of the random nature of the Monte Carlo property. To be as unbiased as possible, each analysis should only ever be run once and the result reported. If a STRmix™ result has been generated for a DNA profile at case management stage, then that same result should be the one used for statement writing. If additional reference samples are received in the case, the reference sample(s) should be run against all original deconvolutions for all samples in the case where mixtures are present. The exception to this is when an analysis has produced a result that requires further investigation and hence further analysis or if the underlying assumptions made about the profile have changed (eg. a two-person mix is reassessed as being a three-person mix).

Consequently, if at review or at a subsequent stage in reporting it is decided that a different number of contributors better fits the DNA profile, the deconvolution for that sample can be rerun using the new assumption. Case-managers/Reporters should discuss any decision to change a reviewed result with the original operator/s. For High Priority samples, if a rework after a result has been released, this will need Managing Scientist or Executive Director approval (see 6.3.6).

If multiple analyses have been conducted, then only the STRmix™ results from the most appropriate analysis should be reported (e.g. the higher number of acceptances or the more appropriate number of contributors). If there are printouts of the STRmix™ results in the casefile, the previous results will need to be removed.

The electronic STRmix™ results from the multiple analyses that are not used must be moved into a sub-folder labelled “Do not use” in the case folder in the STRmix™ results folder.

Use of Ignore Loci function

In certain circumstances a particular locus or loci may be dropped from the interpretation. These include where a Tri-allele pattern has been observed in a reference profile and inconsistent sizing of an allele is observed. See QIS 35007.

If a case has a reference sample with a mutation, all scene profiles within the case (except single-source profiles that do not match the reference sample in question) should have the loci removed from the interpretation. If the reference sample is received after the initial deconvolution was performed, the deconvolutions should be repeated with the relevant locus/loci ignored.

Amended Results

If an amended result is required to be released, this should be accompanied by an Intelligence Report (in most circumstances as per QIS 33773) and cleared by the Managing Scientist or Executive Director prior to release.

6.4.1.3 Profiler Plus interpretation

Since January 2018, Profiler Plus DNA profiles were no longer produced by Forensic DNA Analysis. Samples may still be added to statements (if requested) and reported in a binary fashion. This difference should be explained in the statement of witness.

Samples that are processed with Profiler Plus are not interpreted using STRmix™ as this system has not been validated for use with Profiler Plus data. Interpretation of Profiler Plus samples is outlined in QIS 17168 and 25302.

See QIS 33773 for the use of the FR in reporting Profiler Plus DNA profile interpretation results.

6.5 Report Results

All results are to be communicated as outlined in QIS 23968 and 34308.

Statements and intelligence reports are to be prepared according to QIS 34006 and 34308.

For cases processed and previously reported via AUSLAB, all new items received and/or updated interpretations should be reported via the FR.

If a sample cannot be explained by one of the result lines available, an intelligence letter should be sent to QPS to outline the interpretation. See QIS 34308.

When reporting 4p mixture interpretations where the LR is in the in the range 2-1million favouring contribution, a result is acceptable to be reported via Request/Task in the FR by using the following process:

- PDA Reviewer to ask for the Request/Task when reviewing the sample,
- Using a template (below), case manager/reporter to direct a Task to the reviewer with the information,
- PDA Reviewer directs to Sgt DNA Results Management Unit at same time as reviewing.

- Template to use:
- *Sample barcode: XXXXXXXXX*

- *Result reported: Mixed DNA profile*
- *LR reported: Mix – Support for contribution 2 to 1 million: Person barcode YYYYYYYYY*
- *Actual LR: [number]: Person barcode YYYYYYYYY*

6.5.1 Exhibit Result lines

See QIS 33773 and 34006 for details on how to report result lines in the FR.

For urgent/Priority 1 samples only, an interim exhibit report may be entered.

6.5.2 Exhibit Result line updates and amendments

Exhibit result lines may require updating after additional information is available or additional testing has been completed. Commonly, these lines are updated after a reference sample for the case has been received and new information needs to be sent back to QPS eg. the profile is now to be 'conditioned'.

If the DNA profile has undergone further work and the result line 'SUFP: sample undergone further processing' has been used, the final interpretation result lines need to be added to the FR at the same time and supersede the previous result lines. This means all lines need to be added that are relevant to the updated DNA profile interpretation.

If an incorrect result is needing to be reported, the result line must be marked as incorrect by Senior Scientists or Team Leaders. See QIS 33773 and 34006.

The correct result should be added and reviewed at the same time as marking the previous result as 'incorrect', (see QIS 34006).

If an Intelligence Report is required to be sent to the QPS Inspector of DNA Management Unit to explain and incorrect or amended result, this report needs to be initially sent to the Managing Scientist for awareness. See 34308 for a template for this report.

6.5.3 Suspect checks

If a suspect check has been requested by QPS for a reference sample profiled in Profiler Plus and the sample is not intuitively excluded from the mixture, the reference sample needs to be reworked in PP21 to increase the amount of data available for comparison.

Instructions for reworking reference samples are documented in QIS 34245.

Suspect checks have reserved Exhibit result lines for reporting; refer to QIS 34229.

LR reports from STRmix™ for Suspect Checks need to be retained in the FR. These can be attached as a sample notations for the crime scene sample, or attached to the Result line pertaining to the LR outcome for the comparison.

6.5.4 Samples with undetermined quantitation values or insufficient DNA

It is understood by QPS that samples reported post-quant as 'No DNA Detected' or 'DNA Insufficient for further processing' can be requested for processing at any time.

This request for further processing is made by the QPS sending a Request/Task to the Senior Scientist of the Analytical section to reactivate the sample for processing.

Similarly, case managers may at their discretion order a rework in cases where the only results are low quant samples.

6.5.5 Paternity Samples

For paternity cases, results are reported via the barcode for the child (see QIS 33773).

If the putative father sample is an intelligence sample, the relevant result line would be 'Intel report required for further Interpretation'. The Intel Report is issued as per QIS 34308.

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6.5.6 Using Coronial samples as Reference Samples in Exhibit results.

If a sample has been processed with casework conditions is to be used as a reference sample, it needs to be deconvoluted in STRmix™ because there is no homozygote threshold. This deconvoluted DNA profile is used as the reference in all comparisons.

6.5.7 Using Covert samples to compare to DNA profiles

Covert samples are ones that have been identified by the QPS as being taken in lieu of a official reference sample. Covert samples are treated as crime scene samples and can present to the laboratory as items such as straw swabs, swabs of drink containers and cigarette butts, among others.

The DNA profiles obtained from these covert samples may be requested to be compared to specific, or all crime scene samples. The results of these comparisons should be entered in an Intelligence Report and issued to QPS DNA Management Section, unless specifically informed otherwise.

See QIS 34308, 33773 and 34006.

7 NCIDD

Case managers are responsible for choosing a representative profile for each unique profile seen within a case for upload to NCIDD. These profiles must have at least 12 alleles for NCIDD matching.

To upload an allele to NCIDD for PP21 samples, a 99% deconvolution is required at a locus as per the Statistics Project Working Group (StatsPWG) recommendations.

- ≥99% deconvolution at all PP21 loci is known as a 'full' NCIDD load
- ≥99% deconvolution at ≥ 12 PP21 loci is known as an 'Intel' NCIDD load.

In certain circumstances, a profile with less than 12 alleles (including sub-threshold information) can be loaded to NCIDD, and any matches will be reported back to QPS via an Intelligence report written by the case scientist or Intelligence Team member. This is an intel/upload process and is not for court purposes. Intel/NCIDD work does not get heard in court unless special authorisation is given by the Judge/Justice due to potential to prejudice court.

Only one representative DNA profile is loaded to NCIDD for a person in a case. Profiles that match known deceased persons or complainants in sexual assault cases are not to be uploaded to NCIDD. By the same rationale, unknown DNA profiles previously loaded to NCIDD that match known deceased and sexual assault victims are also removed from NCIDD. Refer to QIS 34246 and 33773.

7.1 Conditioned DNA profiles loading to NCIDD

After a mixed DNA profile has been conditioned in STRmix™, the deconvolution will list that each conditioned allele has been deconvoluted to 100%, a conditioned component of a mixed DNA profile can be loaded to NCIDD provided that :

- The upload alleles are able to be visually separated (i.e. major or minor)
- Upload matching alleles in an even mixture where there is a strong representation

Do not upload contributions from low level mixed minors where we may be confident enough to condition but not load to NCIDD.

8 Peer review

All results must be peer reviewed prior to release to the QPS. Peer review can be at a sample level or case level, Technical or Administrative (see QIS 34322 and 34006).

Peer review of 'No DNA detected' and 'DNA insufficient for further processing' is usually performed by a competent Analytical Section staff member.

9 Reference sample management

Refer to QIS 34245.

10 Case Managing a file with a 'Just in Case' SAIK

'Just in Case' (JIC) kits are sexual assault investigation kits that are distributed to Pathology Queensland (PQ) Laboratories and are used in instances where a patient has disclosed a sexual assault but are not ready to involve police. A forensic examination can be requested "Just in Case" a police complaint may be made at a later date.

The JIC kits include swabs in a tamper evident bag (similar to standard SAIKs), pathology request form, JIC consent form and chain of custody form.

The JIC kits are registered in AUSLAB (Pathology) by Pathology Queensland and received at Forensic Property Point (FPP), FSS within AUSLAB (Pathology) and electronically tracked.

FSS will hold the JIC kits for 12 months, at which time they will be destroyed if the complaint has not progressed.

If the complaint progresses, the JIC kits will be registered in the Forensic Register (FR) by the Queensland Police Service using a barcode allocated by FPP. This may be different to the Pathology Queensland allocated barcode, as FR cannot currently accept the series 2 ten digit barcodes. The AUSLAB audit trail and notation in the FR will link these barcodes. FPP will enter the delivery officer details as per the initial AUSLAB (Pathology) entry, with appropriate notes regarding the date and time the samples were originally received in the FR. The AUSLAB (Pathology) audit trail will be scanned to the FR. NB. the test code "TRAIL" in AUSLAB will output the entire audit trail for the case into a report.

Testing will proceed through standard examination and analysis within Forensic DNA Analysis.

The consent form, pathology request form and Chain of Custody form will be scanned into the FR.

Refer to https://qheps.health.qld.gov.au/hsq/forensics/response-to-sexual-assault for more information.

11 File compilation

11.1 Suggested order of pages (from top to bottom)

1. Case file particulars page (QIS 34307)
2. Copy of final statement (if written)
3. Most recent printout of casefile notations, emails*
4. Exhibit Register list
5. Reference samples – receipt page then profile
6. QP127 (if available)
7. Examination notes:
    i. Description of item
    ii. Diagrams
8. Photos/photocopies/packaging/envelope images*
9. DNA profiles (EPGs)
10. Statistical calculations (if applicable)#

* these items are not required to be printed if the case is not going to court
# STRmix™ v2.6.0 (and beyond) deconvolution and likelihood Ratio reports are not necessary for casefiles. The PDA page may be substituted as it displays the LRs.

11.2 Page numbering

Only cases that are going to court (Statements of Witness or Evidentiary Certificates) need to be page numbered. Assistance is available from the Administrative Team for page numbering.

1. The Case File Particulars page is always Page 1 (except upon reactivation when the additional Case File Particulars page will be numbered page 1 and the original Case File Particulars page will be renumbered as the next consecutive number in the case file).
2. Case Files are numbered from the back of the case file to the front.
3. Number and initial each page, including the reverse of the page if both sides have been used.
4. Ensure the Case number is recorded on each page.
5. Write the total number of pages on the front of the case file and initial and date as indicated.

For those cases that aren't going to court, the total number of pages simply needs to be counted and noted on the front of the case file, that is, each individual page does not need to be numbered.

11.3 Statement compilation

Refer to QIS 34006 for the correct format for statements or reports issued by Forensic DNA Analysis.

11.4 Preparing a case file for peer review

Prior to submitting a case file for final review or prior to a statement being issued, the following is required:

- Ensure that all items/exhibits have been examined or prioritised appropriately.
- Ensure that appropriate reworks have been performed.
- Establish whether further testing needs to be performed
- Ensure that all samples are finalised
- Samples that have been reported as 'No DNA detected' or 'DNA insufficient for further processing' need to be documented in the case file. This can be done by either printing the PDA page, annotation of the receipt or annotation of the packaging image.
- All profiles have been printed and included in the case file. It is not necessary for EPGs within a casefile to be labelled, instead a copy of the PDA page can be printed to accompany the EPG(s). The PDA page contains all of the sample and interpretation information and can be associated with the EPG via its barcode.
- Ensure that appropriate profiles have been selected for upload to NCIDD. Only one example of each profile is to be loaded to the database.
- Ensure that the reference sample receipt is printed for each evidence sample (AUSLAB only).
- If there are multiple EPGs for a particular reference sample, only the reported profile need be printed and annotated as the final profile.
- Ensure that all evidence samples associated with the case are present.
- STRmix™ printouts for all cases that used this program for statistical calculations. It is not necessary to print the report for STRmix™ v2.6.0 (or beyond) as it contains a large number of pages; a printout of the PDA page and EPG is sufficient.
- For Profiler Plus cases: if a statement has been requested, ensure that profiles requiring a genotype frequency have had the statistical calculation performed through the Kinship program (see QIS 25368) and that the results are printed and included in the file. Any mixture interpretation pages, including Popstats where appropriate, must be included in the casefile.

12 Working Remotely

See QIS 34006 for writing and reviewing statements from a location other than at work (eg. working from home).

In these situations, printed casefiles with all contents may not be necessary unless a court requirement eventuates. Casefiles will be needed to be created to contain, at the very least, the hard-copy of the Statement of Witness to enable tracking to occur in the FR.

At times where actions are performed (or not performed) that differ to the standard approach to casefile compilation, these actions should be recorded as casefile notations in the FR.

13 Case file management off-site

When case files are required for court appearances they should be tracked to the Reporting Scientist in the FR.

If a file is taken off-site (in exceptional circumstances eg. flight for court evidence outside Brisbane), then a casefile notation should be added to the FR to detail this occurrence.

14 Reactivated cases and case requiring updated interpretations and testing in other labs

14.1 Reactivated and Cold Case Management

On occasion, some cases require further work after they have been finalised and reviewed. In compiling cases that were previously managed with AUSLAB, it is recommended to print UR notes and any associated communications for the reactivated case, and commence tracking within the FR (QIS 33773).

An assessment of previously reported and uploaded profiles should be undertaken. In July 2007, it was decided (in conjunction with QPS) that all crime scene profiles (except Known Deceased and complainants in sexual assault cases) would be uploaded. Prior to this any crime scene sample that matched a complainant profile for any case type was uploaded to NCIDD.

New evidence samples received for a case which has been profiled using Profiler Plus will be profiled using PP21. It should be discussed with a Senior Scientist or Team Leader and in consultation with DNA Management as to whether the case is transitioned to PP21 profiling.

Any interstate person samples submitted for analysis by the DNA Management Section (QPS) that have been obtained from people located interstate are to be treated as Evidence samples (as per advice from the QPS).

If a case is reactivated for attention, a Request/Task is usually sent to the Team Leader. The case may already have been allocated to an existing staff member or can be considered for allocation to a new case manager.

The reactivation may be for a number of reasons including, but not limited to:

- Check into property holdings at FSS;
- Check into any remnants of testing still held at FSS (ie. spin baskets, extracts);
- Check into what volumes of extracts may remain for consideration of profiling at FSS, or at an external facility;
- Seeking advice on potential for external testing (extract volume and reference sample dependent);
- Request for a copy of the casefile as held at FSS (QIS 34248).

If samples were quantified prior to 04 November, 2015, they would not have been processed with Quant-Trio. These samples would benefit from a re-Quant with Quant-Trio so that the indicators of Degradation and Y-Quant are obtained.

If new samples are received for these Cold Cases, these are usually accompanied by a request for 'Quant and Hold' (see QIS 33773 and 34006).

In some instances, it may be possible upon consultation with QPS Homicide Cold Case Investigation Team Forensic Co-Ordinator to pool samples from the same parent item. Consideration of whether to pool prior to profiling, or after profiling can be discussed. DNA profiling of the sample/s may be before, or after a microcon post-extraction step. Pooling samples may hinder the ability to obtain a usable DNA profile if one sample is complex, or has raised a Quality Flag.

14.2 Testing in other laboratories

Consideration of further profiling interstate or overseas can be made:

- Highly sensitive DNA profiling, using Minifiler and LCN technology, may assist degraded or low-level DNA profiles. The Institute of Environmental Science and Research (ESR) in New Zealand offers this testing.

- Y-STR profiling is performed in most other Australian jurisdictions, and in New Zealand. This technology may be useful if there are male reference DNA profiles, and the DNA profile has a quant value associated to the Y-Quant from Quant-Trio.

- Mitochondrial DNA profiling may be useful if the sample is likely to be single-sourced. This technology is useful for samples that are highly degraded or aged eg. recovered skeletal remains. Currently, Victorian Institute of Forensic Medicine (VIFM) offer this profiling service. This technology may be useful if there are males or females from the same maternal lineage.

If testing for certain samples has been approved to be conducted in other jurisdictions, the appropriate discussions and authorisations with QPS DNA Management should be retained in the FR.

Approvals and packaging process is outlined in QIS 30917.

If a casework sample is processed in another jurisdiction, it should be reported in a statement by that testing laboratory. Reference sample data (including EPG) may be requested by this reporting jurisdiction, which can be sent via DNA Management Unit.

If a casework sample is processed in QLD and Reference sample data is received from another jurisdiction, this should be reported to DNA Management Unit via Intelligence Report.

15 Records

1. Case file records – the location of paper case files is recorded in the FR, or for pre-FR cases, this is recorded in AUSLAB.
2. Paperless case examination notes - all but the current folder is stored in Block 3 Reporting.
3. Batch paper records - Filing Storage area (room 6112) or the Exhibit Room (room 6106)
4. DAD-Prior to AUSLAB Batch Functionality, all results obtained were loaded into an Excel spreadsheet known as DNAmaster. In 2008 these results were transferred to the DNA Analysis Database (DAD).
5. AUSLAB
6. Electropherogram pdf/jpeg files for samples:
   - Genotyper profiles are located in J:\User3100\Results Finalised\PRE-LIMS and I:\User3100\AAARESULTS FINALISED\POST-LIMS
   - As of the 16th February 2009, results have been analysed using GeneMapper ID-X. GeneMapper ID-X profiles are located in P:\Profile PDFs and only accessible from computers with GeneMapper ID-X installed (contains all DNA profile results from 16th February 2009 until June 2012).

- As of July 2012, all DNA profile results are located in O:\Profile PDFs (accessible from all network PCs).

7. STRmix™ result files are stored on a network drive - I:\STRmix Results\

16 Associated Documentation

QIS: 17168 – Procedure for Single Source DNA Profile Statistics

QIS: 23968 – Forensic DNA Analysis Communications Procedure

QIS: 25302 – Interpretation of Mixed DNA (STR) Profiles using Profiler Plus

QIS: 25368 – Kinship Software – Genotype Frequency Module

QIS: 25581 – Kinship Software - Paired Kinship and Paternity Trio/Missing Child Modules

QIS 30917 – Forensic DNA Analysis – Procedure for external transfer of samples and subsamples

QIS: 32139 - STRmix™ Report macro

QIS: 33744 – Forensic Register Training Manual

QIS: 33773 – Procedure for Profile Data Analysis using the Forensic Register

QIS: 34006 – Procedure for Release of Results using the Forensic Register

QIS: 34045 - Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit.

QIS 34307 – Forensic DNA Analysis - Case File Particulars

QIS: 34112 – STR Fragment Analysis of PowerPlex 21 profiles using GeneMapper ID-X software – FR

QIS: 34229 - Explanations of Exhibit Results for FR

QIS: 34245 – Reference Sample Result Management

QIS: 34246 – Uploading and Actioning on NCIDD - FR

QIS: 34248 - Administrative Team - Case File related duties using the Forensic Register

QIS 34308 – Procedure for Intelligence Reports and Interstate/Interpol Requests in the Forensic Register.

QIS 34322 – Technical and Administrative Review of Records Created in the Forensic Register

QIS 35007 – Use of STRmix v2.7.0 software

QIS 35008 – Allele specific stutter threshold worksheet

QIS 35406 – STRmix Stutter Calculator

17 References

Brisotto P, Ryan L, & Scott K. (2020). Observed Reduction in Volume Post-PCR May 2020.

Caunt E, Morgan R, Gardam T, Howes J & Allen C. (2014) Verification and implementation of STRmix™ V2.0.1

Caunt E, Morgan, R, Howes, J & Allen, C. (2015) Assessment of the Number of Contributors for Mixed PowerPlex® 21 DNA Profiles within Forensic DNA Analysis_version 2

Caunt E, McNevin A, Howes J & Allen, C. (2018) Interpretation of four person mixtures using STRmix v2.0.6

Caunt E, McNevin A, Howes J & Allen, C. (2018) Validation of STRmix™ V2.6.0.

Caunt E, Pattison H, McNevin A, Howes J & Allen, C. (2019) Validation of STRmix™ V2.7.0.

McNevin A, Caunt E & Allen C. (2019) Verification of STRmix™ V2.6.2.

Morgan, R., & Caunt E. (2015) Development of Guidelines for Mixed PowerPlex® 21 DNA Profiles within Forensic DNA Analysis_version 2 (Change Management #149).

National Association of Testing Authorities (NATA). Refer to NATA website: http://www.nata.com.au

Nurthen T, Mathieson M & Allen C. (2013) Amplification of Extracted DNA validation v2.0

Nurthen T., Mathieson M., Scott K. & Allen C. (2012) PowerPlex® 21-Direct Amplification of Reference FTA® samples validation.

Parry R, Caunt, E & Allen C. (2012) Verification of the DNA profile module of STRmix™ using the Promega PowerPlex® 21 system.

Parry R, Caunt, E & Allen C. (2013) Verification of the DNA profile module of STRmix™ for Full Volume Amplifications using the Promega PowerPlex® 21 system.

Parry R, Howes J, & Allen C. (2014) The determination of the threshold number of alleles, above which single source DNA profiles can confidently be ascribed a likelihood ratio in excess of 100 billion.

Parry R, Caunt E, & Lloyd A. (2020) 4p Mixture Discussion Paper

Police Powers and Responsibilities Act 2000, Current as of 22 September 2014

Police Powers and Responsibilities Regulation 2012, Current as of 22 September 2014

18 Amendment History

Revision	Date	Updated By	Amendments
1	11 Nov 1998	V Ientile	
2	28 Mar 2001	V Ientile	
3	11 Jun 2001	V Ientile	
4	18 Jul 2001	V Ientile	
5	08 Jan 2002	V Ientile	9(3) – Completed case codes for FACTS
6	21 Nov 2002	V Ientile	Changes to section 9, completing a case
7	19 Nov 2003	V Ientile L Freney	Refer to AUSLAB. Remove FACTS in many places
8	07 Jun 2005	M Gardam	Included requirements for paperwork in case file ie No loose pages
9	03 Aug 2006	M Gardam	List of reference articles added
10	25 Sep 2006	M Gardam	Off site case file management, compilation of case file, case management.
11	13 Feb 2007	L Weston	Update with processes for AUSLAB
12	Apr 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
Version	**Date**	**Updated by**	**Amendments**
12	10 Apr 2008	J Connell	Transferred section on preparing case file for presumptive EXR/EXH validation to Examination of Items SOP
13	12 Feb 2009	K Lee	Major rewrite; Inserted subheadings and table of contents; changed order of information to reflect current processes; expanded on reworking information and other processes undertaken as part of case management; added information regarding dilutions and requesting processing of samples sub-sampled in analytical; summarised finalisation requirements for samples with extra barcodes; added examples for entering final EXR lines. Hyperlinked associated documents.
14	28 Oct 2009	K Lee	Updated with reference to GeneMapper *ID-X* software; changed “Pre/Post LIMS” references

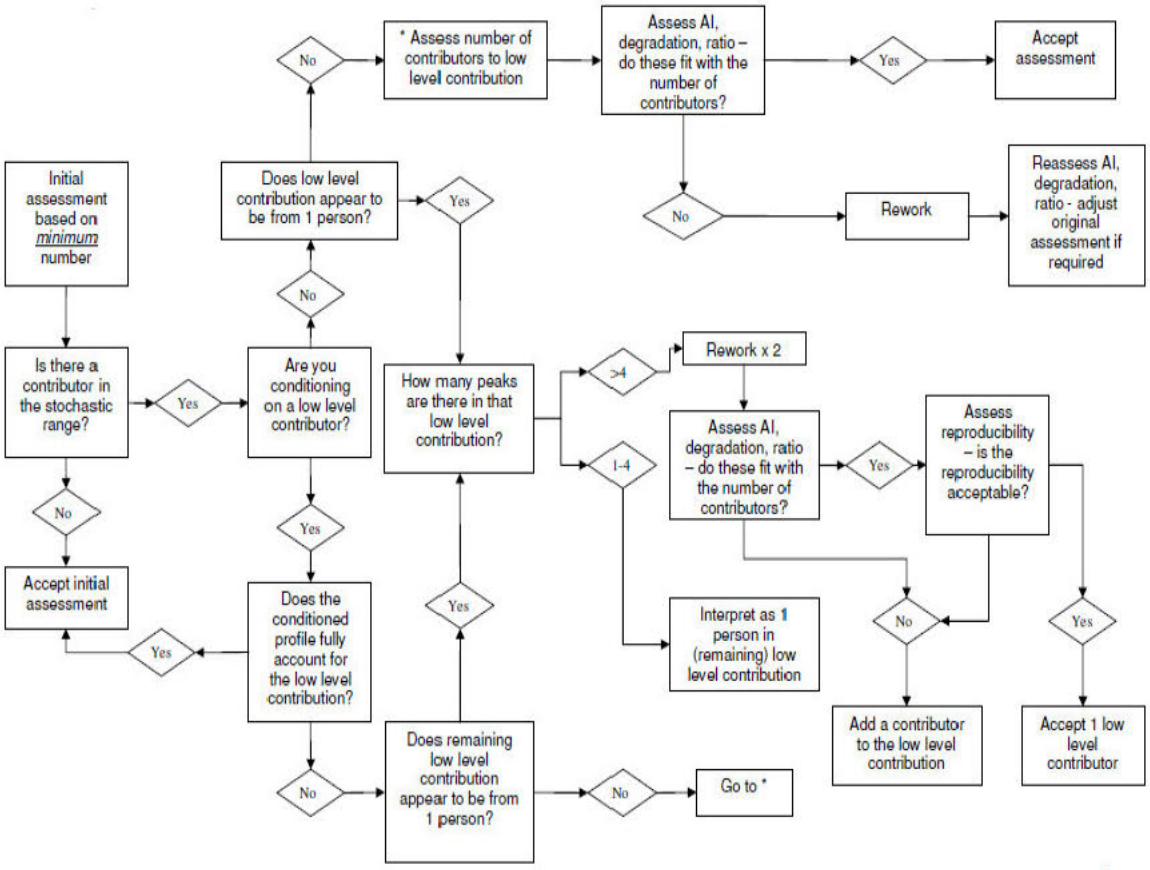
			to “Pre/Post AUSLAB Batch Functionality”; removed unnecessary flow charts; updated hyperlinks and associated documents; introduced paperless case management; re-arranged for better flow and grammatical correctness; Introduced more definitions; included instruction on locating profiles for printing.
15	27 Jan 2012	K Pippia	Introduced new worklists; added section on reworking evidence samples; added VOLUND process; addressed changes in processes since last update; removed references to re-Genescanning and introduced references to re-reads; updated hyperlinks; addressed comments raised against last revision; updated FBNLR process
16	12 Nov 2012	Alicia Quartermain, Emma Caunt, Justin Howes	Updated all processes to include implementation of PowerPlex®21 and STRmix™
17	Jan 2015	Thomas Nurthen	Incorporation of updated workflows, major rewrite , New template
18	August 2015	Thomas Nurthen	Fixed typos, referenced new document for number of contributors, additional steps for FBNLR process, added NCIDD removal process, updated STRmix versions, NCIDD load requirements
19	07 April 2017	Justin Howes	Changed example on p41 to [9, NR]; added information to 5.4 regarding strmix instructions; added eg Profiler Plus to PP21 to 9.3; section 6.3.6 – added info on Profiler Plus and microcon instructions; changed LOD Quant from 0.00214ng/uL to 0.001ng/uL; added information to 6.5.3 re incorrects; added first line to Table 6;added information to 6.2.5 on no further work process; added Appendix 3 – Intuitive Exclusion Guide and details to 6.4.1.2; changed 19977 to 33407; fixed title of 24126 and hyperlinking throughout; edited amendment history versions/revisions to align with QIS.
20	24 December 2018	Justin Howes	Major revision due to implementation of FR and other new SOPs (for the FR).
21	17 February	Justin Howes	Updated definition list; changed

	2021		EXH to result; changed statswg to statsPWG; added 35406 and 35008 to associated docs and details to 6.3; updated title of no. contributors guidelines document; added details to 6.3.1; 6.3.4 edited to remove the requirement for reamps;added authorisations to 6.3.6; removed App 17.2 (intuitive exclusion guide); replaced 're-run' with re-CE'; added 35007 and 30917 to assoc docs, removed 31523; removed details on no. iterations for STRmix in 6.4.1.2; edited the title of mixed profiles to include four-person mixtures; added Sections on remote, cold cases and off-site; added info on broad peaks to 6.3.3; 6.5.2 added info on further processing; added information on increasing iterations; removed 17038 and replaced with 34307; added reference to Intel Report template for amended results in 6.5; updated formatting, added information to section 4.4 and removed numbers; edited 11.1 to remove AUSLAB references; removed checklist (was App 19.1); added contributors workflow to appendix; added reworking strategies to appendix; add information to 6.3.6 and 6.1.1, updated reference list, updated workding in 6.4.1.2; added section 6.5.7, edited wording in section 12 (remote working),6.1.1 and 6.5.3.

19 Appendices

1 Determination of Number of Contributors workflow
2 Considerations in assessing samples for reworks

19.1 Determination of Number of Contributors workflow



19.2 Considerations in assessing samples for reworks

Reworks are required for case work samples for several reasons including optimisation of profiles, confirming information and assessing the impact of quality issues.

Below is a brief set of options to consider when deciding to rework a sample and choosing an appropriate rework strategy. This set of options will not cover every scenario and each sample should be considered on its own merit and within its own case. Samples may exhibit more than one issue that might warrant a rework. In this case select the one that will overcome the majority of issues in one go for maximum efficiency.

Problem/Profile Type	Rework Strategy/Considerations
Quality Issue noted in Batch Notes - Reduced Volume Post PCR - Other batch issue affecting the sample	Refer to the Report on Observed Reduction in Volume Post-PCR (Brisotto et al 2020). The wells commonly affected are A01, A012, H01 and H012. A reduced remaining volume may impact on the rework able to be ordered. If a suboptimal amplification (amp) is obtained due to reduced amp volumes, consider a re-quantification (quant) or re-amp as an appropriate strategy. Only rework if necessary in order to confirm a profile after a quality issue has been found to impact the sample. The best rework strategy will be dependent on the issue affecting the batch and the possible implications of the batch issue itself. Consider that re-extracting the spin basket may be best option. If the profile is considered unsuitable for interpretation, a rework or re-extraction may not assist. Consult a Senior Scientist if in doubt.
Quantification - Quant issue	If the profile seems inconsistent with the quant value or if the quant value is unexpected given other results or testing (such as numerous spermatozoa present), consider a re-quant as the best option. A profile with an inaccurate quant might be able to be identified in a sample with a strong quant with low degradation however with a poor quality or low level profile. Check the quant batch to assess the IPCCt value. A particularly low value (< 27) can be a contributing factor as this does not flag (as it does if it is a high IPCCt). If IPCCt value is low and degradation high, a re-quant should be ordered. If the IPCCt value appears to be low, a Nucleospin clean-up is still an available option for reworking.

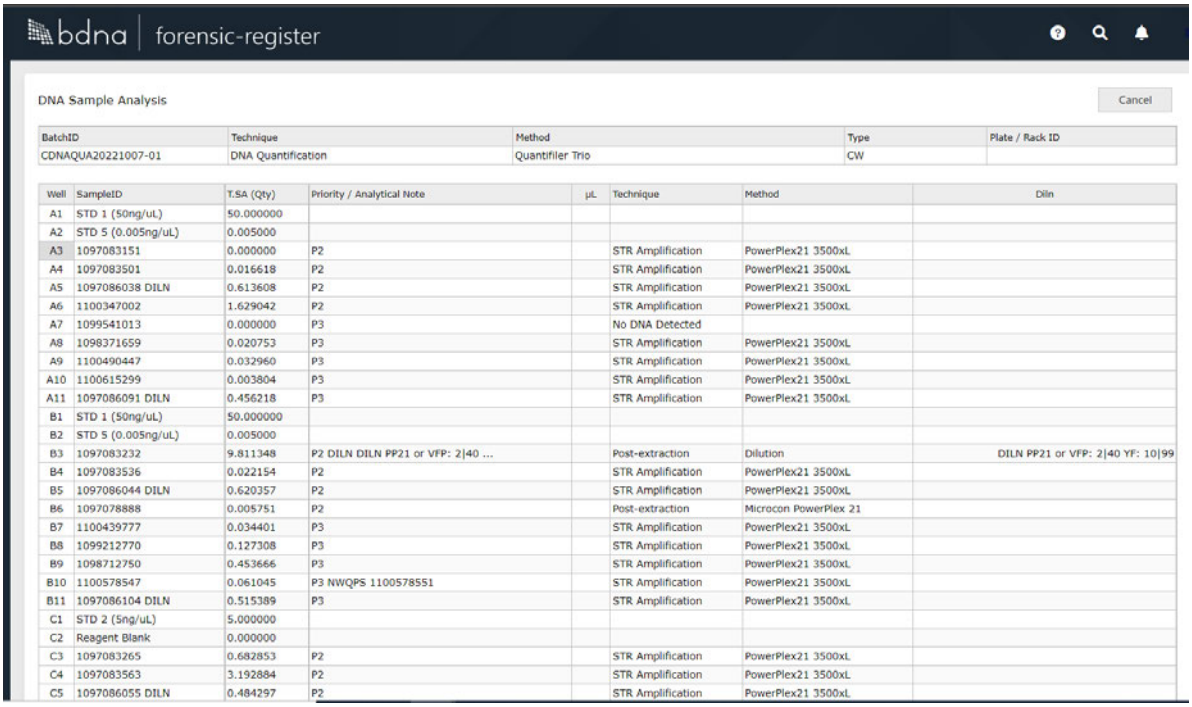
	Note that Quantification of samples is only an estimation of the amount of DNA present within a sample and the true value can vary. A re-quant will use less extract and is more likely to obtain an accurate profile. Microconning a sample with an incorrect quant value can consume the entire extract and potentially obtain an uninformative profile that is unsuitable for interpretation.
- Low quant	A profile displaying limited information due to the low level of DNA present might benefit from a re-amp at maximum volume. If the sample has already been amplified at the maximum volume, consider concentrating the sample via microcon to 35ul (a microcon to full can be a helpful option for low level single source profiles). When considering a microcon, bear in mind that the optimal amplification DNA input is approximately 500pg or 0.033ng/ul quant value. A sample with a quant value less than 0.03 is more likely to benefit from a microcon. The presence of multiple peaks at loci in a low quant profile does not in itself mean that the microconned profile will be complex, it could lead to a clean mixed profile that might be interpreted. This should be considered within the case context.
CE issues	
- Poor Baseline and/or Pull Up	A profile with an unclean baseline can create difficulty in interpretation particularly if pull-up is interfering with true alleles and causing uncertainty as to the number of contributors to the profile. A re-CE is the best first option. A re-amp might be useful if the re-CE doesn't fix the issue.
- Artefacts such as ULPs or VARs etc.	It is no longer policy within DNA Analysis to confirm unlabelled peaks or variant alleles unless there are questions raised as to their accuracy. A re-CE can confirm whether they are truly present however a re-amp will confirm the allele designations.
- Broad Peaks	Broad peaks are peaks considered to be wider than standard. Broad peaks can interfere with STRmix™ deconvolutions of mixed profiles. A mixed DNA profile with labelled broad peaks will require a re-CE before being processed through STRmix™. A re-CE is preferable due to reduced costs and faster turn arounds however a re-amp is a second alternative. If the profile is considered complex or unsuitable for

	interpretation, a rework is not necessary. Note that a single source profile displaying broad peaks that also requires STRmix™ deconvolution does not necessarily require a rework. This is because STRmix™ will assign the broad peaks correctly to the one contributor without much penalty. If the sample has broad peaks and is not being reworked, add a sample note on the PDA page that broad peaks have been observed however are not affecting the overall interpretation.
Degradation	Degradation of a sample can vary from nil to extreme. The greater the degradation, the less the certainty of the interpretation or number of contributors to the profile. Degradation can be identified by taking the quant value into account along with the severity of the slopes of peaks from left to right of the profile. Provided inhibition has not been detected (low/high IPCCt value), re-amplifying using above optimal volume input (but below what might saturate the amplification) may assist. If the Degradation Index is significant, consider if the IPCCt value is appearing satisfactory. A re-quant may be necessary.
Amplification Issues - Preferential Amplification	Preferential amplification is noted by the ski slope effect from left to right across the profile in conjunction with an indication of degradation as per the Degradation Index. Whilst this is relatively rare within casework samples, it can be negated by re-amplifying at slightly lower volumes than previous.
- Poor Amplification	Poor amplifications might occur for a number of reasons including bad injections or pipetting issues. They can generally be identified after a good quality profile followed by a poor quality profile after a re-amp. First consider a re-CE or else re-amp at the same volume. A poor amp can be used for information but may not be particularly useful as part of a STRmix™ deconvolution.

Determination of Number of Contributors	
- Single Source Profiles	Consider that single source profiles only require 12 alleles and preferably as many P+ alleles as possible to be loaded to NCIDD. Therefore a partial single source may not require reworking depending on the sample and case. If the profile is low level and falls within the stochastic range, a re-amp might be beneficial to confirm any high stutters or potentially interfering sub threshold information.
- Two Contributor Profiles	Refer to the Number of Contributor Guidelines (Morgan R and Caunt E, 2015 – Change Management #149) for reworking to determine the number of contributors to a profile. In general terms, re-amps are the most appropriate rework for reproducibility. However if both contributors are clearly present across all loci, there may be no need to rework unless the profile is within stochastic range or STRmix™ might have a better chance at deconvolution with extra runs.
- Three Contributor Profiles	Refer to the Number of Contributor Guidelines for reworking to determine the number of contributors to a profile. In general terms, re-amps are the most appropriate rework for reproducibility. If a profile is assessed as 3 contributors, a re-amp might help to assess if drop out has occurred.
- Four Contributor Profiles	Refer to the Number of Contributor Guidelines for reworking to determine the number of contributors to a profile. In general terms, re-amps are the most appropriate rework for reproducibility
- Uncertain Contributor Profiles	Refer to the Number of Contributor Guidelines for reworking to determine the number of contributors to a profile. In general terms, re-amps are the most appropriate rework for reproducibility. Two additional re-amps (if necessary) are considered appropriate.
- Complex profiles	Complex profiles should not be reworked unless it is considered that the profile is complex due to other amplification or quantification issues.
- General Mixed profiles	There is NO NEED to rework a profile unless there is good reason to do so. Consider the risks of doing so. Does the number of contributors assessed correlate with the appearance of the profile, rather than just counting the number of peaks? If not, consider a rework to see if an extra contributor might be involved or to allow STRmix™ more certainty. Remember that

	the assumption of the number of contributors to a mixed profile is the minimum number of contributors to reasonably explain the DNA profile. Note that the Number of Contributor Guidelines are GUIDELINES ONLY and interpretation can occur without added reworks.

PB143



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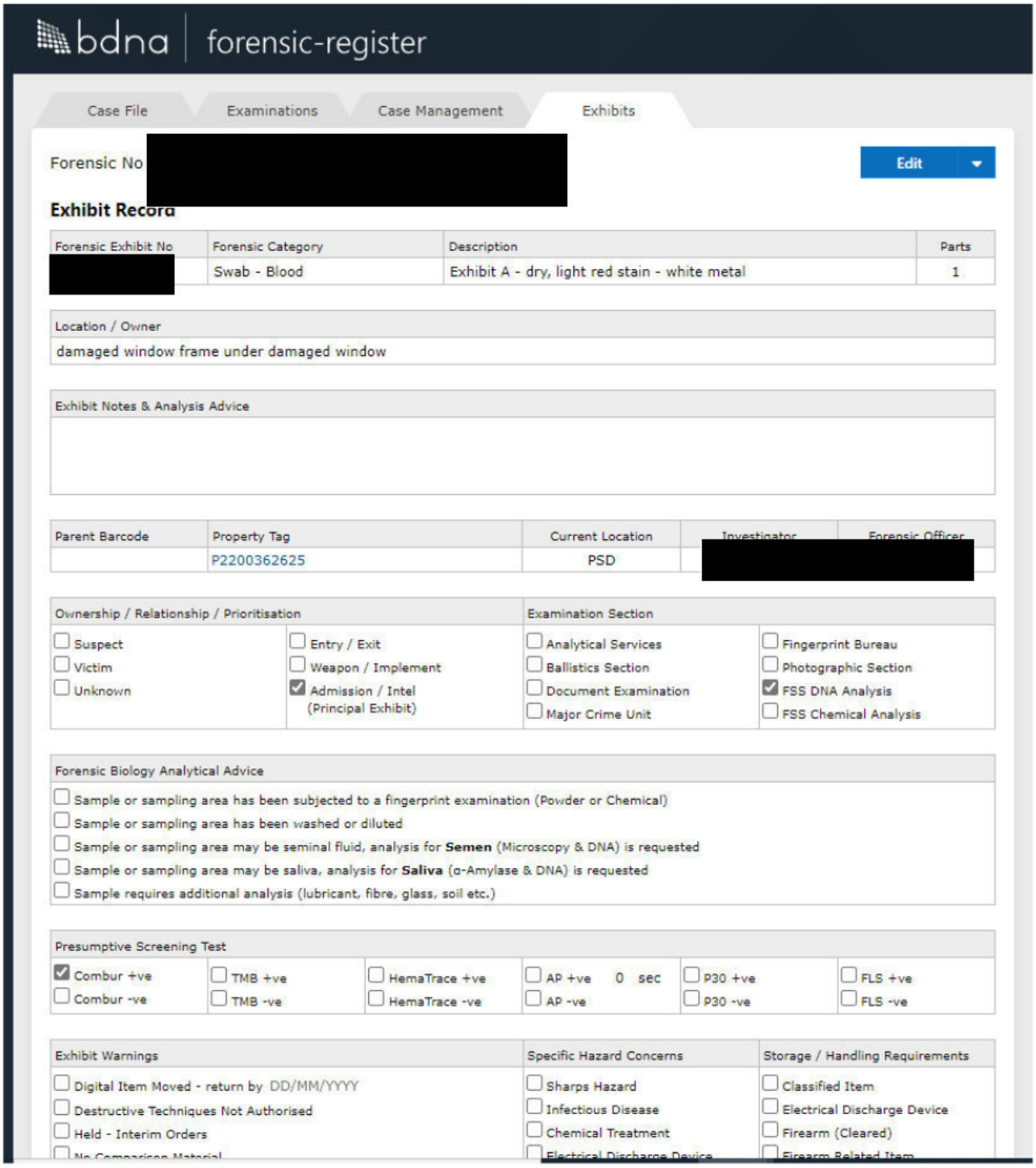
DNA Sample Analysis

Cancel

BatchID	Technique	Method	Type	Plate / Rack ID
CDNAQUA20221007-01	DNA Quantification	Quantifiler Trio	CW	

Well	SampleID	T.SA (Qty)	Priority / Analytical Note	µL	Technique	Method	Diln
A1	STD 1 (50ng/uL)	50.000000					
A2	STD 5 (0.005ng/uL)	0.005000					
A3	1097083151	0.000000	P2		STR Amplification	PowerPlex21 3500xL	
A4	1097083501	0.016618	P2		STR Amplification	PowerPlex21 3500xL	
A5	1097086038 DILN	0.613608	P2		STR Amplification	PowerPlex21 3500xL	
A6	1100347002	1.629042	P2		STR Amplification	PowerPlex21 3500xL	
A7	1099541013	0.000000	P3		No DNA Detected		
A8	1098371659	0.020753	P3		STR Amplification	PowerPlex21 3500xL	
A9	1100490447	0.032960	P3		STR Amplification	PowerPlex21 3500xL	
A10	1100615299	0.003804	P3		STR Amplification	PowerPlex21 3500xL	
A11	1097086091 DILN	0.456218	P3		STR Amplification	PowerPlex21 3500xL	
B1	STD 1 (50ng/uL)	50.000000					
B2	STD 5 (0.005ng/uL)	0.005000					
B3	1097083232	9.811348	P2 DILN DILN PP21 or VFP: 2|40 ...		Post-extraction	Dilution	DILN PP21 or VFP: 2|40 YF: 10|99
B4	1097083536	0.022154	P2		STR Amplification	PowerPlex21 3500xL	
B5	1097086044 DILN	0.620357	P2		STR Amplification	PowerPlex21 3500xL	
B6	1097078888	0.005751	P2		Post-extraction	Microcon PowerPlex 21	
B7	1100439777	0.034401	P3		STR Amplification	PowerPlex21 3500xL	
B8	1099212770	0.127308	P3		STR Amplification	PowerPlex21 3500xL	
B9	1098712750	0.453666	P3		STR Amplification	PowerPlex21 3500xL	
B10	1100578547	0.061045	P3 NWQPS 1100578551		STR Amplification	PowerPlex21 3500xL	
B11	1097086104 DILN	0.515389	P3		STR Amplification	PowerPlex21 3500xL	
C1	STD 2 (5ng/uL)	5.000000					
C2	Reagent Blank	0.000000					
C3	1097083265	0.682853	P2		STR Amplification	PowerPlex21 3500xL	
C4	1097083563	3.192884	P2		STR Amplification	PowerPlex21 3500xL	
C5	1097086055 DILN	0.484297	P2		STR Amplification	PowerPlex21 3500xL	



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Case File | Examinations | Case Management | Exhibits

Forensic No [redacted] Edit

Exhibit Record

Forensic Exhibit No	Forensic Category	Description	Parts
[redacted]	Swab - Blood	Exhibit A - dry, light red stain - white metal	1

Location / Owner: damaged window frame under damaged window

Exhibit Notes & Analysis Advice:

Parent Barcode	Property Tag	Current Location	Investigator	Forensic Officer
	P2200362625	PSD	[redacted]	

Ownership / Relationship / Prioritisation

- ☐ Suspect
- ☐ Victim
- ☐ Unknown
- ☐ Entry / Exit
- ☐ Weapon / Implement
- ☑ Admission / Intel (Principal Exhibit)

Examination Section

- ☐ Analytical Services
- ☐ Ballistics Section
- ☐ Document Examination
- ☐ Major Crime Unit
- ☐ Fingerprint Bureau
- ☐ Photographic Section
- ☑ FSS DNA Analysis
- ☐ FSS Chemical Analysis

Forensic Biology Analytical Advice

- ☐ Sample or sampling area has been subjected to a fingerprint examination (Powder or Chemical)
- ☐ Sample or sampling area has been washed or diluted
- ☐ Sample or sampling area may be seminal fluid, analysis for **Semen** (Microscopy & DNA) is requested
- ☐ Sample or sampling area may be saliva, analysis for **Saliva** (α-Amylase & DNA) is requested
- ☐ Sample requires additional analysis (lubricant, fibre, glass, soil etc.)

Presumptive Screening Test

☑ Combur +ve ☐ Combur -ve	☐ TMB +ve ☐ TMB -ve	☐ HemaTrace +ve ☐ HemaTrace -ve	☐ AP +ve 0 sec ☐ AP -ve	☐ P30 +ve ☐ P30 -ve	☐ FLS +ve ☐ FLS -ve

Exhibit Warnings

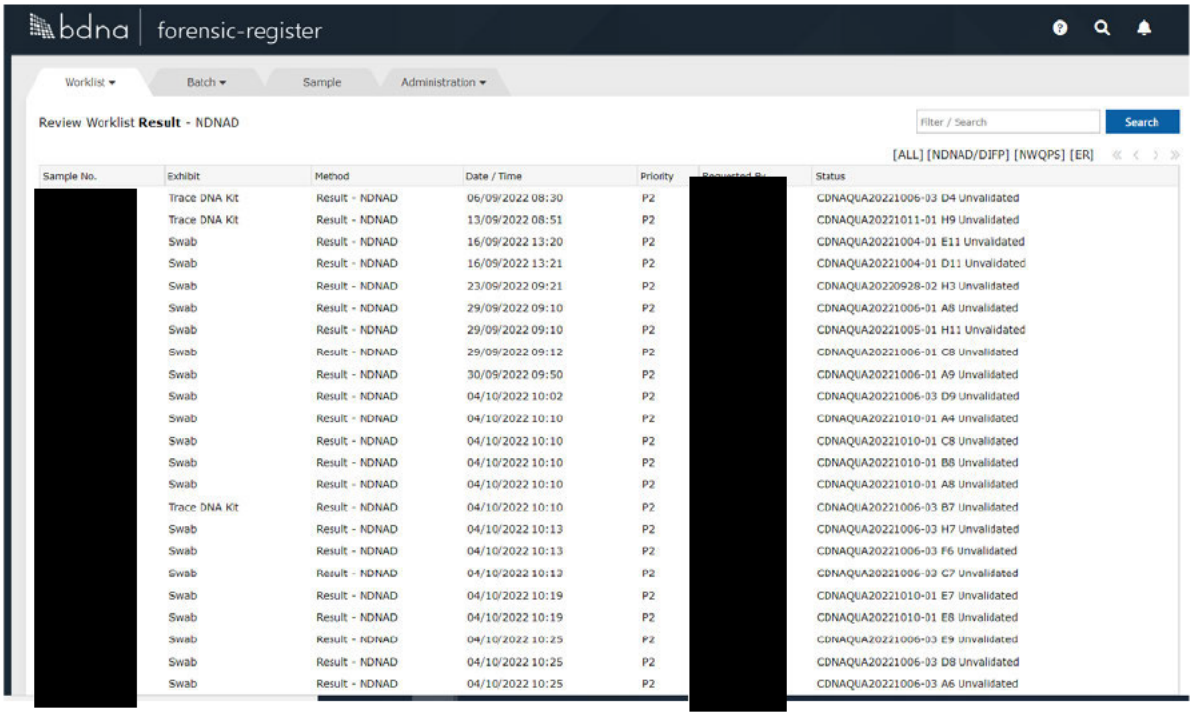
- ☐ Digital Item Moved - return by DD/MM/YYYY
- ☐ Destructive Techniques Not Authorised
- ☐ Held - Interim Orders
- ☐ No Comparison Material

Specific Hazard Concerns

- ☐ Sharps Hazard
- ☐ Infectious Disease
- ☐ Chemical Treatment
- ☐ Electrical Discharge Device

Storage / Handling Requirements

- ☐ Classified Item
- ☐ Electrical Discharge Device
- ☐ Firearm (Cleared)
- ☐ Firearm Related Item


bdna | forensic-register
Worklist
Batch
Sample
Administration
Review Worklist Result - NDNAD
Filter / Search
Search
[ALL] [NDNAD/DJFP] [NWQPS] [ER]
Sample No. | Exhibit | Method | Date / Time | Priority | [redacted] | Status
Trace DNA Kit | Result - NDNAD | 06/09/2022 08:30 | P2 | CDNAQUA20221006-03 D4 Unvalidated
Trace DNA Kit | Result - NDNAD | 13/09/2022 08:51 | P2 | CDNAQUA20221011-01 H9 Unvalidated
Swab | Result - NDNAD | 16/09/2022 13:20 | P2 | CDNAQUA20221004-01 E11 Unvalidated
Swab | Result - NDNAD | 16/09/2022 13:21 | P2 | CDNAQUA20221004-01 D11 Unvalidated
Swab | Result - NDNAD | 23/09/2022 09:21 | P2 | CDNAQUA20220928-02 H3 Unvalidated
Swab | Result - NDNAD | 29/09/2022 09:10 | P2 | CDNAQUA20221006-01 A8 Unvalidated
Swab | Result - NDNAD | 29/09/2022 09:10 | P2 | CDNAQUA20221005-01 H11 Unvalidated
Swab | Result - NDNAD | 29/09/2022 09:12 | P2 | CDNAQUA20221006-01 C8 Unvalidated
Swab | Result - NDNAD | 30/09/2022 09:50 | P2 | CDNAQUA20221006-01 A9 Unvalidated
Swab | Result - NDNAD | 04/10/2022 10:02 | P2 | CDNAQUA20221006-03 D9 Unvalidated
Swab | Result - NDNAD | 04/10/2022 10:10 | P2 | CDNAQUA20221010-01 A4 Unvalidated
Swab | Result - NDNAD | 04/10/2022 10:10 | P2 | CDNAQUA20221010-01 C8 Unvalidated
Swab | Result - NDNAD | 04/10/2022 10:10 | P2 | CDNAQUA20221010-01 B8 Unvalidated
Swab | Result - NDNAD | 04/10/2022 10:10 | P2 | CDNAQUA20221010-01 A8 Unvalidated
Trace DNA Kit | Result - NDNAD | 04/10/2022 10:10 | P2 | CDNAQUA20221006-03 B7 Unvalidated
Swab | Result - NDNAD | 04/10/2022 10:13 | P2 | CDNAQUA20221006-03 H7 Unvalidated
Swab | Result - NDNAD | 04/10/2022 10:13 | P2 | CDNAQUA20221006-03 F6 Unvalidated
Swab | Result - NDNAD | 04/10/2022 10:13 | P2 | CDNAQUA20221006-03 C7 Unvalidated
Swab | Result - NDNAD | 04/10/2022 10:19 | P2 | CDNAQUA20221010-01 E7 Unvalidated
Swab | Result - NDNAD | 04/10/2022 10:19 | P2 | CDNAQUA20221010-01 E8 Unvalidated
Swab | Result - NDNAD | 04/10/2022 10:25 | P2 | CDNAQUA20221006-03 E9 Unvalidated
Swab | Result - NDNAD | 04/10/2022 10:25 | P2 | CDNAQUA20221006-03 D8 Unvalidated
Swab | Result - NDNAD | 04/10/2022 10:25 | P2 | CDNAQUA20221006-03 A6 Unvalidated

PB144

HealthSupport
Queensland
Forensic and Scientific Services

The Examination for and of Spermatozoa

1 Purpose

The presence of spermatozoa is a confirmatory test for the presence of semen. This document describes the method by which a scientist performs microscopic examination for the presence of spermatozoa which includes the preparation of microscopic slides from exhibits, staining of slides and interpretation of the microscopic smears/slides for spermatozoa and other cellular material.

2 Scope

This Standard Operating Procedure (SOP) applies to all scientists performing the examination of items for the presence of semen.

3 Definitions

In this document, where reference is made to spermatozoa, it refers to human spermatozoa unless otherwise specified.

4 Background

The investigation of sexual assault cases may require the testing of exhibits collected as part of a forensic medical examination or scene examination for the presence of semen. Within the laboratory the detection of spermatozoa confirms the presence of semen. A reliable and accurate staining method is essential to aid the examining scientist the ability to differentiate between cellular types; most significantly spermatozoa from epithelial, yeast and white blood cells.

Currently the Haematoxylin and Eosin (H&E) stain is adopted for this process. The H&E staining method has been used for this purpose within the laboratory for many years. The haematoxylin (basic stain) stains the deoxyribonucleic-acid (DNA)/histone rich base of the spermatozoa head deep purplish-blue. The eosin (acidic stain) stains the acrosomal cap pink and the tail pink if the spermatozoa are intact (N.B. because Forensic DNA Analysis uses a water based eosin stain, the acrosomal cap often appears very light pink or clear). The use of counterstaining differentiates spermatozoa from most cellular debris.

Confusion with yeasts, especially monilia, can occur and extreme care must be taken when monilial infections such as thrush are suspected. With experience, spermatozoa and yeasts can be distinguished by size and/or the presence of cell walls.

Haematoxylin is a natural dye. Its active colouring agent is haematin, which is formed by the oxidation of haematoxylin. This oxidation process or "ripening" occurs when haematoxylin solutions are allowed to stand for several days. However, the process can be accelerated with the introduction of an oxidising agent such as sodium iodate. During

oxidation the haematoxylin loses two atoms of hydrogen, and its formula changes from $C_{16}H_{14}O_6$ to $C_{16}H_{12}O_6$. Sufficient haematoxylin should be left unoxidized in the solution so that natural oxidation can continue thus prolonging the shelf life and useability of the stain. Completely oxidized haematoxylin becomes colourless. As the oxidation process occurs when haematoxylin is exposed to light and continues over the life of the solution, haematoxylin should be stored in dark bottles until ready for use. Haematoxylin is an excellent nuclear stain. Haematin, via the aluminium ion mordant, binds to the anionic sites in the nuclei (a mordant is a substance that causes certain staining reactions to take place by forming a link between the tissue and the stain). At this stage the nuclei stain red, which is then converted to the blue-black colour when the pH is raised (by a weak alkali wash such as Scott's tap water substitute in some H&E staining methods) known as "blueing".

To avoid stain precipitation on the slide, the haematoxylin solution must be filtered. It should be changed immediately if staining quality deteriorates.

Eosin is an acid dye which combines electrostatically with the acidophilic tissue components such as cytoplasm. (an anionic dye that stains the cationic tissue components). Alcoholic and acidified solutions of eosin tend to stain much more vividly than do the aqueous solutions. With water soluble eosin, rinse in water very quickly or else eosin will wash out.

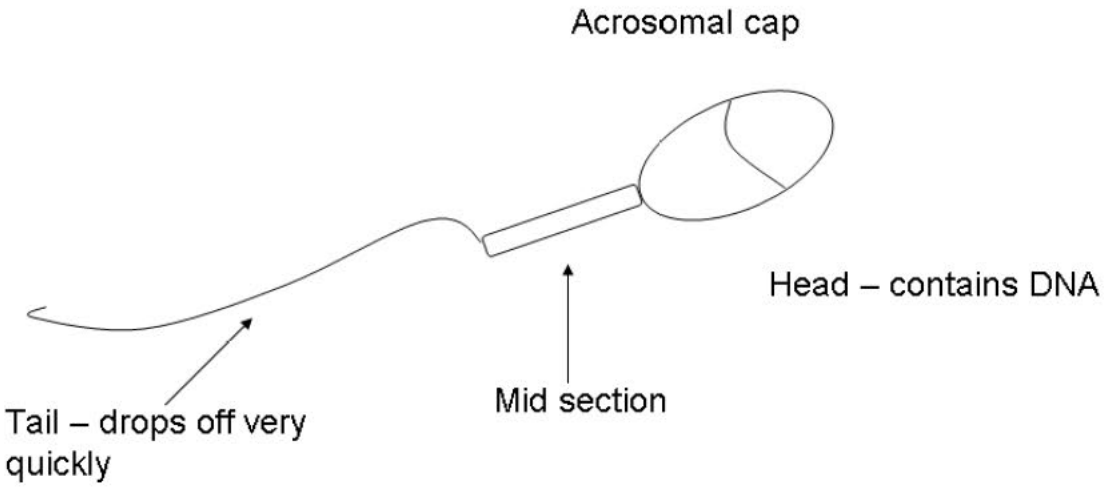


Figure 1 Spermatozoa

5 Actions- Staining procedure

5.1 Slide Preparation

Use new slides and clean with ethanol. Label with the sample ID, date, case number and sampler's initials using a pencil only. Use clean, flamed instruments.

Create a suspension from the exhibit by one of the following methods,

1. Scrape the stained area into a 1.5/2ml tube. Add between100-300µl nanopure water with a POVA pipette to the tube until the scraping is moist. Vortex thoroughly.

2. Excise the stained area and cut into small pieces. Place pieces into a 1.5/2ml tube and add between100-300µl nanopure water with a POVA pipette to the tube until the pieces are moist. Vortex thoroughly.

3. If slide is being prepared from a swab, excise the material from the swab and cut the material into small pieces. Place the pieces of material into a 1.5/2ml tube and add between100-300µl nanopure water with a POVA pipette to the tube until the pieces are covered (approx 200µl). Vortex thoroughly.

Add a drop of the recently vortexed suspension to the labelled slide.

Dry the slide on a heat block. If a heat block is not available, heat-fix the slide by passing it over a flame with the material to be stained uppermost once the suspension has dried.

5.2 Slide Staining

Microscopic slides are stained using Haematoxylin and Eosin (H&E). The method for preparing the staining reagents, and performing manual staining is as per procedure detailed in Appendix 1.

Note: registration of the reagent and association to individual exhibits is detailed in QIS 24469 (Batch Functionality in AUSLAB)

5.3 Microscopic Examination

Examine slide using the x40 or x100 objective using the oil immersion, or the x40 objective using the dry microscope. Score the number of spermatozoa observed (use the standard microscopy form, QIS 17037 (Microscopy of Smears) or the Sexual Assault Investigation Kit form, QIS 17032 (Sexual Assault Investigation Kit).

0	(0)	None seen
<+	(<1+)	Very hard to find (Use England Finder Graticule)
+	(1+)	Hard to find
++	(2+)	Easy to find
+++	(3+)	Very easy to find
++++	(4+)	Abundant

Note whether spermatozoa are intact (heads and tails) or non-intact (heads only). Look for epithelial cells and whether there are bacteria or yeast present. Human spermatozoa are distinguished from non-human mammalian sources by their morphology and by their behaviour toward H&E, resulting in a purplish/blue head and light pink/clear cap (see Section 5.4).

If less than ten spermatozoa are located, for at least one spermatozoa, note the location on the slide with the use of the England Finder Graticule (see Appendix 3).

5.4 Animal Semen

Animal spermatozoa are morphologically different to human spermatozoa and react differently to staining. Where suspected spermatozoa are located which are morphologically different to human spermatozoa, the examining scientist should consider the possible presence of animal spermatozoa. N.B. Forensic DNA Analysis does not identify or characterise animal spermatozoa.

5.5 Spermatozoa Interpretation

If slides are stained properly spermatozoa should be easily distinguished from epithelial cells, cellular debris, fibres etc. Spermatozoa heads can look similar in shape and colour to yeasts. If in any doubt consult an experienced examiner.

The recovery of semen is dependent on a number of factors but not limited to

- The amount of spermatozoa in the ejaculate
- The amount of ejaculate
- The environment the ejaculate is deposited on
- Washing
- Douching
- Menstruation
- Efficiency of the sampling process
- Time between ejaculation and sampling
- Storage of the samples
- Natural drainage or degradation of spermatozoa in certain environments

With respect to the above influences, the time since ejaculation has occurred can only be estimated. A number of studies have been conducted regarding the persistence of spermatozoa in the vagina. References to these studies can be found in Appendix 2.

6 Records

Nil

7 Quality assurance/acceptance criteria

Controls are used to test the quality and validity of the staining reagents prior to use. A positive control slide should be tested prior to the staining of slides for microscopy, (once daily), each time a new batch of Haematoxylin and Eosin solution is prepared and when positive control slides are prepared.

A positive control slide is a known sample of human semen, which has been diluted.

7.1 Creation of H&E control slides

Collect human semen in a sterile green-capped “Falcon” tube. The tube is to be labelled with the following information:

- Sperm donor number
- Date and time of collection

The semen is to be stored within a freezer until required to create H&E positive control slides using the following process:

1. Clean heating block using bleach and 70% ethanol solution.
2. Clean frosted microscope slides with ethanol and label with white label (H&E Pos Ctrl: Sperm donor number; Lot No.).
3. Spread slides out on heating block to heat before use.
4. Clean automatic pipette with bleach and 70% ethanol solution.
5. Using a new filtered pipette tip, add 20µL of the neat semen to 10.0ml nanopure water using a clean 10ml tube. Vortex.
6. Add 20 µL of the diluted semen solution to each slide.

7. Heat fix the slides on a heating block at 50ºC for approximately 30 minutes.
8. Store the slides in labelled plastic slide box "Unstained H&E Positive Control Slide Storage", and store the box in Rm 6124.

7.2 Testing and interpretation of control slides

The following process is used to test, interpret and record control slide results:

1. Remove a H&E control slide from slide box , label with date, initials, lot number and batch, and stain with H&E using the method in Appendix 1.
2. Dry slide on heating block at 50ºC.
3. Coverslip slide using mounting medium.
4. Examine slide microscopically in Rm 6119 as per section 5.3.
5. Completed control slides get transferred to a plastic box labelled H&E Control Slide Storage box #
6. Once a slide box is full of completed positive control slides, transfer the box to Rm 6106B, 'Exhibit Room' for long term storage.
7. Add audit entry to document that the control slide has passed control and what box it has been stored in (i.e. 2)

Acceptance of the reagents is based on the interpretation of the Positive control slide. The following criteria must be met before passing the reagent for use:

1. Spermatozoa head stains a deep purplish-blue.
2. Acrosomal cap stains light pink/clear.
3. Tail stains pink.

In the event the control slide fails the following process is to be completed:

1. Repeat the staining procedure with a new control slide and assess as above.
2. If the control slide fails again then discard the in-use staining batch and stain a new slide using a fresh batch from the stock solutions and assess slide as above.
3. If the control slide fails then a new batch of stock solutions must be prepared and the old solutions discarded, and assess the validity of the reagents as per this section.

8 Associated Documentation

QIS: 17037 Microscopy of Smears Form

QIS: 17142 Examination of Items

QIS: 17185 Detection of Azoospermic Semen in Casework Samples

QIS: 17186 The acid Phosphatase Screening Test for Seminal Stains

QIS: 20080 Digital Imaging in Forensic DNA Analysis

QIS: 23849 Common Forensic DNA Analysis Terms and Acronyms

QIS: 30800 Investigating Adverse Events in Forensic DNA Analysis Unit

QIS: 24469 Batch Functionality in AUSLAB

QIS: 32106 Examination of Sexual Cases

9 References

1 *Biology Methods Manual*, Metropolitan Police Forensic Science Laboratory, Great Britain, 1978.

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3 Allery, J.P., Telmon, N., Mieuset, R., Blanc, A., Rouĝe, D. (2001). "Cytological Detection of Spermatozoa: Comparison of Three Staining Methods." *Journal of Forensic Sciences* V46(2): 349-351.

4 Brown, G. (1978) "An Introduction To Histo Technology"

5 Chiasson, D.A., Vigorito, R., Lee, Y.S., Smialek, J.E. (1994). "Interpretation of postmortem vaginal acid phosphatase determinations." *American Journal of Forensic Medicine and Pathology* 15(3): 242-246.

6 Collins, K.A., Bennett, A.T. (2001). "Persistence of Spermatozoa and Prostatic Acid Phosphatase in Specimens from Deceased Individuals During Varied Postmortem Intervals." *American Journal of Forensic Medicine and Pathology* 22(3): 228-232.

7 Khaldi, N., Miras, A., Botti, K., Benali, L., Gromb, S. (2004) "Evaluation of Three Rapid Detection Methods for the Forensic Identification of Seminal Fluid in Rape Cases." *Journal of Forensic Sciences* July; 49(4):749-753.

8 Leong, A S-Y. (1996)" Principles And Practice of Medical Laboratory Science Volume 1 Basic Histotechnology"

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10 Montagna, C.P. (1996). "The recovery of seminal components and DNA from the vagina of a homicide victim 34 days postmortem." *Journal of Forensic Sciences* July 41(4): 700-702.

11 Randall, B. (1987). "Persistence of vaginal spermatozoa as assessed by routine cervicovaginal (Pap) smears." *Journal of Forensic Sciences* May 32(3): 678-683.

12 Ricci, L. R., Hoffman, S.A., (1982). "Prostatic acid phosphatase and sperm in the post-coital vagina." *Annals of Emergency Medicine* 11(10): 530-534.

13 Silverman, E. M., Silverman, A.G. (1978). "Persistence of spermatozoa in the lower genital tracts of women." *JAMA: The Journal of the American Medical Association* 240(17): 1875-1877.

14 Willott, G.M. and Allard, J.E. (1982). "Spermatozoa - their persistence after sexual intercourse." *Forensic Science International* 19(2): 135-154.
Example

10 Amendment History

Revision	Date	Author/s	Amendments
0	Unknown	Unknown	Unknown
1	Unknown	Unknown	Unknown
2	Unknown	Unknown	Unknown
3	27 Nov 2002	V Ientile	Format updated, manual staining to appendix. Removed notes on examination of swabs, removed unpublished paper, as work wasn't completed.
4	19 Nov 2003	L Freney	Updated references
5	12 Jul 2006	J Howes/A Williamson	"Reference" put after "Actions".
6	05 Aug 2006	J Howes	Added in Sexual Assault Investigation Flowcharts, examination of SAIK Swabs, Photograph or Witness required for ++ (1+) sperm and PSA test.
7	23 Oct 2006	J Howes	Reporting results Eg. ++ or 2+
8	25 Jun 2007	J Howes	Unified grading scale comments. Added Crimelite flowchart.
Version	**Date**	**Updated By**	**Amendments**
9	13 Mar 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
10	16 July 2010	A Lloyd	Removal of Crimelite in scope and the Crimelite flowchart. Changed section 2.2 to include use of suspensions. Removal of section 2.8 – Vaginal Secretions. Changes to section 2.10 to remove AP testing on smears positive to spermatozoa. Photograph or locations required for smear with 1 or 2 sperm seen. Clarification of flowchart regarding previously screened items by QPS. Changes to SAIK flowchart. Removal of animal sperm diagrams and insertion of photographs of animal sperm.
11	03 Feb 2011	A Lloyd	Amended use of vernier for slides to use of the England Finder Graticule.
12	31 Oct 2013	A Lloyd J Seymour-Murray	Removed animal sperm photos. Amended workflow charts, changed headings from CASS to HSSA. Change H&E solutions and staining, add England Finder information. Updated some hyperlinks.
13	03 July 2015	J Seymour-Murray	New template, update hyperlinks, some formatting updates and minor wording changes.

11 Appendices

11.1 Appendix 1: Preparation of H & E Stain and Manual Staining Procedure

11.1.1 Chemical Hazards

Eosin (yellowish)
WARNING: Eosin (yellowish) can cause serious damage to the eyes. Avoid contact, wear PPE and eye protection.

Haematoxylin
WARNING: Haematoxylin: the toxicological properties have not been investigated. Prevent contact with skin and eyes. Do not inhale or ingest. Wear PPE and eye protection.

Sodium iodate (NaIO3)
WARNING: Sodium iodate causes burns and is harmful if inhaled or swallowed. Protect eyes and skin.
Wear PPE and eye protection.

Glacial acetic acid
WARNING: Acetic acid is extremely corrosive and is harmful if inhaled or swallowed. Protect eyes and skin. Wear PPE and eye protection.

Pertex Mounting medium
WARNING: Pertex is irritating to eyes, respiratory system and skin.

Xylene
WARNING: Flammable. Harmful by inhalation and in contact with skin. Irritating to skin.

NOTE: All reagents prepared in the laboratory shall bear a label:

(*enter details eg* 10% NaOH)….
Prepd from Lot/batch:……..
Date: ../../.. Initials: ……..
Expires:../../.. Store at:….ºC
WARNING: Contains ……
Or an individual label printed by the Bar-One Lite system (B1Lite on short cut).

11.1.2 Preparation of Eosin

Eosin –Water soluble (CI 45380)

- Eosin 5.0g
- Nanopure water 500mL

Procedure

1. Weigh 5.0g Eosin and add 500mL nanopure water in a flask.
2. Mix on the magnetic stirrer until completely dissolved.
3. Label the reagent bottle according to laboratory standards.

11.1.3 Preparation of Haematoxylin

Haematoxylin (CI 75290)

- Haematoxylin 2.5g

- Sodium iodate (NaIO3) 0.2g (Must be accurate)
- Potassium aluminium sulphate dodecahydrate (KAl(SO4)2.12H2O (potassium alum) 25.0g
- Nanopure water 350mL
- Glycerol 150mL
- Glacial Acetic acid 10mL

Procedure

1. Weigh 2.5g Haematoxylin and dissolve in 350mL nanopure water in a flask. Mix on the magnetic stirrer. (Haematoxylin must be fully dissolved before adding the other reagents)
2. Weigh 25.0g potassium aluminium sulphate dodecahydrate and add to haematoxylin solution, continue stirring.
3. Weigh 0.2g sodium iodate and add to Haematoxylin solution, continue stirring until reagents are dissolved.
4. Measure 10mL glacial acetic acid and add to haematoxylin solution, continue stirring.
5. Measure 150mL glycerol and add to haematoxylin solution, continue stirring for 5 minutes.
6. Filter into a clean dark reagent bottle. (Haematoxylin oxidises)
7. Label the reagent bottle according to laboratory standards.

11.1.4 Register lot details of reagent

- Register lot details using QIS 24469 Batch Functionality in AUSLAB and QIS 17165 Receipt, Storage and Preparation of Chemicals, Reagents & Kits (Appendix 3).

11.1.5 Manual Staining Procedure

Staining is performed in the staining fumehood in Rm 6124, main laboratory, Forensic DNA Analysis.
Procedure:

1. Place slide on staining rack over sink, stain with haematoxylin for five minutes (add one volume and let rest)
2. Wash with nanopure water.
3. Stain with eosin for one minute.
4. Wash with nanopure water (quick wash).
5. Allow to dry on hot plate.
6. Mount coverslip in Pertex

11.1.6 Staining Quality Controls

The following quality steps should be implemented:

- Haematoxylin should be filtered before use as the crystals in solution can result in stain deposit on the slides which affects the reading of the slide. (Once a week)
- Haematoxylin differentiates better if it is matured for 3 to 4 days before use.
- Eosin should be filtered once a week.

When stored in dark bottles, haematoxylin may keep for up to 12 months and eosin for up to 12 months (dependent on control slide result).

11.2 Appendix 2: Persistence of Spermatozoa in the Vagina

Literature provides a range of time periods for the persistence of non-motile spermatozoa in the vagina:

- Up to 24 Hours[1]
- Up to 3-4 days[2]
- Up to 9 days or 12 days in the cervix, sometimes after menstruation[3]
- Up to 3 to 4 days, but may be longer[4]

Literature provides a range of time periods for the persistence of motile spermatozoa in the vagina:

- The number of motile spermatozoa discernible in the vagina may be normal after one hour and markedly decreased after 2 hours; after 3 hours normally no spermatozoa are found. Menstruation often prolongs motility in the vagina to as long as 4 hours compared with the normal period of 30 to 45 minutes.[5]
- Spermatozoa remain motile in the vagina for 2 to 3 hours and in the cervix for 48 to 110 hours[6]
- Normally 10% of the spermatozoa are alive in the vagina at the end of 2 hours post coitum. Variations in number and motility depend upon the pH of the vagina and semen, quantity of semen deposited, bacteria and flora of the vagina and the time examined post-coitally. The author has seen motile spermatozoa in the vaginal pool after 8 hours.[7]
- In several cases in which repeated examinations were possible before conception occurred, all motility ceased within one hour after intercourse. A fall of motility to 10% within 30 minutes is compatible with fecundity. On the other hand, spermatozoa may continue to move for 3 hours in a normal untreated vagina.[8]
- The motility of the spermatozoa in the specimen may give a clue to their length of stay as they remain motile from 30 to 60 minutes after deposition in the vagina.[9]

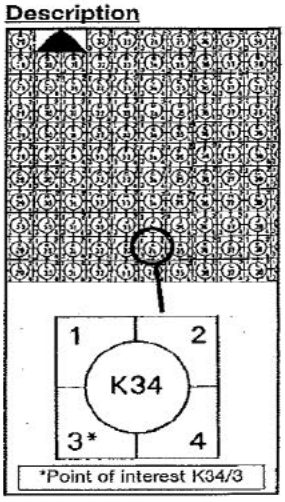
[1] O.J. Pollack. 1963 Arch. *Pathology* 35 p140-184
[2] Gordon, Turner and Price 1965 *Medical Jurisprudence*
[3] Morrison 1972 *Brit. J. Vener. Dis* 48 p141
[4]Gordon, Turner and Price 1965 Medical Jurisprudence
[5] O.J. Pollack. 1963 Arch. *Pathology* 35 p140-184
[6] Weisman 1941 *Spermatozoa and Sterility*
[7] Wm.Heinmann Medical Books Ltd 1945 *Fertility in Women*
[8] Hamish Hamilton Medical Books 1948 *Sterility and Impaired Fertility*
[9] Gonzales, Vance, Helpern and Umberger 1954 *Legal Medicine*

11.3 Appendix 3: England Finder Package Insert

The England Finder

The England Finder is a glass slide marked over the top surface in such a way that a reference position can be deduced by direct reading, the relationship between the reference pattern and the locating edges being the same in all finders. The object of the Finder is to give the microscopist an easy method of recording the position of a particular field of interest in a specimen mounted on a slide, so that the same position can be re-located using any other England Finder on any microscope.

Description



The England Finder, a section of which is illustrated, consists of a glass slide 3" x 1" marked with a square grid at 1mm intervals. Each square contains a centre ring bearing reference letter and number, the remainder of the square being subdivided into four segments numbered 1 to 4. Reference numbers run horizontally 1 to 75, and letters vertically A-Z (omitting I). The main locating edge is the bottom of the slide which is used in conjunction with either the left or right vertical edge of the slide, according to the fixed stops of the stage of the microscope, all three locating edges being marked with arrow heads. The label on the finder should always appear visually at the bottom left corner when through most microscopes the reference image will appear correct.

In the illustration (part shown), the point of interest is marked with a cross, and will be seen to lie in the third segment of the square of reference K34, hence the England Reference is K34/3.

Method of Use

1. Mark the specimen slide with a label on the left indicating with arrows which sides are to be used for location. Place the slide on the stage of the microscope bringing the bottom long edge in contact with the base stops of the stage and then sliding either left or right into contact with the vertical fixed stops as appropriate. It is important always to obtain the main location of the slide and finder on the base stops first.

2. Having examined the specimen in the normal way and found a point of interest, bring this to the centre of the field of view (a crosswire in the eyepiece is useful in this respect).

3. Taking care not to alter the position of the fixed stops of the stage, remove the slide and replace with the England Finder, again bringing the bottom edge in contact first and sliding to the appropriate vertical stop, the label of the Finder being at the bottom left corner.

4. The reference pattern of the Finder will now be seen through the microscope (adjusting the focus if necessary). The reference number of the main square is recorded followed by an oblique stroke and the number of the segment in which the centre of the field of view lies (1 to 4 or 0 if in the centre circle). The boundary lines of the main squares are easily distinguishable as these are the only continuous straight lines of the pattern.

5. The reverse procedure is adopted to re-locate the point of interest, The England Finder is placed on the stage as outlined above and the stage is adjusted until the appropriate reference square and segment appear in the centre of the field of view. Remove the finder and replace with the specimen slide with label to left and appropriate vertical slide in contact with the fixed stop, when the point of interest will appear in the centre of the field of view.

11.3.1 England Finder Graticule Use

Before use ensure stage slide holder is in correct position (slide holder should be in the correct position as it is not removed on cleaning but if not-hold with one hand push holder back to full extent against the screws, tighten screws while holding and check for correct positioning).

1. Place graticule on stage with labelled corner at LHS front and clear edge against back of slide holder

2. Using the 10x objective (and Kohler illumination) – locate co-ordinates

3. Proceed to 40x objective and adjust focus as required (using oil if applicable). Locate co-ordinates and revert back to the 10x objective.

4. Taking care not to alter the position of the fixed stops of the stage, remove the graticule and replace with the slide of interest.

5. Proceed stepwise to 40x objective (oil or dry as applicable)

6. Adjust focus and locate sperm

NB: If the stage has moved repeat from step 1.

PB145

17-02-2017 #181 PMB, EJC KDR

KDS, EJC and MOH came to my office to discuss dilutions for #181. EJC + ARM had meeting earlier - issue - Allan was not willing to budge on addition of 5ul of 1/5 semen (as done previously in initial samples that were halted and thrown) Concern was allan was not listening and ~~adamant~~ adament that 5ul was right.

Said I would talk to Allan.
Spoke to Allan (separately) and he was surprised EJC ~~was~~ had issue with 5ul, as he thought she agreed with him. He said she raised issue and he explained why it would not impact/change result, and she was okay with it. Allan showed me reasons why 5ul of 1/5 semen lep. was used - referred to in Experiment design of project #181 (SOP #25874). - made ~~up~~ ~~it~~ similar to pos diff lysis control.
Looked at pos control d/l data for QC AAA: Analytical all previous ctls, and saw generally 5-8ul of 1/30 - 1/55 dilution was used to give ~cell count of ~7000 sperm. +1-+2 min as we intended to used based on this info 5ul of 1/5 dilu, it would give (theoretically) 20000 - 80000 sperm/ul. Said I would discuss with EJC + KDR.

Called EJC in office (as she walked past) and phoned KDR to come over.
~~Showed~~ Before KDR arrived, discussed meeting

between EJC & ARM, and how Allan was surprised as thought EJC agreed with him. She said that she was frustrated, over project, ~~had~~ and did not want to argue so just left it. She said she was feeling overwhelmed by everything (#181, FR, 3500) as was not in the right frame of mind.
I showed her table of QC data, explained it, as she said 'why didn't he show me this before' and that she was happy with dilin. I said the info was generally in the exp. design and could have found the info if we went looking but was not easy to quickly ~~review~~ view. Took Kyle through data on arrival - she was happy will dilin & reasoning.

Talked about EJC being overwhelmed, and how that may have impacted on her interactions with Allan. Said she was not willing to 'argue' (but later changed it to robust discussion) with Allan as he wasn't budging. I said I appreciate how she felt, and my understanding from Allan is that she raised issue, he explained his reasoning, and she accepted. I said given all that was going on around the project, FR etc, maybe what normally would have been an okay discussion did not go well. I suggested that if she felt this way, maybe it would be best to post pone meeting, or preface it to say it was not a good day. She agreed that on a different day, she would have been happy to have robust discussion with Allan, but today was overwhelming.

DR suggested that Allan didn't need to be so immovable (:) . I reiterated that he had thought emma agreed with him. Suggested maybe if something similar occured to get me or JAH to offer opinion if weren't in agreement, instead of giving in as 'over project' and wanted to move forward as completely overwhelmed. Said both Allan & EJC frustration with project may have contributed to discussion . . . ?

Talk about sperm issues, modified process for 0 sperm, KDR concern about auditing results prior to modified process.
Shared EJC & KDR data mining (so far) from 0 sperm to diff slides. Said from my perspective, did not show major failure of process (0 → 2+ 1.1%, all of which P30 pos)
EJC & KDR both said they thought process before modification still ~~and~~ a concern, as ER staff may be more diligent now they think there is a problem with micro slide. Said from feedback from ER I believe they would not be changing their technique pre/post modification, as they believed to difference in micro was due to diff in conc. between ER to diff lysis process.

KDR raised concern re: audit of cases that may be affected by problem. We needed to go back and review and inform QPS of issue. I again asked what samples / cases were the issue. - those where sperm 0, AP/PSA -ve - NFA. EJC also said those that should have gone for diff lysis, not cells.

I said the latter weren't the issue, as did obtain DNA result and with DTT would have lytic sperm (if present). Nothing further could be done with those samples

Also said that ~~project~~ issues (errors) had not yet been identified, and until project complete and some outcome, we wouldn't know what cases may be affected. We are only at initial stages of assessment, and if any errors identified they would be addressed in the outcome/audit

EJC also said part of frustration was no direction to #181. I said that the way it was set up, in my opinion, was an initial assessment (currently agreed to with dil'n) as no further steps were planned, as we couldn't do this until initial project complete. Once we got to the next step we would determine how big / small project was, and if EJC needed to be replaced due to FR commitments. Advised to conc. on first step, not future as already overwhelmed.

EJC talked about SSOO project, and decisions not being made. KDR & I discussed that next step was baseline & BAP working on this. Also said if they felt decisions not progressing, suggested EJC, KDR meet with JAH weekly / fortnightly to discuss progress of projects

Talked briefly about new lets - Globalfiler/ ID plus, and talked about possible direction for validation - PP21 for P3, ID+ for P3, or GF for all,

Asked if everything was okay and if they wanted to discuss anything else.
They both said no. Thanked me for helping

During conversation, I encouraged them both to speak with Justin ~~raised~~ if they had any concerns about projects, workload etc.

* additional note:
During talk on project, KDK said updates from Allan could have been provided in managemet team meetings. I said, for most part, there were no updates as due to workload in ER, experiment did not progress for a while - detailed in meeting. KDK referred to tables, spreadsheets etc. I said all info was there at Experimental design sign-off. So nil updates until commencement of lab work, and then last update by me (16/02) regarding redoing the dilutions.

PB146

Paula Brisotto

From: Matthew Hunt
Sent: Thursday, 26 November 2020 3:14 PM
To: Allan McNevin; Kirsten Scott; Paula Brisotto; Justin Howes; Cathie Allen; Kylie Rika; Sharon Johnstone; Luke Ryan; Wendy Harmer
Cc: Chelsea Savage
Subject: Feedback from Project #181 -Implementation
Attachments: Project 181 Implementation FRIT.pptx

Hi,

Justin suggested I document the feedback received from the two Reporting team meetings following my presentation (attached) of an overview of Project #181 Implementation.

R1
Tom asked about whole items where an area has been found to be AP+ve, but then micro neg and p30 neg. He said we've sometimes seen samples where an AP+ and p30 neg can still give male DNA. He suggested we could quant and hold these subsamples, and if the Y-Target and the SAT are sufficient (and similar to each other) then there may be sufficient male DNA to be worth proceeding to amp/profile.

Tom also queried whether we had quantified the amount of sperm being retained on the swab heads following initial ER microscopy. After the meeting we discussed that we had not looked at that during the project, although section 6 (Part 2 of the Final Report) includes a result table showing the sperm head count, including for the swabs retained in spin baskets for the alternative ER process we were considering early on.

Jacqui expressed concern about cases with allegations of digital penetration which she has seen processed for Diff Lysis. I suggested that she make note of any future examples and then we can pass these on to ERT for feedback if the examination performed was felt to unsuitable for the case circumstances.

Adrian asked whether we had considered other methods such as adding buffered solution instead of water to help preserve and improve recovery of sperm cells. I said that this was discussed in the earlier stages of the Project, and that we read articles discussing methods used in other jurisdictions (for example Cellmark, UK who use proprietary buffers for this purpose). Project #95 concluded using buffers was not found to confer any particular advantage when using p30 kits.

R2

Josie wondered if there were particular cases in which TSI information was likely to be especially pertinent, then QPS could flag these in advance so that we could potentially process these differently, to allow for us to still be able to assess whether or not whole sperm (with tails) were present. We discussed that improving the microscopy sensitivity and obtaining an informative DNA profile result were of a higher priority to the potential for TSI information, which is not part of our standard reporting and rarely comes up in testimony.

I had mentioned that for white stains on fabric that could potentially be aspermic semen, if the high dose hook effect were suspected (due to micro neg, p30 neg) then ERT could retest a dilution for p30. Claire asked whether this would also apply to a swab of pure semen. Would this get picked up if we didn't have the stain to examine?

Neither team expressed strong opinions against dropping AP for presumptive screening (except for locating semen stains).

Thanks,

Matthew Hunt
Scientist - Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream
Forensic & Scientific Services, Health Support Queensland, Queensland Health

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PB147

Paula Brisotto

From: Paula Brisotto
Sent: Thursday, 26 November 2020 12:05 PM
To: Kylie Rika; Allan McNevin; Allison Lloyd; Cathie Allen; Justin Howes; Kirsten Scott; Luke Ryan; Sharon Johnstone
Cc: Chelsea Savage; Matthew Hunt
Subject: RE: Project #181 Implementation decision point required

Hi everyone,

Thanks Kylie for raising this. Some of my thoughts are below.

One of my concerns is storage space. We currently have storage issues, and this could become our greatest risk in the future. This is not to say we will discard anything we need to retain. It means storage does need to factor into our considerations.

My thinking on retention of the supernatants:

My understanding is that we currently do not retain these supernatants (or any supernatants) from our processes, so this would be a new process and substrate to retain. If it is considered necessary to retain supernatants for sperm detected samples, would we potentially need to do this this for all case types? If this is the case, we would need to consider how this would be managed, and by whom. This may become quite complicated.

I think if the technology makes advancements and there becomes products available in the future that could be suitable for such testing, it could be considered then, but for now I think the risk is greater for storage space and ability to manage the cases for potential retention from all sample types.

These are just my musings and some questions that popped into my head as I was thinking it through. Happy to hear other thoughts.

Thanks,
Paula

From: Kylie Rika <[redacted]
Sent: Thursday, 26 November 2020 11:26 AM
To: Allan McNevin <[redacted] Allison Lloyd <[redacted] Cathie Allen <[redacted] Justin Howes <[redacted] Kirsten Scott <[redacted] Luke Ryan <[redacted] Paula Brisotto <[redacted] Sharon Johnstone <[redacted]
Cc: Chelsea Savage <[redacted] Matthew Hunt <[redacted]
Subject: RE: Project #181 Implementation decision point required

Thanks Allan

Even if sperm were detected, it is not to say that the profile obtained is the only profile of interest in the case. This all very much depends on the case circumstances which is why I think some sort of review process on what cases might become a cold case would help us in preserving potentially useful material.

I agree with you that spin baskets are probably a better source than SN's but in the cold cases where we have exhausted even the spin basket option, we could look at the SN – who knows what technology and applications (not to mention increased sensitivity) there will be in the future.

Thanks
Kylie

From: Allan McNevin <[redacted]
Sent: Thursday, 26 November 2020 10:23 AM
To: Kylie Rika <[redacted] Allison Lloyd <[redacted] Cathie Allen <[redacted] Justin Howes <[redacted] Kirsten Scott <[redacted] Luke Ryan <[redacted] Paula Brisotto <[redacted] Sharon Johnstone <[redacted]
Cc: Chelsea Savage <[redacted] Matthew Hunt <[redacted]
Subject: RE: Project #181 Implementation decision point required

Hi all,

The supernatants that we propose to discard are from those samples where spermatozoa were detected microscopically, for all samples where there were no sperm seen, the supernatant will be consumed in testing. Spin baskets would be a better source of secondary material if further testing on was to be considered down the track.

Cheers
Al

Allan McNevin
Senior Scientist – Evidence Recovery

Evidence Recovery Team, Forensic DNA Analysis
Forensic & Scientific Services, Health Support Queensland, Queensland Health

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From: Kylie Rika <[redacted]
Sent: Thursday, 26 November 2020 9:22 AM
To: Allan McNevin <[redacted] Allison Lloyd <[redacted] Cathie Allen <[redacted] Justin Howes <[redacted] Kirsten Scott <[redacted] Luke Ryan <[redacted] Paula Brisotto <[redacted] Sharon Johnstone <[redacted]
Cc: Chelsea Savage <[redacted] Matthew Hunt <[redacted]
Subject: RE: Project #181 Implementation decision point required

Hi all and thanks Allan,

I am really sorry I forgot to vote – I remember at the time reading the information and thinking that I need to think on this so will come back to it. Apologies again for missing the deadline.

I would just like to raise a concern I have of not keeping the supernatants indefinitely.

At first thought, it would seem that there would be little chance of the supernatants containing anything useful for the future (for cold cases). However, unless we have done testing or have information from journal articles demonstrating that the supernatants don't contain trace amounts of nDNA, mRNA, mtDNA etc… then there exists a risk that we could be throwing something valuable away.

Often with cold cases we are asked if there is anything at all we can go back to. Recently, we got a really useful profile from a completely empty DNA extract tube – 10 or 20 years ago who would have anticipated that?!

We don't know what we don't know yet, so perhaps we could have some kind of review process for cases we think may turn into cold cases and for those ones, we could keep absolutely everything? This would be in line with Allan's project of trying to get sperm off cover slipped slides etc..

I hope that my late thoughts can be considered.

Thanks
Kylie

From: Allan McNevin <[redacted]
Sent: Thursday, 26 November 2020 8:11 AM
To: Allison Lloyd <[redacted] Cathie Allen <[redacted] Justin Howes <[redacted] Kirsten Scott <[redacted] Kylie Rika <[redacted] Luke Ryan <[redacted] Paula Brisotto <[redacted] Sharon Johnstone <[redacted]
Cc: Chelsea Savage <[redacted] Matthew Hunt <[redacted]
Subject: RE: Project #181 Implementation decision point required

Hi all,

I have received votes from almost everyone

So far the votes are as follows:

Retain for three months: 5 votes
Retain for 12 months: 1 vote
Don't retain at all: 1 vote
All other options received no votes

3 months was the time frame the project team were considering most appropriate as well

Given that we have a clear majority for 3 months, that is the process we will implement from Monday

Cheers
Al

Allan McNevin
Senior Scientist – Evidence Recovery

Evidence Recovery Team, Forensic DNA Analysis
Forensic & Scientific Services, Health Support Queensland, Queensland Health

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From: Allan McNevin <[redacted]
Sent: Wednesday, 18 November 2020 8:14 AM
To: Allan McNevin <[redacted] Allison Lloyd <[redacted] Cathie Allen <[redacted] Justin Howes <[redacted] Kirsten Scott <[redacted] Kylie Rika <[redacted] Luke Ryan <[redacted] Paula Brisotto <[redacted] Sharon Johnstone <[redacted]
Cc: Chelsea Savage <[redacted] Matthew Hunt <[redacted]
Subject: Project #181 Implementation decision point required

Hi all,

Looking back through the workflow notes we had made, we have realised that a decision needs to be made regarding one element of the new process. This decision is not critical prior to go live, however it would be neater if I had answer before implementation.

As a reminder here is a simple overview of the new process:

Exhibit for semen testing is sampled into a tube in ER
Nanopure water added, mixed and incubated, then some supernatant is removed and stored frozen for possible later testing
Exhibit submitted to Analytical – Diff Lysis extraction
Post extraction EFRACs are processed to quant etc. or held as per current processes
Post extraction, SFRACs are held, microscopy on diff slide performed

- Spermatozoa detected – process to quant etc.
- No sperm seen, p30 is performed on the retained supernatant from above
    - p30 pos – process to quant etc..
    - p30 neg – sample is considered semen neg, processing halted

The decision point required.

For all of the samples where spermatozoa are detected microscopically, no testing will be performed on the supernatant that was retained by ER at the start of the process.

So, how long should we retain those supernatants (remembering they will be spermatozoa detected microscopically)?

(Note: it is expected that we could be producing upwards of 1,000 supernatants that do not require testing each year, so could become a storage burden relatively quickly)

Please use the voting options and vote by COB Wed next week (25/11); if choosing “other” please also reply-all with your suggestion

Thanks for your attention
Cheers
Al

Allan McNevin
Senior Scientist – Evidence Recovery

Evidence Recovery Team, Forensic DNA Analysis
Forensic & Scientific Services, Health Support Queensland, Queensland Health

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PB148

Paula Brisotto

From: Luke Ryan
Sent: Monday, 23 November 2020 3:37 PM
To: Adam Kaity; Alanna Darmanin; Amy Cheng; Belinda Andersen; Biljana Micic; Generosa Lundie; Lai-Wan Le; Lisa Farrelly; Maria Aguilera; Megan Mathieson; Melissa Cipollone; Nicole Roselt; Pierre Acedo; Sharelle Nydam; Tara Prowse; Tegan Dwyer
Cc: Kirsten Scott; Allan McNevin; Chelsea Savage; Paula Brisotto
Subject: Project 181 Implementation

Afternoon All

Project 181 is being implemented on Monday 30th November. This main outcome of this project was to move the presumptive screening tests ER perform to after the DNA Extraction (DLYS) has been performed. This will limit sample consumption during presumptive testing as only one slide will be made – the Analytical DLYS slide (and ER will only be reading one slide per sample).

ER will make and retain a supernatant for all DLYS samples and submit for extraction. Analytical will do the DLYS extraction and make the DLYS slide as normal which will be transferred to ER for reading. ER will do the following:

- Slide Sperm pos – ER add to Quant WL (no P30 test required)
- Slide Sperm Neg – P30 pos – ER add to Quant WL
- Slide Sperm neg and P30 neg - NFA

The change for Analytical relates only to transition of SFRACs at the end of the DLYS extraction. Please blank out all SFRACs so they do not transition to Quant. ER will be responsible for ordering Quants on samples once they have completed their presumptive testing as above. There is no change to how the EFRACs are processed. The only exception to this rule is for P1s which will progress through to quant after the DLYS. An FR enhancement has been logged to automate this process in the future (based on ER's validated presumptive results).

Allan has kindly created three sturdy plastic slide boxes (see below). Please track your DLYS slides into these boxes at completion of your DLYS. These boxes will be used to transfer slides to ER and then they will be returned empty.

To facilitate the smooth transition, on Friday afternoon please add all DLYS samples on the extraction WL to an extraction batch. This means that all samples added to the list on Monday 30th morning will be processed according to the new protocol. I appreciate this means we will have a partial DLYS batch, but this is unavoidable.

Any questions please come and see me.

Thanks

Luke

Luke Ryan
Senior Scientist – Analytical Team

Forensic DNA Analysis, Forensic and Scientific Services
Health Support Queensland, Queensland Health

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PB149

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PowerPlex®21 – Amplification of Extracted DNA Validation

Megan Mathieson, Thomas Nurthen & Cathie Allen

DNA Analysis, Forensic & Scientific Services

December 2012

Queensland Government

Document details

Contact for enquiries and proposed changes

If you have any questions regarding this document or if you have a suggestion for improvements, please contact:

Contact officer: Thomas Nurthen

Title: Senior Scientist Quality & Projects

Phone: [redacted]

Email:

Version history

Version	Date	Changed by	Description
1.0	14/12/2012		1st Issue

Document sign off

This document has been **approved** by:

Name	Position	Signature	Date
Cathie Allen	Managing scientist	[redacted]	17.12.12.

Approval provided verbally on 6.12.12.

This document has been **endorsed** by:

Name	Position	Signature	Date
Justin Howes	Team Leader FRIT	[redacted]	14.12.12
Paula Brisotto	Team Leader ER&Q		14-12-12
Sharon Johstone	Senior Scientist Intell Team		14/12/12
Amanda Reeves	Senior Scientist Reporting 1		14/12/12

Name	Position	Signature	Date
Emma Caunt	A/Senior Scientist Reporting 2	[redacted]	14/12/12

Name	Position	Signature	Date
Adrian Pippia	A/Senior Scientist ER	[redacted]	14-12-12

Name	Position	Signature	Date
Allan McNevin	Senior Scientist Analytical	[redacted]	14/12/2012

Name	Position	Signature	Date
Thomas Nurthen	Senior Scientist Q & P	[redacted]	17/12/2012

Contents

1 Abstract

To meet Queensland legislative requirements and core business needs, DNA Analysis has validated the PowerPlex® 21 system DNA profiling Kit. All Australian jurisdictions are expected to implement a new DNA profiling kit by the end of 2012. This project came about through the Australian and New Zealand Policing Advisory Agency (ANZPAA).

The loci within the AmpFℓSTR® Profiler Plus® and AmpFℓSTR® COfiler® kits, which are currently used in DNA Analysis, are represented within the PowerPlex® 21 system loci. This allows concordance of the kit for direct comparison and matching against existing AmpFℓSTR® Profiler Plus® crime scene and reference DNA profiles.

This validation has demonstrated that the PowerPlex® 21 system kit is fit for purpose for the amplification of extracted DNA samples processed in the DNA Analysis Unit. A limit of reporting threshold of 40RFU will be adopted for analysis of extracted DNA samples amplified at either 25µL or 12.5µL total PCR volumes.

The sensitivity of this next generation STR kit has greatly increased, however the increased sensitivity does not necessarily result in increased information. The results of this validation indicates that Promega's PowerPlex® 21 system is a very sensitive STR amplification kit, but to reduce the risk of type 2 errors (calling a heterozygous locus homozygous[1]) consideration needs to be given to restricting the range of DNA template added. Single source samples with DNA templates of greater than 0.5ng overload the PowerPlex® 21 system resulting in DNA profiles being unable to be interpreted. Generally samples with lower templates (reaching the often termed 'low copy number' level of 100-150pg) tend to exhibit enhanced stochastic effects as one would expect. Therefore, it should be considered whether samples around this input template level should be amplified given that interpretation of the results could be unwieldy. It would be possible to increase the template levels of samples that fall into this category by post extraction concentration or increase the total PCR volume.

At a total DNA input template of 0.5ng, for 25µL and 12.5µL total PCR volumes, all alleles were detected for the mixtures with ratios of 1:1, 2:1 and 5:1.

The results from this validation support that the Promega PowerPlex®21 System is suitable for analysis of short tandem repeats (STR).

2 Introduction

To meet Queensland legislative requirements and core business needs, DNA Analysis has validated the PowerPlex® 21 system DNA profiling Kit. All Australian jurisdictions are expected to implement a new DNA profiling kit by the end of 2012. This expectation has been directed by ANZPAA, which comprises a Police Commissioner from each jurisdiction.

The initial plan endorsed by the members of the Biological Specialist Advisory Group (BSAG) involved a series of experiments designed to enable each jurisdiction to choose an appropriate STR amplification kit but using the same methodology (national approach to STR kit validation)[2].

This plan included:

1. Sensitivity and amplification volume determination
2. Population studies
3. Concordance
4. Mixture studies
5. Baseline determinations, peak balance, stutter thresholds, minimum reporting threshold and probability of drop in. This last series of experiments were devised by the Statistics Scientific Working Group (StatSWG)[3].

The plans created by BSAG and StatSWG are a significant development with respect to STR validation and interpretation within Australia. In line with current research, these plans involve the move away from a binary approach to DNA profile interpretation to a continuous model. To achieve this, a new DNA profile interpretation software (STRmix™) has been developed by forensic DNA experts & statisticians from Australia and New Zealand forensic laboratories. The validation of the STRmix™ software will be covered in the STRmix™ validation document to be issued subsequent to this report.

The PowerPlex® 21 system[4] is a new short tandem repeat (STR) kit made available to the Australian forensic laboratories in early 2012. The kit has all of the nine loci amplified in AmpFℓSTR® Profiler Plus®[5] and the six loci amplified in AmpFℓSTR® COfiler®[6] and an additional seven loci. See Table 1 for kit loci.

Table 1 - Comparison of loci in three different kits

(dye colour indicated by colour text)

PowerPlex® 21 System	AmpFℓSTR® Profiler Plus®	AmpFℓSTR® COfiler®
AMEL	AMEL	AMEL
D3S1358	D3S1358	D3S1358
D1S1656		
D6S1043		
D13S317	D13S317	
Penta E		
D16S539		D16S539
D18S51	D18S51	
D2S1338		
CSF1PO		CSF1PO
Penta D		
TH01		TH01
vWA	vWA	
D21S11	D21S11	
D7S820	D7S820	D7S820
D5S818	D5S818	
TPOX		TPOX
D8S1179	D8S1179	
D12S391		
D19S433		
FGA	FGA	

The scope of this validation is to determine for the PowerPlex® 21 system, the limit of detection (LOD), limit of reporting (LOR), the optimal total PCR amplification volume, the range of DNA template, ensure concordance of the PowerPlex® 21 system against the AmpFℓSTR® Profiler Plus® and COfiler® kits, observe the performance of mixed DNA samples and create population datasets required for statistical calculations. Secondary to this, this validation provides the data necessary for STRmix™ validation.

3 Materials

The following materials were used within this validation:

- BSD Duet 600 Series II (BSD Robotics, Brisbane, QLD,AU)
- STORstar instrument (Process Analysis & Automation, Hampshire, GB)
- MultiPROBE II PLUS HT EX with Gripper Integration Platform (PerkinElmer, Downers Grove, IL, US)
- Sterile conductive filtered Roborack 25µL disposable tips (PerkinElmer, Downers Grove, IL, USA)
- 5804 centrifuge (Eppendorf AG, Hamburg, DE)
- 5424 centrifuge (Eppendorf AG, Hamburg, DE)
- Thermomixer (Eppendorf AG, Hamburg, DE)
- MixMate (Eppendorf AG, Hamburg, DE)

- Vortex (Ratek Instruments Pty Ltd, Melbourne, VIC, AU)
- Micro centrifuge (Tomy, Tokyo, JP)
- 1.5mL screw-cap tubes (Axygen Inc. Union City, CA, US)
- Pipettes (Eppendorf, Hamburg, DE and Thermo Fisher Scientific(Finnpipette), Waltham, MA, US)
- Pipette tips (VWR International LLC Radnor, PA, US and Molecular Bioproducts Inc., San Diego, CA, US)
- 96-well PCR plates(Axygen Inc. Union City, CA, US)
- 2.0mL sterile screw-cap tubes (Axygen Inc. Union City, CA, US)
- Plate septas (Axygen Inc. Union City, CA, US)
- Adhesive film (QIAGEN, Hilden, DE)
- FTA™ collection kits (Whatman™ GE Healthcare, Buckinghamshire, GB)
- Positive controls (DNA Analysis Unit, Brisbane, QLD, AU)
- TNE (DNA Analysis Unit, Brisbane, QLD, AU)
- Proteinase K (20mg/mL) (Sigma-Aldrich® Corporation, St Louis, MO, US)
- Dithiothreitol (Sigma-Aldrich® Corporation, St Louis, MO, US)
- Trigene (Medichem International, Kent, GB)
- Ethanol (Recochem Incorporated, Wynnum, QLD,AU)
- Bleach (Ionics Australasia Pty Ltd., Lytton, QLD, AU)
- Amphyl (Rickitt Benckiser Inc. Parsippany, NJ, US)
- Sarcosyl (Sigma-Aldrich® Corporation, St Louis, MO, US)
- Nanopure water (DNA Analysis Unit, Brisbane, QLD, AU)
- Quantifiler™ Human DNA Quantification kits (Life Technologies Applied Biosystems, Foster City, CA, US)
- AB 7500 Real Time PCR System (Life Technologies Applied Biosystems, Foster City, CA, US)
- GeneAmp® PCR system 9700 (Life Technologies Applied Biosystems, Foster City, CA, US)
- ABI 3130*xl* Genetic Analyzer (Life Technologies Applied Biosystems, Foster City, CA, US)
- Hi-Di™ Formamide (Life Technologies Applied Biosystems, Foster City, CA, US)
- 3130 POP-4™ Polymer (Life Technologies Applied Biosystems, Foster City, CA, US)
- Running Buffer (Life Technologies Applied Biosystems, Foster City, CA, US)

- DNA IQ™ Casework Pro Kit for Maxwell® 16 (Promega Corp., Madison, WI, US)
- Promega PowerPlex® 21 system (Promega Corp., Madison, WI, US)
- Promega CC5 Internal Lane Standard 500 (Promega Corp., Madison, WI, US)
- Promega PowerPlex 5 Dye Matrix Standard (Promega Corp., Madison, WI, US)
- Promega PowerPlex® 21 Allelic Ladder Mix (Promega Corp., Madison, WI, US)
- 2800M Control DNA, 10ng/µl (Promega Corp., Madison, WI, US)

Water amplification grade (Promega Corp., Madison, WI, US)

4 Methods

4.1 Sample Selection

All samples used in this validation were sourced from the internal DNA Analysis staff DNA database, Collaborative Testing Services (CTS) DNA testing samples, or reference samples that had the National Criminal Investigation DNA Database (NCIDD) categories of Volunteer Unlimited Purpose (VUP) or Suspect (SCT). Permission to use reference samples from NCIDD was obtained from the Queensland Police Service (QPS).

4.2 Selection of Sub-Population Samples

4.2.1 Aboriginal and Torres Strait Islanders Sub-Populations

Aboriginal samples:

Aboriginal samples previously profiled as part of the sub-population dataset for the validation of AmpFℓSTR® Profiler Plus® loci were recommended as the best samples to use for compilation of the Aboriginal sub-population dataset for the Promega PowerPlex®21 system. The samples are self-declared Aboriginal ethnicity and were collected over a number of years.

220 Aboriginal samples were randomly selected from the Aboriginal dataset (545 total) previously profiled with AmpFℓSTR® Profiler Plus®. Microsoft Excel RANDBETWEEN function was used and duplicates removed until 220 unique samples were identified for profiling.

These 220 samples were originally extracted using Chelex. The extracts for the 220 samples were viewed for sufficient volume. 201 samples with sufficient volume were identified and given new population dataset barcodes.

Torres Straits Islander samples:

A list of FTA™ samples previously profiled with AmpFℓSTR® Profiler Plus® resulting in a full profile and identified as self-declared Torres Strait Islander ethnicity in AUSLAB were compiled to be used for the Aboriginal sub population dataset.

599 samples were listed and after further filtering, including removing duplicates, 249 Torres Strait Islander samples remained. Of the 249 Torres Strait Islander samples listed 223 samples were randomly selected for processing. Samples were given new population dataset barcodes

4.2.2 Caucasian Sub-Population

A list of FTA™ samples previously profiled with AmpFℓSTR® Profiler Plus® resulting in a full profile and identified as Caucasian ethnicity in AUSLAB were compiled to be used for the Caucasian sub-population dataset.

From this list 210 samples were selected and 208 were selected for processing as two were deemed unsuitable. Samples were given new population database barcodes.

4.2.3 South East Asian Sub-Population

A list of FTA™ samples previously profiled with AmpFℓSTR® Profiler Plus® resulting in a full profile and identified as South East Asian ethnicity in AUSLAB were compiled to be used for the South East Asian population dataset.

157 samples were listed and after further filtering 141 South East Asian samples remained. These 141 samples were given new population database barcodes.

4.3 Collection Procedure for FTA™ Cards

Where staff samples were entirely consumed during processing, additional samples were collected. New FTA™ samples were collected using FTA™ Collection kits. A foam swab was used to collect buccal cells from each cheek for one minute then applied to the FTA™ card[7]. The FTA™ card was stored at room temperature until required.

4.4 FTA™ Punching Method

1. PCR Amplification mix was created as required.
2. 25µL (full) or 12.5µL (half) of PCR amplification mix was added to a clean 0.2mL 96 well PCR plate.
3. Plate was sealed and centrifuged to ensure PCR amplification mix was at the bottom of the wells.

4. Each FTA™ sample was punched with the 1.2mm diameter die into the 96 well PCR plate using the BSD Duet 600 Series II.
5. 1µL of 2800M control DNA was added to the Positive control well.
6. 1 x 1.2mm punch of a blank FTA™ card was added to the blank control well
7. Amplification mix without FTA™ card was used as a negative control.
8. The plate was sealed and centrifuged briefly to pull the FTA™ cards to the bottom of the plate wells.

4.5 FTA® Punching Method 2

1. 7.5µL of amplification grade water was added to the required wells.
2. Plate was sealed and centrifuged to ensure the water was at the bottom of the wells.
3. Each FTA® sample was punched with the 1.2mm diameter die into the 96 well PCR plate using the BSD Duet 600 Series II.
4. 1µL of 2800M control DNA was added to the Positive control well.
5. 1 x 1.2mm punch of a blank FTA® card was added to the blank control well
6. PCR Amplification mix without FTA® card was used as a negative control.
7. PCR Amplification mix was created as required and 5µL added to each well.
8. The plate was sealed and centrifuged briefly to pull the FTA® cards to the bottom of the plate wells.

4.6 Punching for Extraction

FTA™ samples were prepared for extraction by punching four paper spots of 3.2mm diameter into 1.5mL/2mL tubes using the BSD Duet 600 according to standard operating procedure 24823 V4.0 "FTA™ Processing and Work Instructions".

4.7 Extraction

FTA™ samples requiring DNA extraction were processed using the DNA IQ™ Casework Pro Kit for Maxwell®16 according to standard operating procedure 29344 V4.0 "DNA IQ™ Extraction using the Maxwell®16".

4.8 Preparation of DNA Stock Solutions

Samples used to make dilution series required a stock solution to be prepared. FTA™ samples were selected and punched in duplicate for

extraction (as outlined in section 4.6) then extracted (as outlined in section 4.7). The duplicate samples were pooled into a single tube and quantified twice (as outlined in section 4.9).

4.9 Procedure for Creating a Dilution Series

The samples used to make dilution series were diluted with amplification grade water provided with the Promega PowerPlex®21 System. Spreadsheets for calculating the normalisation and dilution series were written to outline the serial dilutions required to obtain the specified concentrations

4.10 Quantification

All preparations of reactions were performed using MultiPROBE II plus HT EX platform according to standard operating procedure 19977 V8.0 "Automated Quantification of Extracted DNA using the Quantifiler™ Human DNA Quantitation Kit".

4.11 Amplification Set up

For the experiments that used extracted DNA, all amplification reactions were performed using a MultiPROBE II plus HT EX platform. A new protocol called PowerPlex 21 amp setup v1.0 was created using WinPrep® software and utilised for amplifications at 25µL and 12.5µL total PCR volumes. The protocol is saved and stored on the C drive of the MultiPROBE II plus HT EX platform computer. Table 2 outlines the components of the amplification mix per sample.

Table 2 - Amplification mix per sample.

Kit components	Volumes (µL)	Volumes (µL)
Master Mix	5.0	2.5
Primer pair	5.0	2.5
Sample	15	7.5
Total Volume	25	12.5

4.12 Amplification Conditions

Table 3 lists the PCR cycling conditions used in this validation. All PCR reactions were carried out in 96 well plates (Axygen Inc.) on GeneAmp® 9700 thermal cyclers

Table 3 - PCR cycling conditions used for PowerPlex®21 system

PowerPlex® 21 Kit	Direct amp	Standard
GeneAmp 9700 mode	Max	Max
	25,26 or 27 cycles	30 cycles
Activation	96°C for 1 minute	96°C for 1 minute
Cycling	94°C for 10 seconds 59°C for 1 minute 72°C for 30 seconds	94°C for 10 seconds 59°C for 1 minute 72°C for 30 seconds
Extension	60°C for 20 minutes	60°C for 10 minutes
	4°C Soak	4°C Soak

4.13 DNA Fragment Analysis

The plates for DNA fragment analysis were prepared as recommended by the manufacturer, using a combination of Hi-Di™ formamide, size standard and sample as outlined below.

Formamide: size standard mixture composed of

[(2.0µl CC5 ILS 500) x (number of injections)] + [(10.0µl Hi-Di™ formamide) x (number of injections)]

Formamide: size standard mixture **12µL**

PCR product or allelic ladder **1µL**

The prepared plate was then centrifuged to remove bubbles, denatured at 95°C for 3 minutes then chilled in an ice block in the freezer for 3 minutes. The prepared plates were then run on a 3130*xl* Genetic Analyzer.

The PCR fragments were separated by capillary electrophoresis (CE) using a 3130*xl* Genetic Analyzer set up according to manufacturer recommendations outlined in Table 4.

Table 4 - CE Protocol conditions.

Injection time	Injection voltage	Run time
5s	3kV	1500s

4.14 Profile Interpretation 1

All DNA profiles were analysed with GeneMapper® ID-X v1.1.1. The analysis panel used was PowerPlex_21_IDX_v1.0. The thresholds were set as follows:

1. Heterozygote threshold was set at 40RFU
2. Limit of Detection (negative controls) was set at 16RFU
3. Individual locus stutter thresholds were set as per Promega PowerPlex® 21 Stutter filter
4. Homozygote threshold was set to 200RFU

4.15 Profile Interpretation 2

All DNA profiles were analysed with GeneMapper® ID-X v1.1.1. The analysis panel used was PowerPlex_21_IDX_v1.0. The rules were set as follows:

1. Samples were analysed at 1RFU.
2. All known alleles, forward and back stutter (+/-4bp or +/-5bp) of known alleles, known artefacts and spectral pull-up were removed. As defined by Promega artefact peaks in the N-2bp and/or N+2bp position at D1S1656, D6S1043, D13S317, vWA, D21S11, D7S820, D5S818, D12S391 and D18S51 loci and in the N-1bp position at Amelogenin were also removed.
3. Any peaks determined to be carry over peaks were also removed. Carry-over is defined as the physical transfer of DNA from one injection to the next.

4.16 Profile Interpretation 3

All samples were analysed with GeneMapper ID-X v1.1.1 with the stutter thresholds set to zero. The analysis panel used was PowerPlex_21_IDX_v1.1.

1. Samples were analysed at 20RFU
2. Loci where the two main alleles were one repeat apart were excluded from analysis.

5 Experimental Design

5.1 Sub-Population Datasets

As part of the national approach to implementation of next generation STR amplification kits, the creation of three national sub-population datasets was undertaken. Each jurisdiction contributed DNA profiles for each sub-population Caucasian, Aboriginal and South East Asian to Jo-Anne Bright (ESR) and John Buckleton (ESR) for analysis.

5.1.1 Aboriginal dataset

In this experiment 201 Aboriginal samples were transferred to appropriate tubes and the DNA concentrations determined as outlined in Method 4.10.

The samples were amplified with the recommended DNA template input of 0.5ng in a 25µL total PCR volume. Three plates were amplified using the PowerPlex®21 system kit with each plate including a positive amplification control (2800M DNA) and a negative amplification control (amplification grade water). The three plates were prepared as per Method 4.11.

Standard amplification cycling conditions, DNA fragment analysis and profile interpretation was followed as outlined in Methods 4.12, 4.13 and 4.14.

5.1.2 Torres Strait Islander dataset

In this experiment 223 Torres Strait Islander samples were punched across three 96 well plates as outlined in section 4.4. Each sample had one spot punched, a total PCR volume of 12.5µL and was directly amplified at 26 PCR cycles.

Amplification cycling conditions, DNA fragment analysis and profile interpretation was followed as outlined in Methods 4.12, 4.13 and 4.14.

5.1.3 Caucasian dataset

In this experiment 208 Caucasian samples were punched across three 96 well plates as outlined in section 4.4. Each sample had two spots punched, a total PCR volume of 25µL and was directly amplified at 25 PCR cycles.

Caucasian samples that did not produce a full PowerPlex®21 profile were punched again using 2 spots, a total PCR volume of 25µL and was directly amplified at 26 PCR cycles.

Amplification cycling conditions, DNA fragment analysis and profile interpretation was followed as outlined in Methods 4.12, 4.13 and 4.14.

5.1.4 South East Asian dataset

In this experiment 141 South East Asian samples were punched across two 96 well plates as outlined in section 4.5. Each sample had one spot punched, a total PCR volume of 12.5µL and was directly amplified at 26 PCR cycles.

South East Asian samples that did not produce a full PowerPlex®21 profile were punched for extraction, extracted, quantified and amplified as outlined in Methods 4.6, 4.7, 4.8 and 4.10.

Amplification cycling conditions, DNA fragment analysis and profile interpretation was followed as outlined in Methods 4.12, 4.13 and 4.14.

5.2 Concordance

155 samples purchased from Collaborative Testing Services (CTS) as external Proficiency Tests were used to test the concordance of the PowerPlex® 21 system. These samples had previously been extracted, quantified and amplified with AmpFℓSTR® Profiler Plus® and AmpFℓSTR® COfiler® kits.

The samples were amplified with the recommended DNA template input of 0.5ng in a 12.5µL total PCR volume. Two plates were amplified using the PowerPlex®21 system kit with each plate including a positive amplification control (2800M DNA) and a negative amplification control (amplification grade water). The two plates were prepared as outlined in Method 4.11.

Amplification cycling conditions, DNA fragment analysis and profile interpretation was followed as outlined in Methods 4.12, 4.13 and 4.14.

The alleles obtained from these samples were compared with the CTS published alleles. Three loci could not be compared as CTS did not publish results for the D12S391, D1S1656 and D6S1043 loci.

5.3 Baseline Determination

To determine the limit of detection (LOD) and the limit of reporting (LOR), the baseline (background) was assessed.

Ten samples from the Caucasian sub-population dataset that exhibited high heterozygosity were used for baseline determination.

The samples were prepared as Methods 4.6, 4.7, 4.8, 4.9, 4.10, 4.11.

Ten samples diluted in ten steps (10x10) outlined in Table 5 were used for the baseline calculations. Each dilution set was amplified at 25µL and 12.5µL total PCR volumes.

50 negative samples were also amplified at 25µL and 12.5µL total PCR volumes.

Table 5 - Total DNA input for each dilution

Dilution	Total DNA (ng)
1	0.500
2	0.447
3	0.394
4	0.342
5	0.289
6	0.236
7	0.183
8	0.131
9	0.078
10	0.025

Amplification, amplification cycling conditions, DNA fragment analysis and profile interpretation was followed as outlined in Methods 4.12, 4.13, 4.14 and 4.15.

The average peak height RFU (μ_{Pk}) for each dye channel was calculated using the AVERAGE function (Arithmetic mean) in Microsoft Excel. The standard deviation (σ_{Pk}) was calculated using the STDEV function in Microsoft Excel.

The thresholds were calculated as follows:

The limit of detection (LOD) was calculated from Equation 1[8].

Equation 1

$$\mathbf{LOD = \mu_{PK} + 3\sigma_{PK}}$$

The limit of reporting (LOR) also known as the analytical threshold (AT) was calculated from Equation 2[8].

Equation 2

$$\mathbf{LOR = \mu_{PK} + 10\sigma_{PK}}$$

5.4 Sensitivity 1

This experiment tested the sensitivity of PowerPlex® 21 system at amplification volumes of 25µL and 12.5µL for DNA template inputs from 4ng to 1pg.

Two staff (one male and one female) with the most heterozygous DNA profile processed with AmpFℓSTR® Profiler Plus® and AmpFℓSTR COfiler® kits were selected for testing[9]. Heterozygous loci provide more information with respect to allele drop out and peak balance.

FTA™ cards were collected, processed, extracted, stock solutions prepared, quantified and dilution series prepared as outlined in Methods 4.6, 4.7, 4.8, 4.9 and 4.10.

Each donor had 9 dilutions prepared as outlined in Table 6. These dilutions were amplified in duplicate with a total amplification volume of 25µL and 12.5µL. Each amplification plate included the kit positive control (2800M DNA) and a negative control (amplification grade water).

Amplification, amplification cycling conditions, DNA fragment analysis and profile interpretation was followed as outlined in Methods 4.11, 4.12, 4.13 and 4.14.

Table 6 - Total DNA input for sensitivity 1

DNA Template Input (ng)
4
2
1
0.5
0.1
0.05
0.01
0.005
0.001

5.5 Sensitivity 2

To assess the differences between the two total PCR volumes with respect to low DNA extract concentrations a second sensitivity experiment was performed.

This experiment tested a dilution series of the same samples used in sensitivity 1 at low DNA templates outlined in table 7. Each dilution was amplified in duplicate at 25µL and 12.5µL.

Amplification, amplification cycling conditions, DNA fragment analysis and profile interpretation was followed as outlined in Methods 4.11, 4.12, 4.13 and 4.14.

Table 7 - Concentration, DNA template input for each dilution.

Concentration (ng/µL)	Volume of sample added to 25 µL reaction volume	Total DNA template input (ng)	Volume of Sample added to 12.5 µL volume reaction	Total DNA template input (ng)
0.01	15	0.15	7.5	0.075
0.005	15	0.075	7.5	0.0375
0.0025	15	0.0375	7.5	0.01875
0.00125	15	0.01875	7.5	0.009375
0.000625	15	0.009375	7.5	0.004688
0.0003125	15	0.004688	7.5	0.002344
0.00015625	15	0.002344	7.5	0.001172
0.000078125	15	0.001172	7.5	0.000586

5.6 Drop In

50 negative samples were amplified alongside the 10 x10 data at 25µL and 12.5µL. Amplification, amplification cycling conditions, DNA fragment analysis and profile interpretation was followed as outlined in Methods 4.11, 4.12, 4.13 and 4.15.

The negative samples were analysed at 1RFU using GeneMapper ID-X v1.1.1 to determine if any peaks above 20RFU were present. Known artefacts, carry-over and pull-up were removed and not included in the analysis.

5.7 Stutter

To determine the thresholds for forward and back stutter peaks 342 samples from the Aboriginal data set, 10 x10, sensitivity 1 and sensitivity 2 were amplified at 25µL and 255 samples from 155 CTS samples, 10 x 10, sensitivity 1 and sensitivity 2 samples were amplified at 12.5µL.

Amplification, amplification cycling conditions, DNA fragment analysis and profile interpretation was followed as outlined in Methods 4.11, 4.12, 4.13 and 4.16.

The stutter ratio (SR) was calculated for each locus as per Equation 3.

Equation 3

$$SR = E_S/E_A$$

SR = Stutter Ratio, E_S = Stutter Height, E_A = Allele Height

The stutter threshold (ST)[4] for each locus was calculated as per Equation 4.

Equation 4

$$ST = \mu_{SR} + 3\,\sigma_{SR}$$

ST = Stutter Threshold, μ_{SR} = average stutter ratio, σ_{SR} = standard deviation of stutter ratio.

The stutter results were also processed with a multiple regression analysis by Jo-Anne Bright for use within the STRmix™ validation and STRmix™ settings[10].

5.8 Peak Balance

The samples from the 10 x10 (section 5.4) were used to calculate peak height ratios and an allelic imbalance threshold to be used for reference samples and as a guide for determining the number of contributors to a mixture.

5.8.1 Peak Height Ratio and Allelic imbalance threshold

Peak height ratios for heterozygote loci (1127 alleles for 12.5µL and 1094 alleles for 25 µL total PCR volumes) were determined by dividing the lower peak height by the higher peak height. Loci where the two main alleles were one repeat apart or were homozygous were excluded from analysis.

The peak height ratio (PHR) was calculated for each locus as per equation 5 [11].

Equation 5

PHR = LPH / HPH

PHR = Peak Height Ratio, LPH = Lower Peak Height, HPH = Higher Peak Height

The average peak heights and standard deviation of peak height ratio were calculated using the Microsoft Excel AVERAGE and STDEV worksheet functions.

The allelic imbalance threshold (AI) was calculated as per Equation 6[12, 13]

Equation 6

$$AI_{TH} = \mu_{PHR} - 3\sigma_{PHR}$$

AI_{TH} = Allelic Imbalance threshold, μ_{PHR}= overall average PHR, σ_{PHR} = standard deviation of the PHR.

5.8.2 Homozygote threshold

The homozygote threshold is the threshold above which you can be confident that a heterozygote locus will not be incorrectly called as a homozygote locus. It was calculated using the following methods

Method 1 – As previously described in the internal validation[14] of peak heights and allelic imbalance thresholds and illustrated below:

Equation 7

$$Th_{Hom} = LOR \times (1 / AI_{TH}) \times 2$$

The LOR used for this calculation is from 5.3 and AI_{TH} was determined in 5.8.2.

Method 2 – As described in the Promega Internal validation guidelines[15] determined from a plot of allelic imbalance versus the lower RFU of a heterozygote pair. The homozygote threshold is assigned at the point at which there is a rapid drop off in peak height ratio.

5.9 Drop Out

To aid in determining the default total PCR volume and template DNA range a series of drop out analyses were performed on the 10 x 10 (section 5.4), sensitivity experiments (sections 5.3 & 5.5) and population datasets (section 5.2).

5.9.1 Drop out 1

The samples from the sensitivity 1 experiment (section 5.3) were used to determine at what RFU the partner of a heterozygote pair drops out. The data was interpreted as outlined in section 4.13. Homozygote peaks, excess samples and no size data were excluded from data analysis. Heat maps were used to summarise the data.

5.9.2 Drop out 2

Samples processed at 25µL and 12.5µL were analysed to determine the threshold when an allele most frequently drops out.

334 DNA profiles amplified at 25µL (from section 5.1.1, 5.3, 5.4 and 5.5) and 279 DNA profiles amplified at 12.5µL (from section 5.2, 5.3, 5.4 and 5.5) were analysed as outlined in Method 4.13.

Homozygote peaks, excess samples and no size data were excluded from both sets of data.

5.9.3 Drop out 3

The samples from the 10 x 10 (section 5.4) and sensitivity experiments (section 5.3 & 5.5) experiments (156 samples) were analysed to record the peak height at which a heterozygote paired allele was lost. The data was interpreted as outlined in Method 4.13.

Homozygote peaks, excess samples and no size data were excluded from data analysis.

5.10 Mixture Studies

In experiment 4 samples, two female and two male samples with high heterozygosity were selected, from the Caucasian dataset and CTS samples, to be combined to make mixed DNA samples. The samples were created as Methods 4.3, 4.4, 4.6, 4.7 and 4.10.

One female sample was combined with one male profile to create a two person mixture, the same female sample was combined with the two male samples to create a three person mixture and two female samples and two male samples were combined to create a four person mixture. The amount of sample required from each contributor to create the mixture ratio was calculated using excel spreadsheets . Varying contributor ratios were made for each of the mixture combinations as outlined in table 8. Each mixture combination was amplified in duplicate at a variety of DNA templates.

Amplification, amplification cycling conditions, DNA fragment analysis and profile interpretation was followed as outlined in Methods 4.11, 4.12, 4.13 and 4.16.

Table 8 - Mixture ratios

Mixture Ratio	Template (ng)
Female:Male	
50:1	0.500
	0.250
	0.125
30:1	0.500
20:1	0.500
	0.250
	0.125
10:1	0.500
	0.125
5:1	0.500
	0.125
2:1	0.500
	0.06
1:1	0.500
Female:Male:Male	
20:10:1	0.500
	0.125
10:5:1	0.500
5:2:1	0.500
	0.125
Female:Male:Male:Female	
5:3:2:1	0.500
	0.125

The mixture ratio was calculated for each DNA profile and compared to the admixture ratio to determine whether there is any variability and whether the mixture ratio can be expected to hold across the profile.

The DNA profiles were analysed to determine at what ratio the minor contributor would be expected to drop out.

6 Results and Discussion

6.1 Population Datasets

Results were tabulated in the following format Unique Sample ID, Race ID, Marker, Allele 1 and Allele 2. Table 9 summarizes the number of profiles for each sub-population submitted for analysis.

Table 9 - Summary of number of profiles for each sub-population submitted.

	Caucasian	Aboriginal	SE Asian
DNA Analysis, FSS	**139**	**309**	**126**
Dataset total	**1707**	**1778**	**990**

Data generated for the three sub-population datasets were analysed by Jo Bright and John Buckleton and used in STRmix™ for statistical analysis[16, 17].

6.2 Concordance

All samples (number of alleles = 4644) tested were found to be concordant to the CTS reported DNA profiles. Table 10 displays the number of times a particular allele was seen at each locus within the laboratory.

Different DNA amplification kits may contain different primers for each locus. Comparison of allele calls (concordance) is required to ensure that each kit gives consistent allele designations, as mismatches or null alleles will affect matching on NCIDD or within a case. The current kits used by the DNA Analysis are AmpFℓSTR® Profiler Plus® and AmpFℓSTR COfiler® DNA amplification kits. Both of these use primers developed by, and manufactured by Life technologies. There are known issues with these kits such as a reverse primer binding mutation at the D8S1179 locus[18], vWA locus[19] and FGA locus[20]. The PowerPlex® 21 kit uses different primer sequences. All alleles tested were found to be concordant. As primer binding mutations and null alleles have been observed within DNA Analysis, any resulting mismatches on NCIDD will need to be retested using PowerPlex® 21.

Table 10 - Observed number of allele concordances

Allele Size	D3S1358	D13S317	Penta E	D16S539	D18S51	D2S1338	CSF1PO	Penta D	TH01	vWA	D21S11	D7S820	D5S818	TPOX	D8s1179	D19S433	FGA
2.2								5									
3.2								2									
5			17					5	1								
6									44					7			
7			32				4	5	75			4	3	4			
8		23	22	4			8	9	42			68	6	133	1		
9		21	10	44			4	48	50			28	13	34	4		
9.3									69								
10		11	25	26	2		69	31	3			80	19	13	11	1	
10.3									1								
11		79	26	83	2		77	45		1		65	91	65	14	6	
11.2																1	
12	1	86	40	78	37		93	51				26	100	11	37	26	
12.2																4	
13	1	48	27	46	30		16	44		3		9	15	1	96	72	
13.2																5	
14	41	20	15	2	38	1	1	8		28			3		71	67	
14.2																9	
15	84		12		42	1		3		43					43	23	
15.2																8	
16	56		13		48	14		1		63					10	5	
16.2																4	
17	67		10		36	46				67					1		
17.2																1	
18	36		6		18	19				57					1		4
18.2																1	
19	4		2		13	33				20							23
20			1		10	28				2							39
20.2																	2
21			2		5	19				2							35
22			2		2	13				1							56
22.2																	3
23					1	20											48
24						13											36
25						22											28
26						8					3						10
27						1					7						4
28											61						
29											47						1
29.2											1						
29.3											1						
30											78						
30.2											10						
31											18						
31.2											22						
32											5						
32.2											25						
33.2											9						
35											2						

6.3 Baseline Determination

The thresholds determined by the baseline experiments are the limit of detection (LOD) and limit of reporting (LOR). The use of thresholds for reporting is essentially a risk assessment[21], if the thresholds are set too low then labelling of artefacts and noise may occur, if set too high then real peaks will not be labelled and information will be lost[1, 11].

Type 1 errors are defined as false labelling of noise peaks. LODs calculated from negative samples may not be optimal for medium-high template samples, as the baseline will differ between positives and negative samples[22].

Type 2 errors are defined as false non-labelling of alleles. If the LOD is set too high, then low level samples may have a heterozygous locus called as a homozygous locus[1, 22-24].

The LOR is the threshold in which a peak can be confidently distinguished from the background fluorescence (baseline). Several methods can be used to determine this threshold.

For the method used here[8] the LOR is derived from the mean baseline plus ten standard deviations (Equation 2).

The LOD is the lowest signal that can be distinguished from the background fluorescence (baseline) and may vary between CE instruments.

Previously in DNA Analysis [14] baseline for the AmpFℓSTR® Profiler Plus® kit was determined using the BatchExtract software v0.16. The LOD was calculated using Equation 1. This approach of using the mean and three standard deviations would account for 99.73% of baseline fluorescence.

The files generated by GeneMapper ID-X v1.1.1 are not compatible with the BatchExtract software without modification. For this validation an equivalent process for measuring the baseline as described by Promega was used with some modifications to the types of samples used. For this validation samples containing DNA were used to determine baseline fluorescence.

Table 11 shows the results determined from the baseline calculations when the samples were amplified at 25µL. The highest average peak height (5.74RFU) and the highest standard deviation (3.21) was in the TMR (yellow) channel from run 2 on 3130xl A. The TMR (yellow) channel for run 2 on 3130xl A also yielded the highest LOD (15.37) and highest LOR (37.84). The LOD was rounded to 16RFU and the LOR was rounded to 40RFU and is to be used for all dye channels for samples amplified using a total amplification volume of 25µL.

Table 11 - Baseline results for amplifications at 25µL

		3130xl A run 1	3130xl A run 2	3130xl B run 1	3130xl B run 2	Overall 3130xl A & B run 1 & 2
Fluorescin (Blue)	μ_{PK}	2.33	2.58	1.90	1.68	2.11
	σ_{PK}	1.55	2.05	1.01	0.89	1.52
	LOD	6.99	8.73	4.93	4.36	6.68
	LOR	17.86	23.07	12.01	10.59	17.35
JOE (Green)	μ_{PK}	3.51	3.83	2.25	2.16	2.94
	σ_{PK}	2.34	2.62	1.04	1.29	2.12
	LOD	10.54	11.68	5.37	6.02	9.30
	LOR	26.94	29.99	12.65	15.02	24.14
TMR (Yellow)	μ_{PK}	5.29	5.74	3.33	3.07	4.32
	σ_{PK}	2.73	3.21	1.27	1.66	2.68
	LOD	13.47	15.37	7.15	8.05	12.37
	LOR	32.55	37.84	16.06	19.66	31.16
CXR (Red)	μ_{PK}	2.22	2.41	2.02	1.78	2.09
	σ_{PK}	1.36	1.54	0.89	1.01	1.35
	LOD	6.29	7.05	4.69	4.81	6.16
	LOR	15.79	17.79	10.93	11.88	15.63
CC5 (Orange)	μ_{PK}	1.76	1.99	1.14	1.36	1.66
	σ_{PK}	1.30	1.80	0.44	1.39	2.44
	LOD	5.68	7.38	2.47	5.52	9.00
	LOR	14.81	19.94	5.58	15.24	26.11
Overall	μ_{PK}	3.41	3.72	2.44	2.22	2.79
	σ_{PK}	2.45	2.80	1.33	1.39	2.29
	LOD	10.76	12.13	6.23	6.40	9.65
	LOR	27.91	31.76	15.54	16.14	25.65

Table 12 shows the results determined from the baseline calculations when the samples were amplified at 12.5µL. The highest average peak height (6.06RFU) was in the TMR (yellow) channel from the run on 3130xl A and the highest standard deviation (4.41) was in the JOE (green) channel from the run on 3130xl A. The TMR (yellow) channel for the run on 3130xl A yielded the highest LOD (18.50) and the JOE (green) channel yielded the highest LOR (48.60). It was noted on 3130xl A the baseline was raised more than expected compared to other baseline runs on the same instrument and baseline runs on 3130xl B. This could be due to a prolonged period between spectral calibrations, aging reagents and arrays and was taken into consideration when setting thresholds. With natural variations, the results from run to run and instrument may vary, by using the mean + 10SD for the LOR, although the baseline itself may shift, the LOR will always be greater than the LOD even if baseline is either increased or decreased on any given run. By using an "over all" result, the standard deviation is increased due to the difference in fluorescence between instruments, and this then gets factored into the overall LOR.

The highest overall LOD (15.70) was in the TMR (yellow) channel and was rounded to 16RFU and the highest overall LOR (42.27) was in the JOE (green) channel and was rounded to 40RFU.

In an effort to eliminate error and confusion a single LOD and LOR value is to be used for both instruments.

Table 12 - Baseline results for amplifications at 12.5µL

		3130xl A 12.5µL	3130xl B 12.5µL	Overall 3130xl A & B 12.5µL
Fluorescin (Blue)	μ_{PK}	3.10	2.19	2.64
	σ_{PK}	3.66	2.72	2.99
	LOD	14.07	10.36	11.59
	LOR	**39.67**	**29.42**	**32.49**
JOE (Green)	μ_{PK}	4.46	2.69	3.62
	σ_{PK}	4.41	2.86	3.86
	LOD	17.70	11.26	15.22
	LOR	**48.60**	**31.28**	**42.27**
TMR (Yellow)	μ_{PK}	6.06	3.58	4.83
	σ_{PK}	4.15	2.43	3.63
	LOD	18.50	10.88	15.70
	LOR	**47.52**	**27.92**	**41.08**
CXR (Red)	μ_{PK}	2.87	2.10	2.49
	σ_{PK}	2.32	1.28	1.93
	LOD	9.84	5.94	8.27
	LOR	**26.11**	**14.90**	**21.75**
CC5 (Orange)	μ_{PK}	2.38	1.66	2.02
	σ_{PK}	2.31	1.87	2.14
	LOD	9.33	7.26	8.84
	LOR	**25.53**	**20.33**	**23.40**
Overall	μ_{PK}	3.94	2.54	3.32
	σ_{PK}	3.87	2.46	3.30
	LOD	15.56	9.91	13.21
	LOR	**42.68**	**27.10**	**36.28**

μ_{PK} = Average peak height, σ_{PK} = Standard Deviation, LOD = limit of detection, LOR = Limit of Reporting

6.4 Sensitivity

All PCR amplification kits are optimised for a particular total reaction volume by the manufacturer; but it is commonplace to reduce the total PCR reaction volume to increase the sensitivity[25-28] and reduce processing costs[27]. Two sensitivity experiments were performed, in addition to the 10x10 (baseline determination) dataset.

To contrast and compare the effect of total PCR volume on DNA profiles, the same dilution series were amplified at two different total PCR volumes (25µL and 12.5µL) using 30 PCR cycles.

The results for the amplification of the two donors at 25µL and 12.5µL are summarised in tables 13 and 14 respectively.

Table 13 - Summary of the 2 donors amplified at 25µL

Donor 1 25µL	Template	Av No. Alleles	Av PH (RFU)	Max PH	Min PH	AV PHR
Donor1	4ng	N/A	NAD XS	N/A	N/A	N/A
Donor1	2ng	N/A	XS	N/A	N/A	N/A
Donor1	1ng	42	2512.56	4661.00	1456.00	90.47
Donor1	0.5ng	42	1347.65	2492.00	172.00	85.58
Donor1	0.1ng	42	277.47	506.00	119.00	78.78
Donor1	50pg	41	153.39	387.00	48.00	67.09
Donor1	10pg	17	46.86	108.00	20.00	79.08
Donor1	5pg	6.5	39.57	78.00	20.50	0.00
Donor1	1pg	1.5	33.83	43.00	27.00	0.00
Donor 2 25µL	**Template**	**Av No. Alleles**	**Av PH (RFU)**	**Av Max PH**	**Av Min PH**	**AV PHR**
Donor2	4ng	N/A	XS	N/A	N/A	N/A
Donor2	2ng	N/A	XS	N/A	N/A	N/A
Donor2	1ng	42	2790.81	5126.00	1461.00	89.19
Donor2	0.5ng	42	1344.10	2878.00	431.00	86.91
Donor2	0.1ng	42	292.72	698.00	88.00	74.55
Donor2	50pg	41.5	157.40	479.00	47.00	68.59
Donor2	10pg	24.5	69.69	171.00	14.25	69.60
Donor2	5pg	5.5	44.95	75.00	23.00	96.79
Donor2	1pg	6	33.62	55.00	20.00	94.85

Av = Average, PH = Peak Height, No. = Number, Max = Maximum, Min = Minimum, PHR = Peak Height Ratio

Table 14 - Summary of the 2 donors amplified at 12.5µL.

Donor 1 12.5µL	Template	Av No. Alleles	Av PH (RFU)	Max PH	Min PH	AV PHR
Donor1	4ng	N/A	NAD XS	N/A	N/A	N/A
Donor1	2ng	N/A	XS	N/A	N/A	N/A
Donor1	1ng	N/A	XS	N/A	N/A	N/A
Donor1	0.5ng	42	3132.96	6719.00	1590.00	84.41
Donor1	0.1ng	42	780.57	2444.00	180.00	74.66
Donor1	50pg	42	346.67	931.00	58.00	68.88
Donor1	10pg	27	91.95	406.00	21.00	49.76
Donor1	5pg	12	48.20	91.50	20.00	71.22
Donor1	1pg	4.5	35.80	51.00	22.00	88.24
Donor 2 12.5µL	**Template**	**Av No. Alleles**	**Av PH (RFU)**	**Av Max PH**	**Av Min PH**	**AV PHR**
Donor2	4ng	N/A	XS	N/A	N/A	N/A
Donor2	2ng	N/A	XS	N/A	N/A	N/A
Donor2	1ng	N/A	XS	N/A	N/A	N/A
Donor2	0.5ng	42	2878.80	6159.00	1281.00	78.29
Donor2	0.1ng	42	742.73	1612.00	140.00	68.12
Donor2	50pg	42	333.38	892.00	93.00	60.88
Donor2	10pg	25	82.33	249.00	21.00	59.05
Donor2	5pg	13.5	51.47	121.00	21.00	67.89
Donor2	1pg	0	0.00	0.00	0.00	0.00

The amplifications at 25µL total PCR volume with DNA templates of 4ng and 2ng for both donors gave excess profiles resulting in the profiles being unable to be interpreted. The results from the excess samples were excluded from the data analysis. The average number of alleles and the

average peak height was similar for both donors when processed with an amplification volume of 25µL.

The amplifications at 12.5µL with DNA templates of 4ng, 2ng, 1ng and one replicate of the 0.5ng for both donors gave excess results. The results from the excess samples were excluded from the data analysis. The average number of alleles and average peak height was similar for both donors when processed with an amplification volume of 12.5µL.

Figure 1 displays the average number of alleles and average peak height ratio obtained for each donor at each template amplified at 25µL and 12.5µL.

Figure 2 displays the average peak height and average peak height ratio at each DNA template amplified for 25µL and 12.5µL.

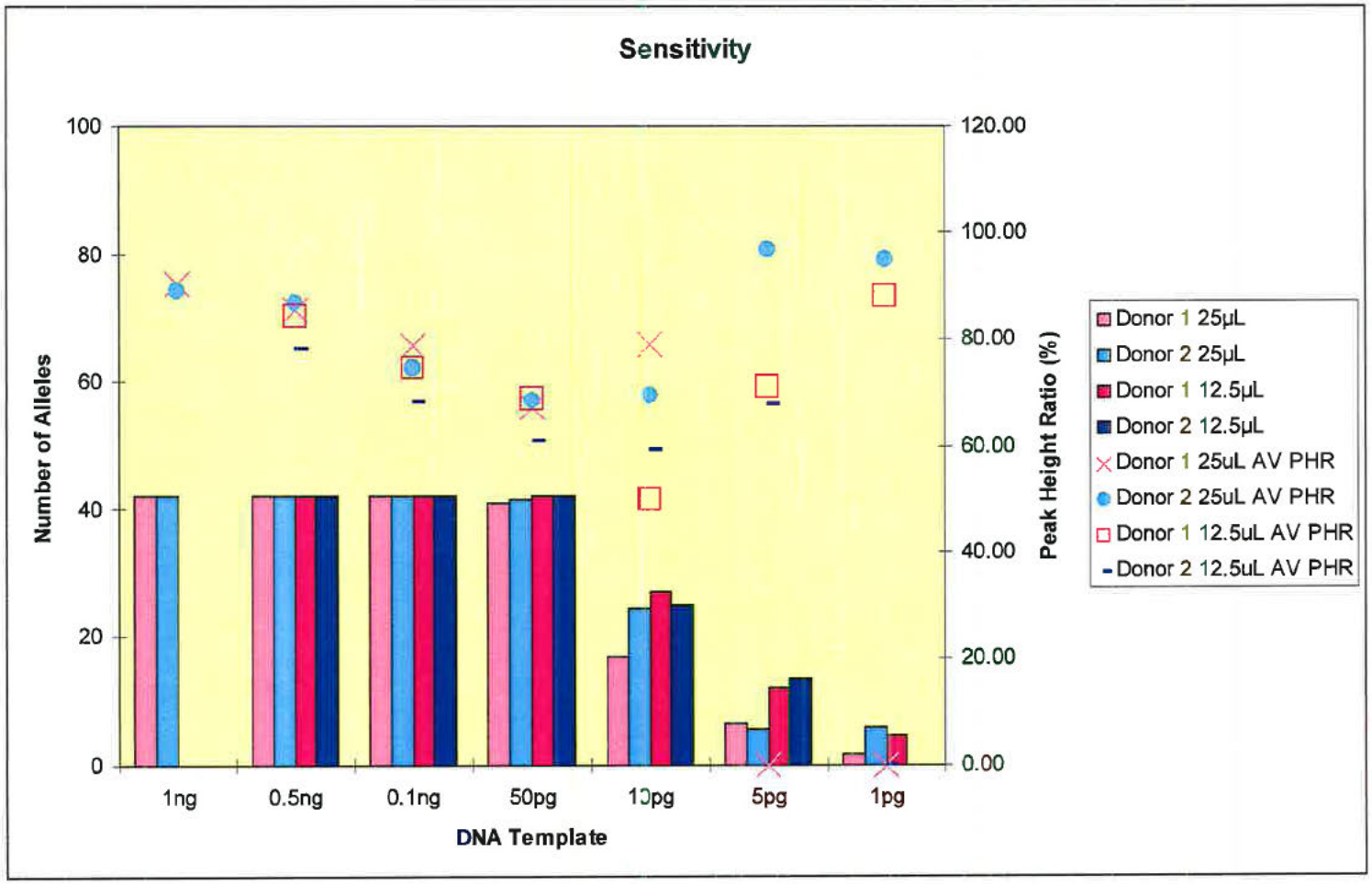

Figure 1 - Average number of alleles for each donor at each DNA template at amplification volumes of 25µL and 12.5µL. AV PHR = Average Peak Height Ratio

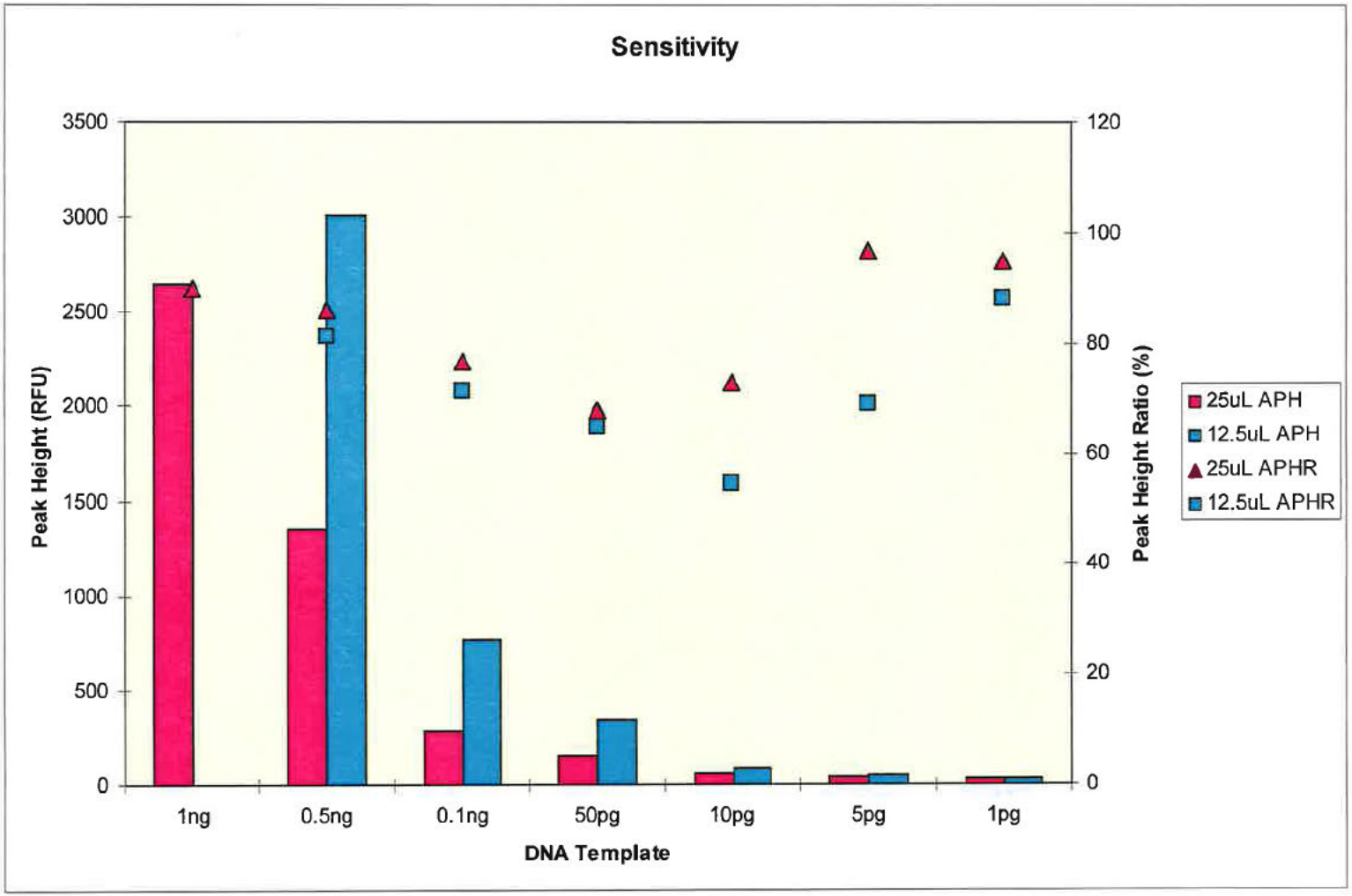

Figure 2 Average peak height and average peak height ratio for each DNA template

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A full complement of alleles in the PowerPlex® 21 system was obtained for both donors at total DNA template inputs of 0.5ng and 0.1ng when amplified at both total PCR volumes. As expected the average number of alleles decreased as the DNA template decreased.

For both total PCR volumes, as the total DNA template decreased, the peak heights also decreased. The 12.5µL amplification gave higher peaks heights at the 0.5ng, 0.1ng and 50pg DNA template inputs compared with the 25µL amplification.

The average peak height ratio decreased as the DNA template decreased to 50pg. Below a DNA template of 50pg less heterozygote pairs were observed (as expected) which resulted in the peak height ratio becoming more variable and drop out being observed.

The samples from the 10x10 dataset ranged from template inputs of 0.5ng to 0.025ng. The results of these experiments are concordant with the first sensitivity experiment.

A full complement of alleles in the PowerPlex® 21 system was obtained for all samples between 0.5ng and 0.132ng DNA template inputs when amplified at both total PCR volumes.

The second sensitivity experiment was undertaken to enable direct comparison of the sample concentration when amplified at a total PCR volume of 25µL and 12.5µL rather than comparing the total DNA template input.

Figure 3 shows the results of low concentration samples amplified at 25µL and 12.5µL total PCR volumes with the vertical red line highlighting the limit of detection[29] (quantification) used for the AB 7500 Real Time PCR system. The numbers of alleles obtained at each concentration were counted using the LOR thresholds determined in section 6.4.

The DNA profiles exhibited increased allelic imbalance across different loci when the sample concentration dropped below 0.025ng/µL.

Overall the PowerPlex®21 system is a very sensitive STR amplification kit capable of detecting DNA amounts below what is generally considered low copy number (LCN). The data analyses indicate that the risk of type 2 errors will increase if the DNA template is too low for both total PCR volumes.

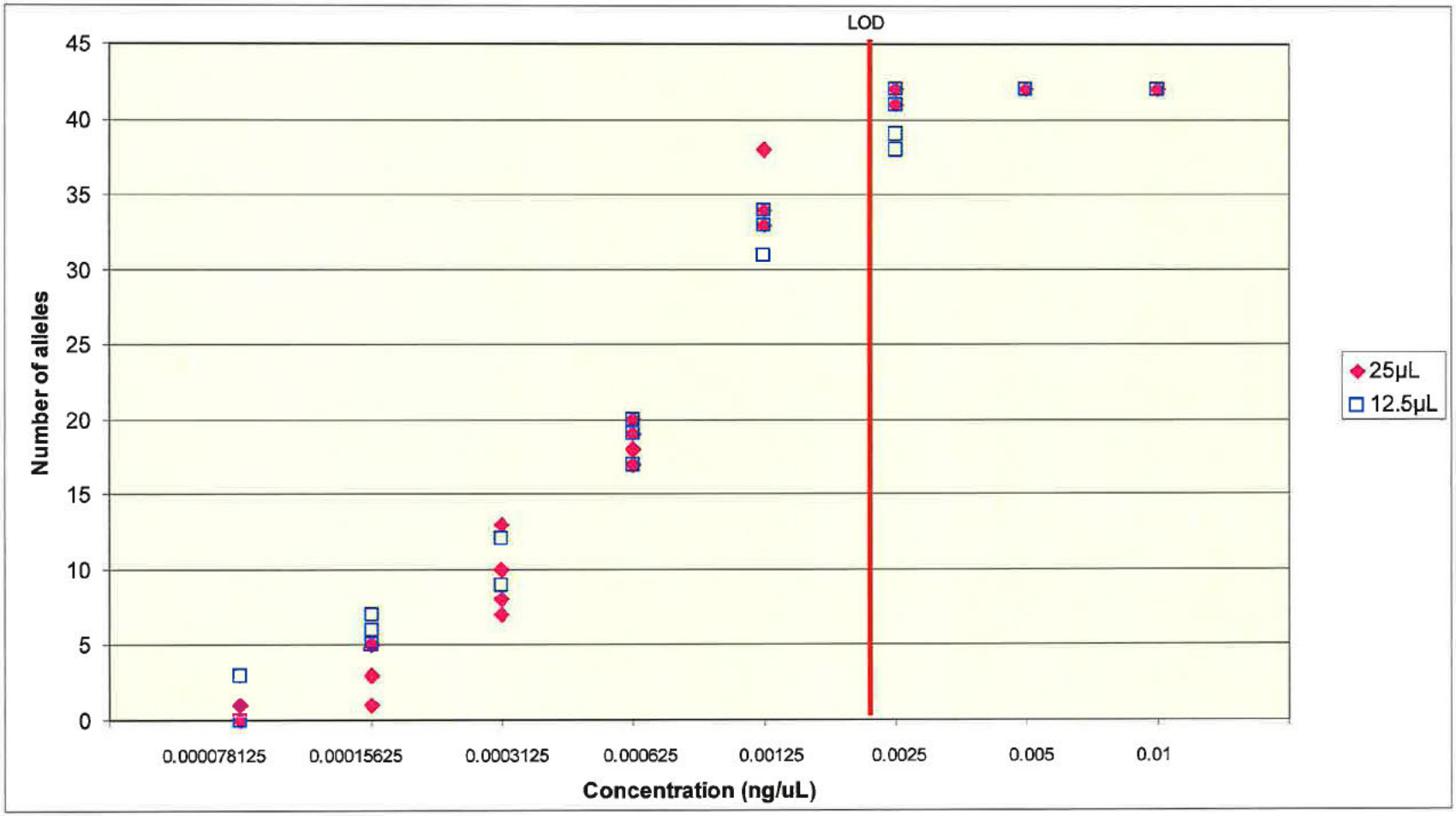


Figure 3 - Comparison of sample concentration vs allele count for 25µL and 12.5µL total PCR volumes.

6.5 Drop In

Allelic drop-in is due to spurious amplification products from unknown DNA, which makes allele drop-in a random event[30, 31]. The phenomenon of allelic drop-in is usually not reproducible and can be detected through testing samples multiple times[32].

For the 25µL amplifications processed on both 3130xl instruments 3 drop in events were noted. True drop-in alleles were seen in three negative controls at D16S539 as a 7 allele at 21RFU, D3S1358 as a 21 allele at 19RFU and at TH01 as a 5 allele at 19RFU.

For 12.5 µL amplifications on both 3130xl instruments no drop in events were noted.

Drop in data was sent to John Buckleton for fit to a Poisson distribution and tested. This data is required for STRmix™ validation and STRmix™ settings.

The rate of drop in events for 25µL volume amplifications (3 events in 1050 alleles above 15RFU) was calculated for STRmix™ by John Buckleton, see figure 4.

STRmix™ uses the model for drop-in ae-bx where the values for a and b are the drop-in parameters in STRmix™. John Buckleton's calculations determined that a=b=0.393. The maximum drop-in seen at any one locus is determined in RFU; this means that if two peaks were seen at one locus the drop-in would be the total height of both peaks. Since only one drop-in peak was observed at any one locus and the highest of these events was 21RFU, then our drop-in setting for STRmix™ would be 21RFU. Since our LOR was determined to be 40RFU, it seemed reasonable to set the drop-in level to 40RFU.

Although no drop-in events were observed for half volume amplifications, the same parameters will be applied.

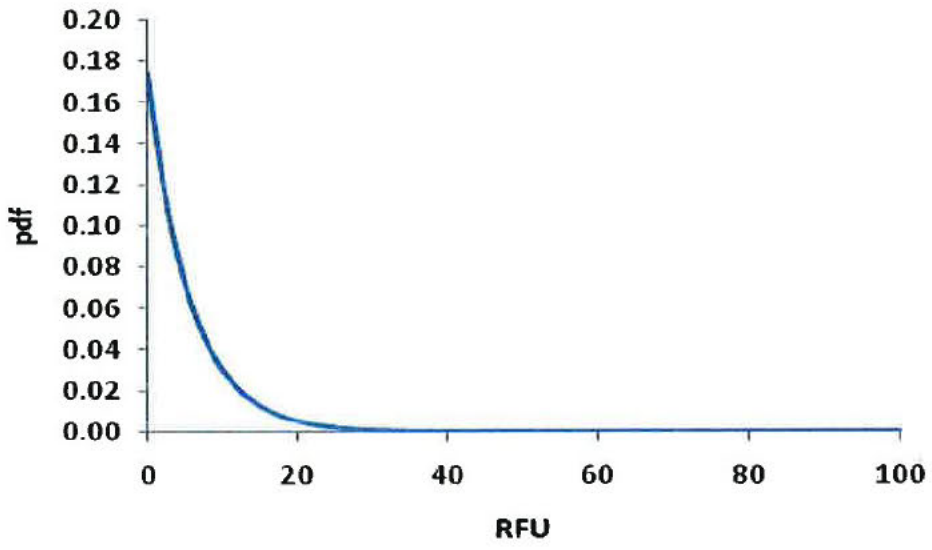


Figure 4 - Probability of Drop in for 25µL total PCR volume.

6.6 Stutter

Stutter peaks are Polymerase Chain Reaction (PCR) artefacts commonly observed in all STR analysis[4, 33]. They are usually observed as a peak one repeat unit smaller in size than the true allele peak[33]. The stutter mechanism has been attributed to slippage of the DNA strand during replication.

Over stutter is observed as a peak one repeat unit more in size than the true allele. Figure 5 shows an example of stutter and over stutter.

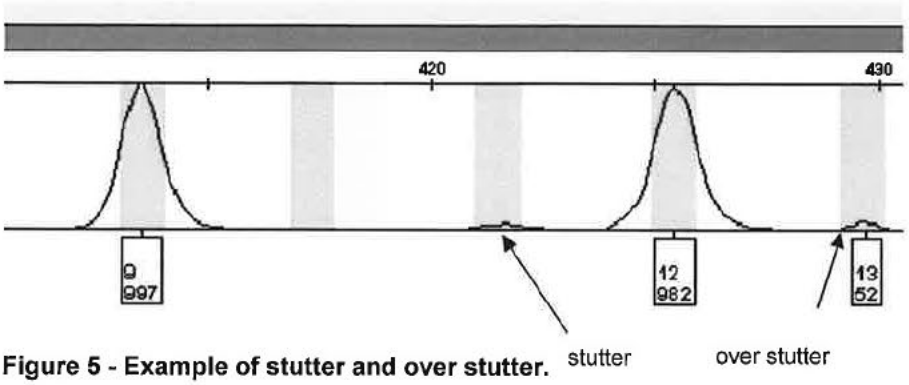


Figure 5 - Example of stutter and over stutter.

Promega supplied a stutter text file (using $\mu + 3\sigma$[4]) for GeneMapper ID-X v.1.1.1. We have used the same calculation as it incorporates 99.73% of the data assuming normal distribution.

The data for the observed stutter ratios (forward and over) for samples amplified at 25µL are listed in table 15 and for 12.5µL are listed in table16.

Over stutter was observed for all loci when amplified at 25µL and therefore a threshold was able to be calculated for each locus. Over stutter was not observed for all loci when amplified at 12.5µL and therefore a threshold was only able to be calculated for those loci at which over stutter was observed. Over stutter will be continued to be monitored until enough data is obtained to review the thresholds set in this validation.

Most calculated stutter thresholds were higher than the Promega supplied stutter filter file both for 25µL and 12.5µL. The exceptions were D6S1043, D18D51, D2S1338, and Penta D for 25µL and D6S1043, Penta E, D18D51, D2S1338, and Penta D for 12.5uL.

When comparing the calculated stutter thresholds for the 25µL and 12.5µL total PCR volumes, they appear to be similar.

Table 15 - 25µL Calculated stutter thresholds.

Locus	μ_{SR}	σ_{SR}	Stutter Ratio (%)	μ_{OSR}	σ_{OSR}	Over stutter Ratio (%)
D3S1358	0.0868	0.0184	14.2	0.0131	0.0100	4.3
D1S1656	0.0910	0.0269	17.2	0.0183	0.0163	6.7
D6S1043	0.0685	0.0171	12.0	0.0164	0.0192	7.4
D13S317	0.0496	0.0228	11.8	0.0185	0.0184	7.4
Penta E	0.0457	0.0203	10.7	0.0113	0.0018	1.7
D16S539	0.0686	0.0173	12.1	0.0133	0.0099	4.3
D18S51	0.0873	0.0244	16.0	0.0144	0.0116	4.9
D2S1338	0.0878	0.0203	14.9	0.0196	0.0150	6.5
CSF1PO	0.0640	0.0244	13.7	0.0155	0.0096	4.4
Penta D	0.0245	0.0190	8.2	0.0306	0.0193	8.8
TH01	0.0325	0.0181	8.7	0.0085	0.0041	2.1
vWA	0.0782	0.0246	15.2	0.0157	0.0135	5.6
D21S11	0.0809	0.0199	14.1	0.0175	0.0177	7.1
D7S820	0.0485	0.0218	11.4	0.0207	0.0124	5.8
D5S818	0.0595	0.0202	12.0	0.0165	0.0132	5.6
TPOX	0.0381	0.0174	9.0	0.0235	0.0130	6.3
D8S1179	0.0790	0.0177	13.2	0.0176	0.0123	5.5
D12S391	0.0948	0.0311	18.8	0.0146	0.0128	5.3
D19S433	0.0666	0.0205	12.8	0.0211	0.0165	7.1
FGA	0.0702	0.0227	13.8	0.0182	0.0135	5.9

Stutter thresholds higher than the recommended stutter thresholds from Promega =

μ_{SR} = mean stutter ratio, σ_{SR} = standard deviation of stutter ratio, μ_{OSR} = mean over stutter ratio, σ_{OSR} = standard deviation of over stutter ratio

Table 16 - 12.5µL Calculated stutter thresholds.

Locus	μ_{SR}	σ_{SR}	Stutter Ratio (%)	μ_{OSR}	σ_{OSR}	Over stutter Ratio (%)
D3S1358	0.0880	0.0194	14.6	0.0113	0.0067	3.2
D1S1656	0.0909	0.0247	16.5	0.0138	0.0055	3.0
D6S1043	0.0738	0.0153	12.0	0.0141	0.0088	4.0
D13S317	0.0544	0.0197	11.3	0.0148	0.0070	3.6
Penta E	0.0389	0.0141	8.1	0.0289	0.0111	6.2
D16S539	0.0690	0.0195	12.8	0.0120	0.0049	2.7
D18S51	0.0827	0.0258	16.0	0.0167	0.0125	5.4
D2S1338	0.0909	0.0218	15.6	0.0298	0.0241	10.2
CSF1PO	0.0721	0.0258	14.9	0.0145	0.0071	3.6
Penta D	0.0262	0.0093	5.4	0.0324	0.0005	3.4
TH01	0.0252	0.0120	6.1	0.0071	0.0000	0.0
vWA	0.0836	0.0212	14.7	0.0149	0.0097	4.4
D21S11	0.0839	0.0199	14.4	0.0256	0.0132	6.5
D7S820	0.0508	0.0232	12.0	0.0250	0.0108	5.7
D5S818	0.0675	0.0230	13.7	0.0163	0.0139	5.8
TPOX	0.0346	0.0179	8.8	0.0145	0.0000	0.0
D8S1179	0.0818	0.0208	14.4	0.0173	0.0125	5.5
D12S391	0.1026	0.0313	19.6	0.0135	0.0083	3.8
D19S433	0.0689	0.0185	12.4	0.0129	0.0032	2.2
FGA	0.0700	0.0218	13.5	0.0192	0.0223	8.6

6.7 Peak Balance

6.7.1 Peak Height Ratio and Allelic Imbalance Threshold

Peak height ratio (PHR) is the ratio between the two peaks in a heterozygous pair. Under optimal conditions the amplification of a pair of alleles should result in equal peak heights however, input DNA, inhibitors and quality of DNA will affect the amplification [34, 35].

The method used in Equation 4 is recommended in the SWGDAM guidelines [11]and well represented in the literature [36], although other methods have been published by Kelly et al [37].

By assigning a threshold of the mean minus three standard deviations, this incorporates 99.73% of the data, resulting in a conservative threshold. This threshold was rounded up to the nearest RFU. Use of this method to produce a threshold is a low risk to reference samples, as samples that deviate would be reprocessed.

Table 17 shows the summary of PHR and AI_{Th} data calculated. The overall average PHR for 12.5µL and 25µL total PCR volumes are 78.9% and 80.4% respectively. These values are consistent with other kits listed in the literature [12, 38]. Although the average peak height ratios are similar to those reported in the literature, given the wide standard deviation

observed in our data, the calculated AI_{Th} of 31.1% for 12.5uL and 38.6% for 25uL reaction volumes are considered low.

Figures 6 and 7 display the data obtained from the 10 x10 experiments for 25µL and 12.5µL total PCR volumes respectively. For both total PCR volumes, as the amount of DNA input is decreased from the recommended 0.5ng template DNA, the average peak height ratio (μ_{PHR}) decreases and the standard deviation of the peak height ratio (σ_{PHR}) increases.

When the mean PHR are calculated for each DNA template, between 0.183ng and 0.5ng inputs there is no significant difference between total PCR volumes although the standard deviation is higher for the 12.5µL total PCR volume, resulting in a much lower threshold. Refer to table 17.

Figures 10 -19 show observed PHR for different template DNA amounts. The PHR range is separated into 0.1 increments plotted against number of allele pairs. Figure 10 is lowest template DNA amount. This shows that at the low template DNA range, the PHR varies unpredictably for both the 25µL and 12.5µL total PCR volumes. As the template DNA amount increases, the PHR converges towards the ideal of 1.0.

The μ_{PHR_25} at 25pg input was 0.736 and at 0.5ng input was 0.851 compared with the $\mu_{PHR\_12.5}$, at 25pg input was 0.598 and at 0.5ng was 0.832.

The results of our validation are consistent with previous published findings referring to low template DNA and reduced volume amplifications [13, 34, 39].

Stochastic effects were obvious in this experiment in data from templates below 0.132ng. Stochastic effects are the result of random, uneven amplification of heterozygous allele pairs from low template samples (SWGDAM 2010 interpretation) which is displayed by low peak heights or allele/locus dropout. At 0.132ng DNA template is approaching what is usually defined as low copy number (LCN) (~0.100ng to 0.150ng).

Supportive experimental data is displayed in Figure 20 AI_{TH} vs input graph, which displays a rapid drop off the AI_{TH} after 0.132ng DNA template. The calculated AI_{TH} drops below 0 for 0.02475ng DNA template because the standard deviation is so large. The rapid drop off is likely to increase the number of type 2 errors if AI_{TH} is used calculated from the entire dataset due to the large standard deviation. Exclusion of data from templates below 0.132ng increases the μ_{PHR} and decreases σ_{PHR}.

A multiple regression analysis was performed by Jo-Anne Bright, Duncan Taylor and John Buckleton to calculate the peak height variance for use in STRmix™[40].

The peak height ratios calculated here are for use with reference samples that have been amplified from extracted DNA and as a guideline to help determine the number of contributors for mixture interpretation as required for STRmix™ analysis.

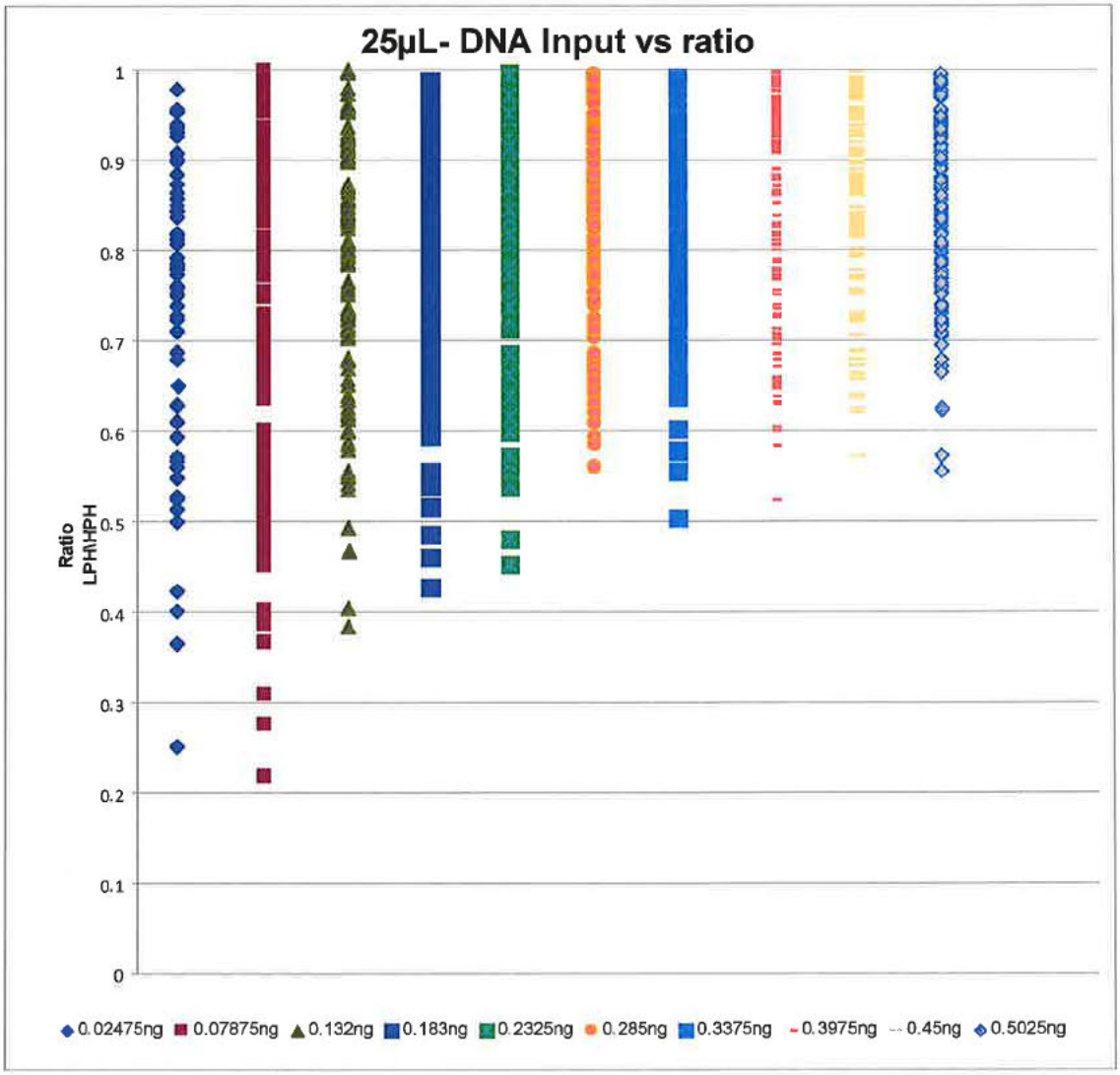


Figure 6 - 25µL total PCR volume, Peak balance vs total input DNA

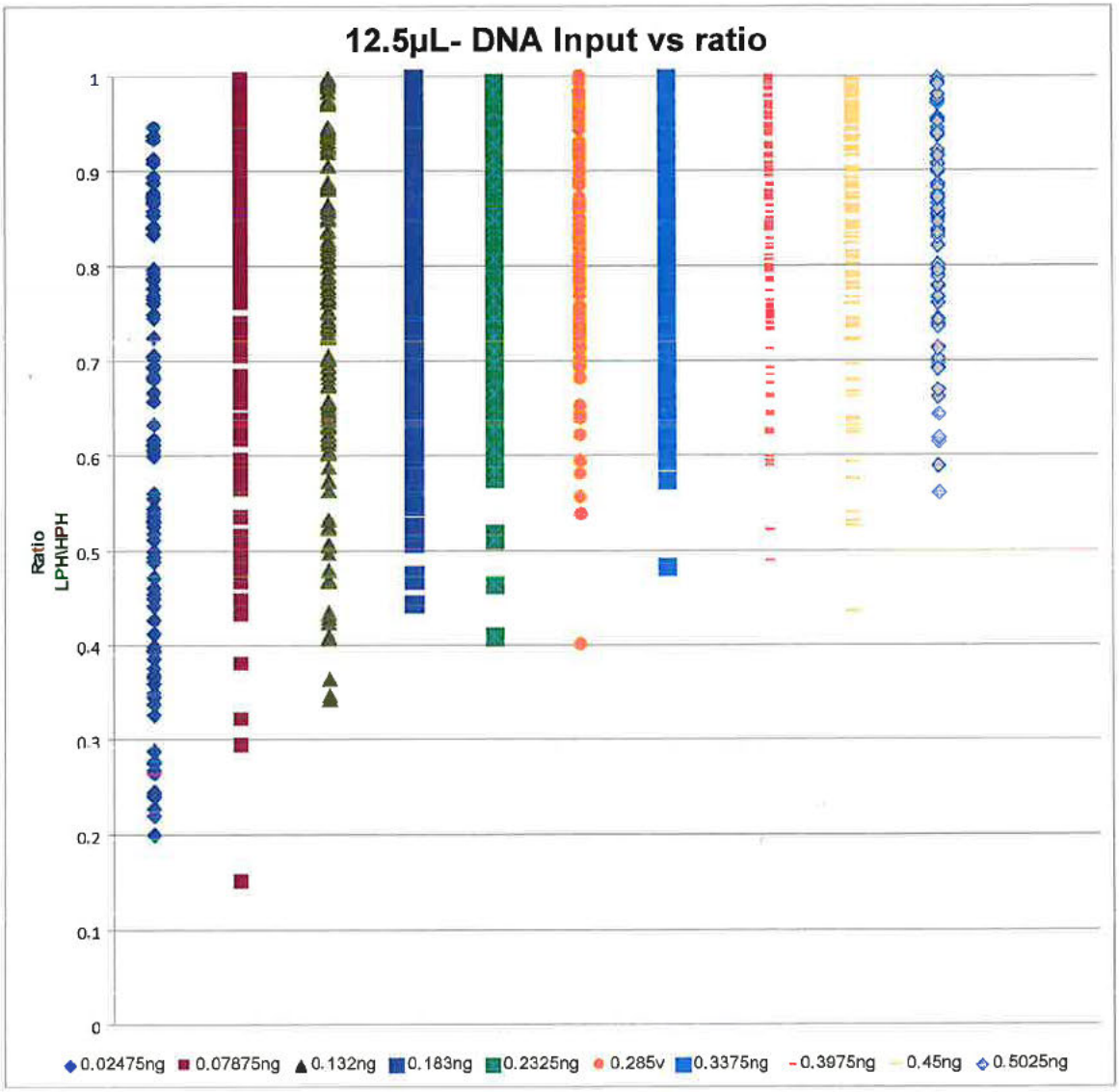


Figure 7 - 12.5µL Total PCR volume - Peak balance vs total input DNA.

Table 17 - Summary of calculated AI_{TH}.

	12.5µL			25µL		
	All Data	0.132 - 0.50	0.183- 0.50	All Data	0.132 - 0.50	0.183- 0.50
µ	0.789	0.814	0.825	0.804	0.824	0.830
σ	0.160	0.134	0.124	0.140	0.123	0.119
AI_{TH}	0.311	0.414	0.452	0.386	0.455	0.472

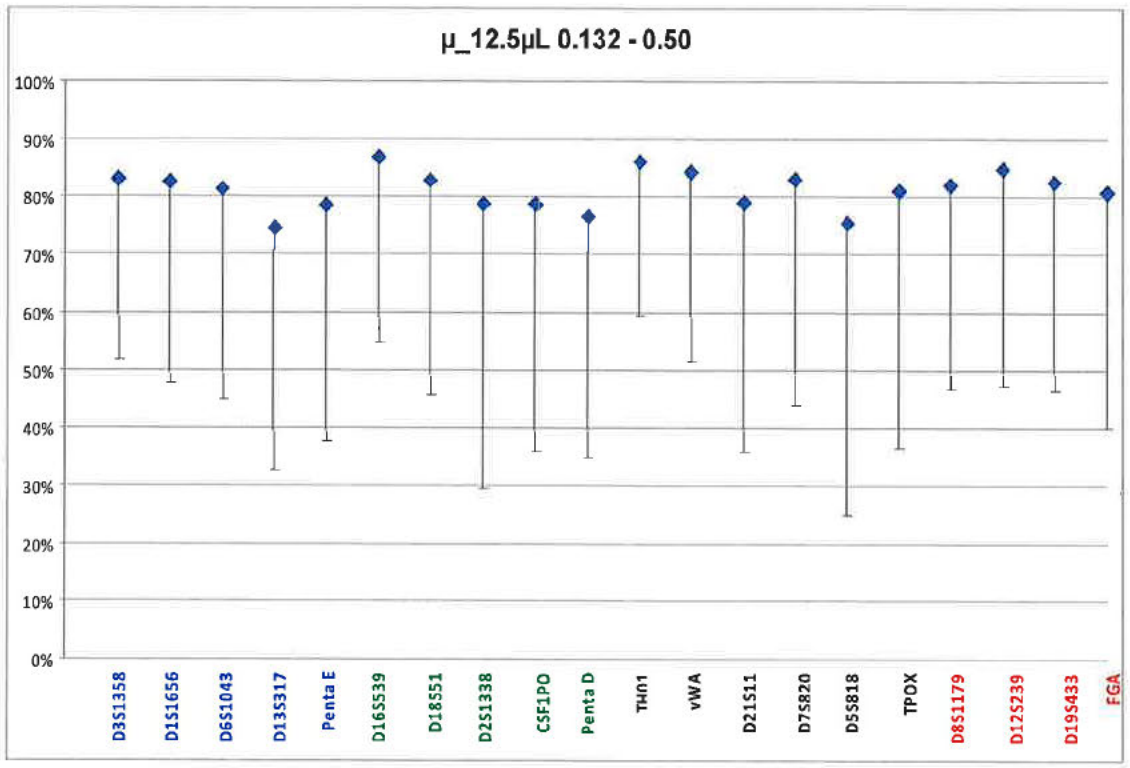


Figure 8 - 12.5µL total PCR volume µPHR per Loci

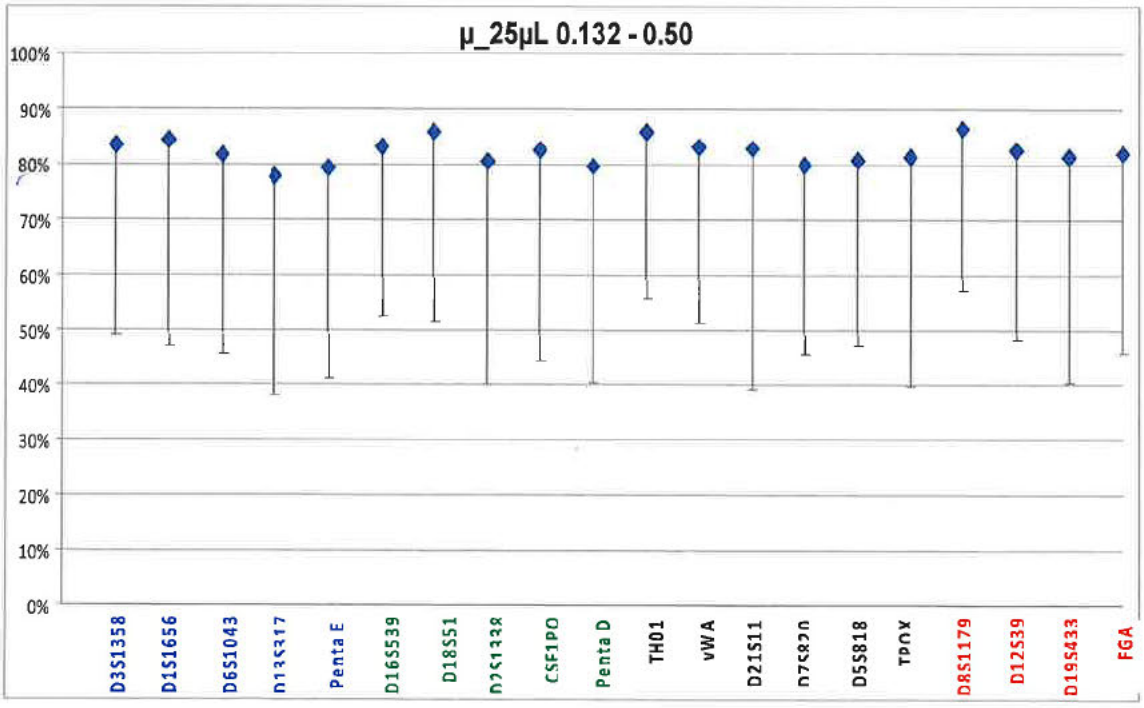


Figure 9 - 25µL total PCR volume µPHR per Loci

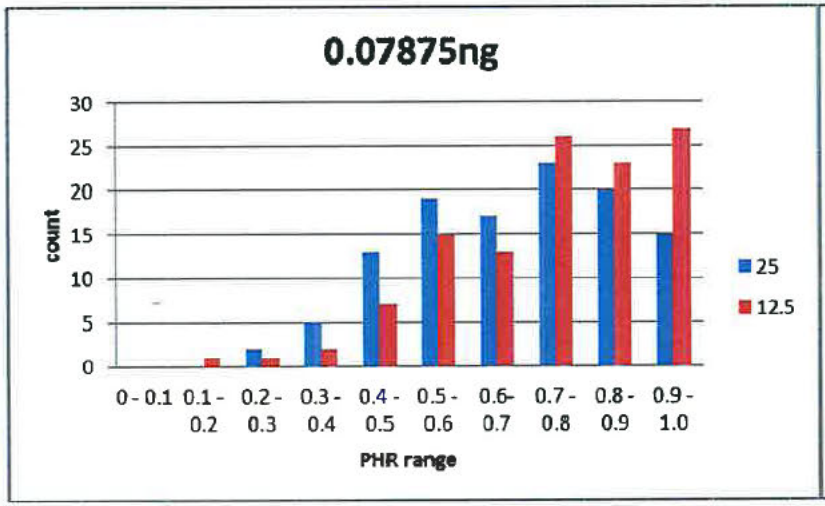


Figure 10 - The count of allele pairs per 0.1 PHR bin for 0.02475ng.

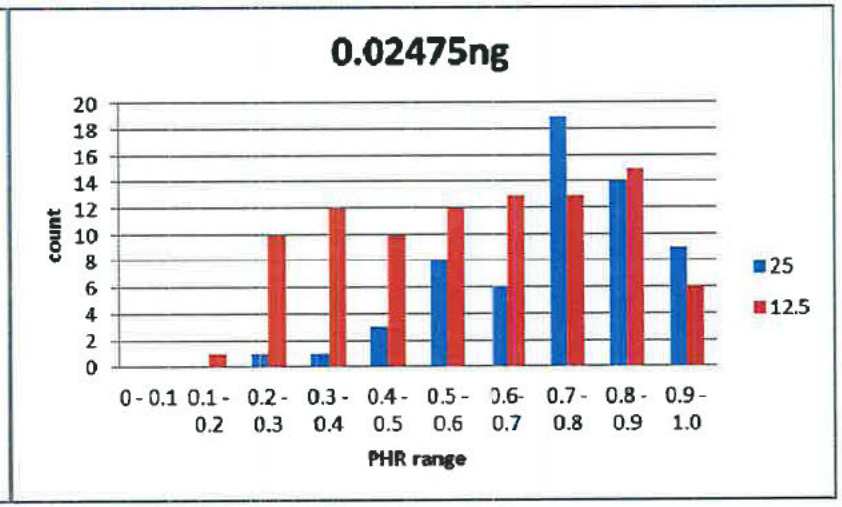


Figure 11 - The count of allele pairs per 0.1 PHR bin for 0.07875ng.

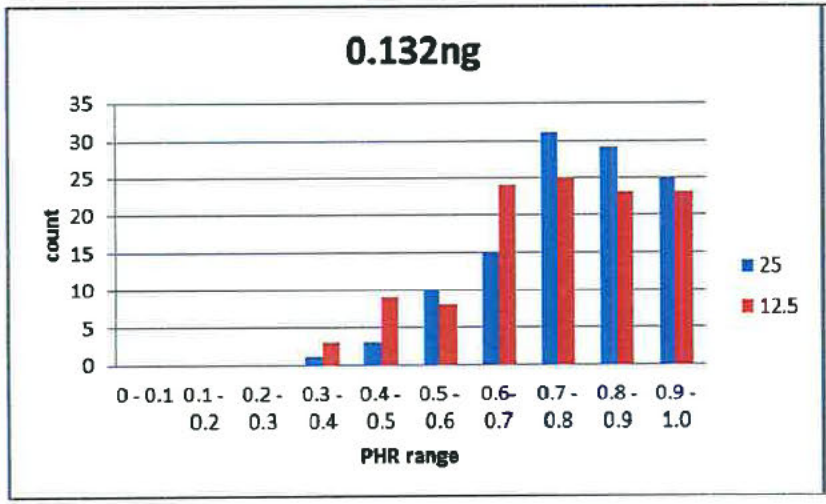


Figure 12 - The number of allele pairs per 0.1 PHR bin for 0.132ng.

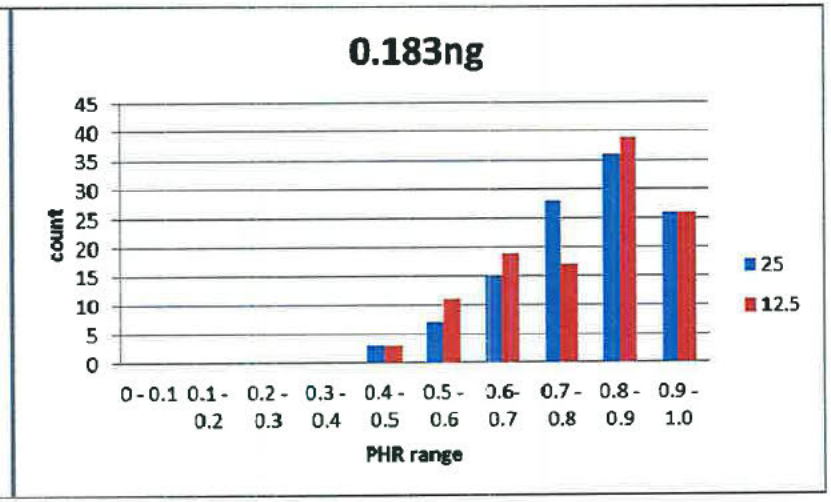


Figure 13 - The count of allele pairs per 0.1 PHR bin for 0.183ng.

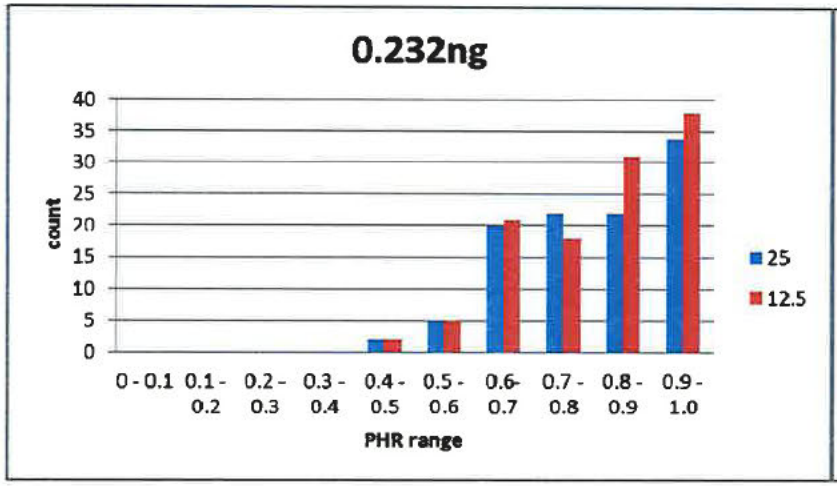


Figure 14 - The count of allele pairs per 0.1 PHR bin for 0.232ng.

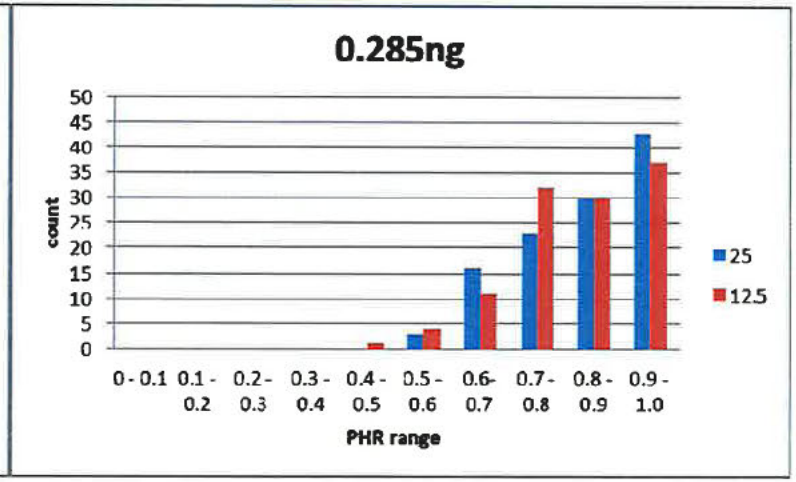


Figure 15 - The number of allele pairs per 0.1 PHR bin for 0.285ng.

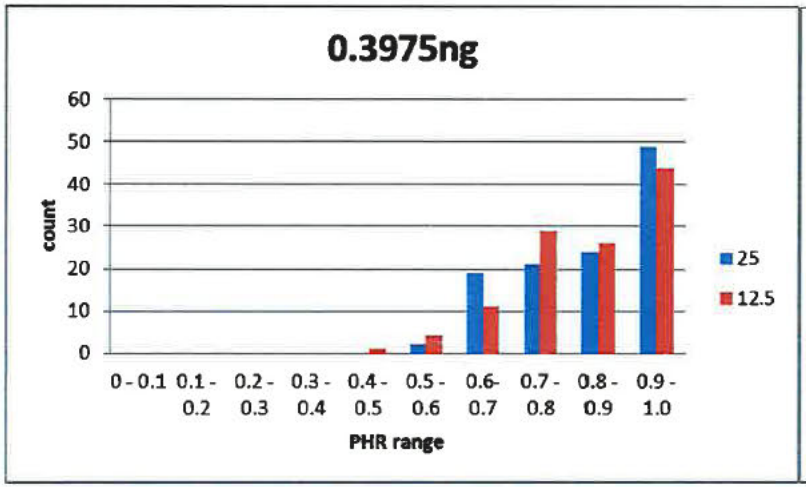


Figure 16 - The count of allele pairs per 0.1 PHR bin for 0.3375ng.

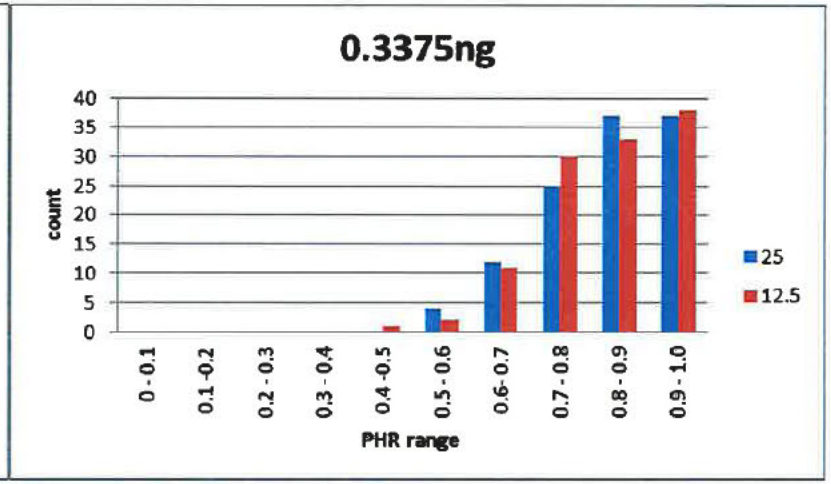


Figure 17 - The count of allele pairs per 0.1 PHR bin for 0.3975ng.

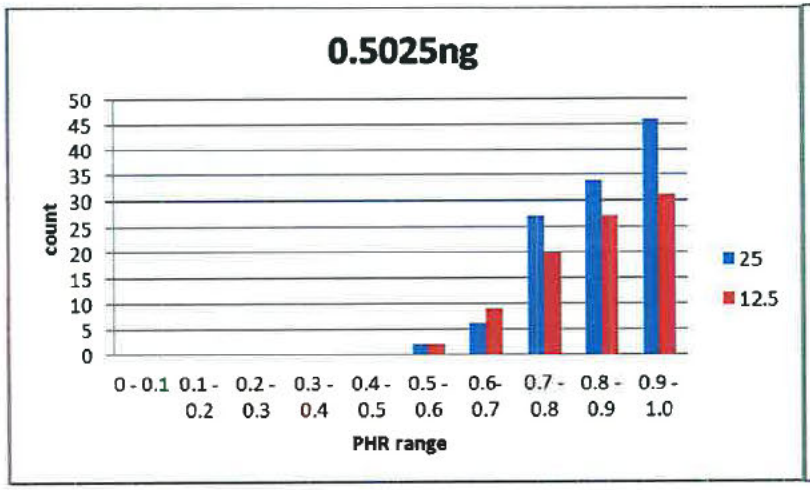


Figure 18 - The count of allele pairs per 0.1 PHR bin for 0.45ng.

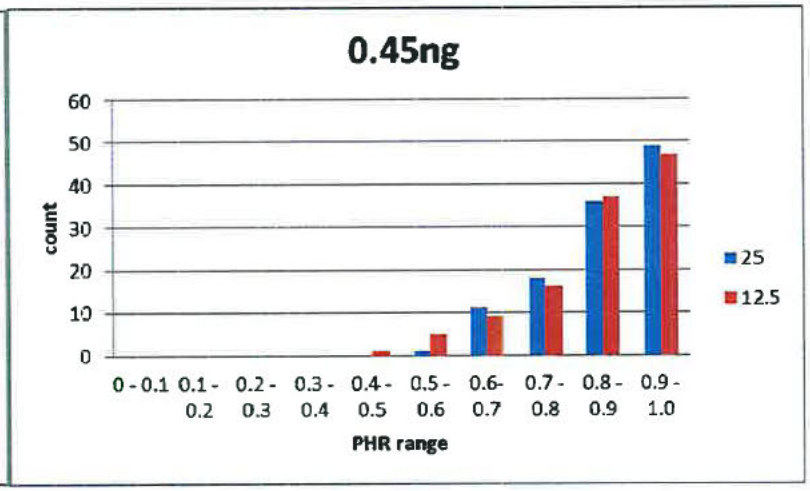


Figure 19 - The count of allele pairs per 0.1 PHR bin for 0.5025ng.

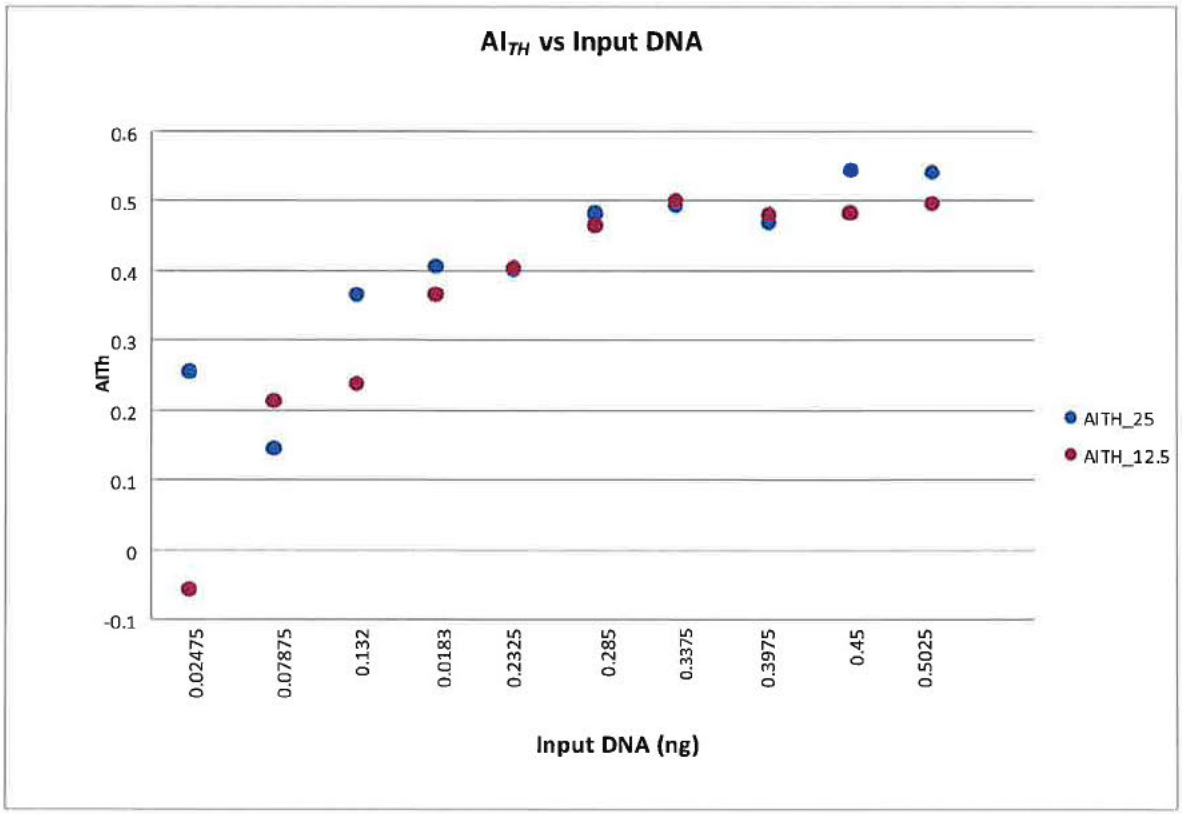


Figure 20 - Calculated AI_{TH} vs DNA template

6.7.2 Homozygote thresholds

The homozygote threshold is the threshold above which you can be confident that a heterozygote locus will not be incorrectly called as a homozygote locus.

Setting the homozygous threshold too high will result in excessive reworking of samples as a partial DNA profile would be called. Conversely, setting the threshold too low could result in false exclusions [1, 11, 23].

The method for determining the homozygote threshold varies in the literature. Traditionally, it had been arbitrarily designated at a particular level above the LOR. As already mentioned the risk of Type 1 and Type 2 errors should be balanced. Literature describes the setting of Th_{Hom} with respect to casework samples [21, 41, 42].

Previously in DNA Analysis, the Th_{Hom} was calculated as described in section 5.10 Equation 7. Using this method a figure of 176RFU for 25µL and 193FU for 12.5µL was calculated. These thresholds have been calculated excluding data below 0.132ng DNA template.

Another method of determining the Th_{Hom} is described in the Promega Internal Validation of STR systems reference manual[15]. This plots the peak height ratio for heterozygous loci against the lower RFU peak. The

threshold is defined as the point at which peak height ratio drops off significantly. Figures 21 and 22 display the data, the average AI_{TH} calculated for the range 0.132ng-0.5ng in section 6.7.1 for 25µL and 12.5µL respectively. An RFU that encompasses the majority of the data that falls below the average AI_{TH} calculated.

Unlike data reported in other publications[21, 43] there is not a rapid drop off of peak height ratios observed in the PowerPlex® 21 system, most likely due to the exclusion of the lower template data that exhibits extreme allelic imbalance. We have observed that the PowerPlex® 21 system loci tend to completely drop out completely compared to partially dropping out.

As both methods used give similar results, it is recommended the homozygote threshold be set at 200RFU for 25µL and 250RFU for 12.5µL.

These methods are subjective but when considered with the observed drop out data in Figures 23-32, Th_{Hom} of 200RFU would result in no type 2 errors. Additionally the threshold is more than three times the LOR threshold so Type 1 errors would also be addressed.

The homozygote threshold calculated in this validation will be used for extracted reference samples as case work samples do not require a homozygote threshold for STRmix™ analysis.

To ensure all of the thresholds set for this validation are appropriate a post implementation review of the thresholds will be performed. If the thresholds are found to be too conservative and have resulted in additional processing the review will provide an opportunity to re-adjust the thresholds based on empirical data.

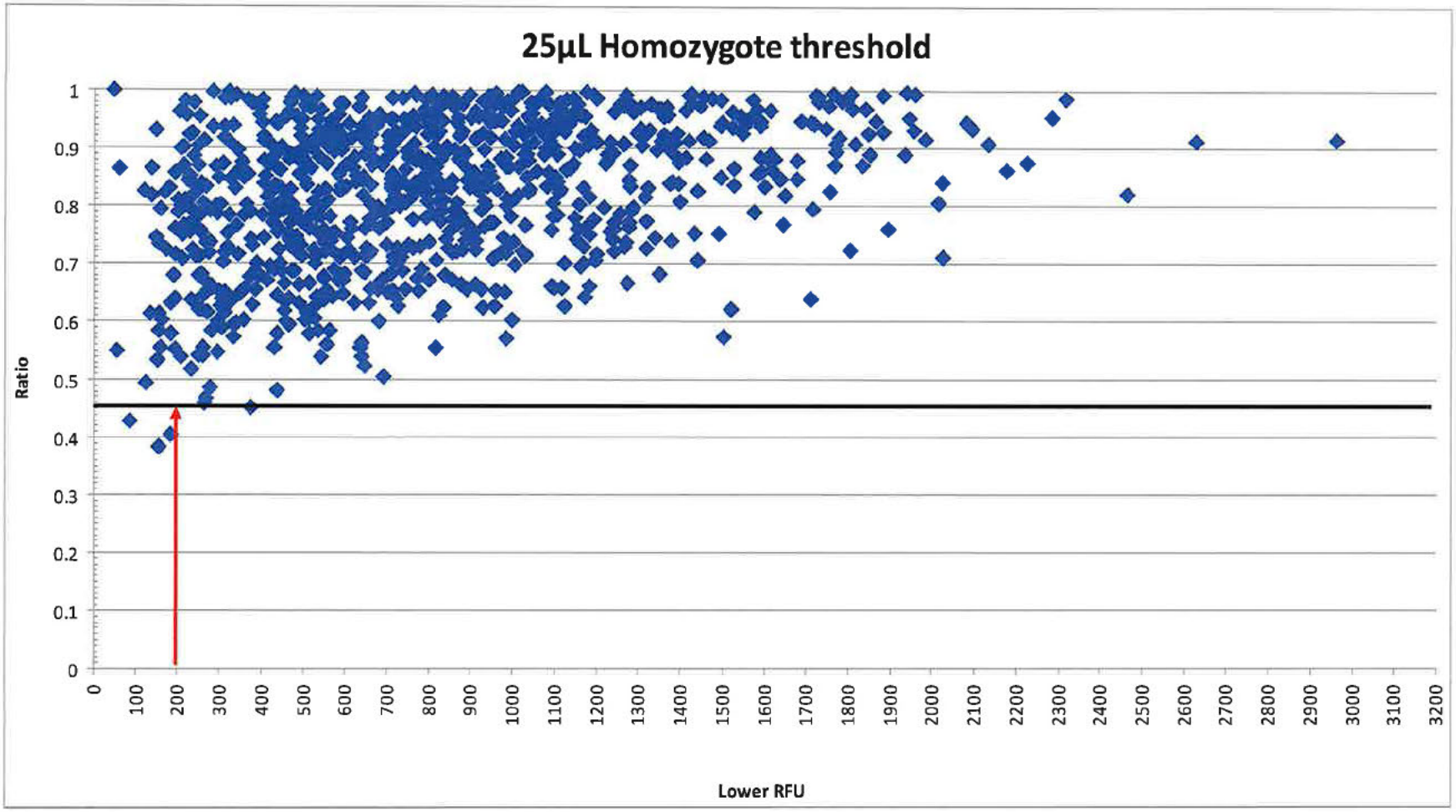


Figure 21 - Plot of the peak height ratio vs RFU of lower peak for 25µL. The black horizontal line is the AI_{TH}. The red vertical line is set to encompass the majority of points that fall below the AI_{TH}.

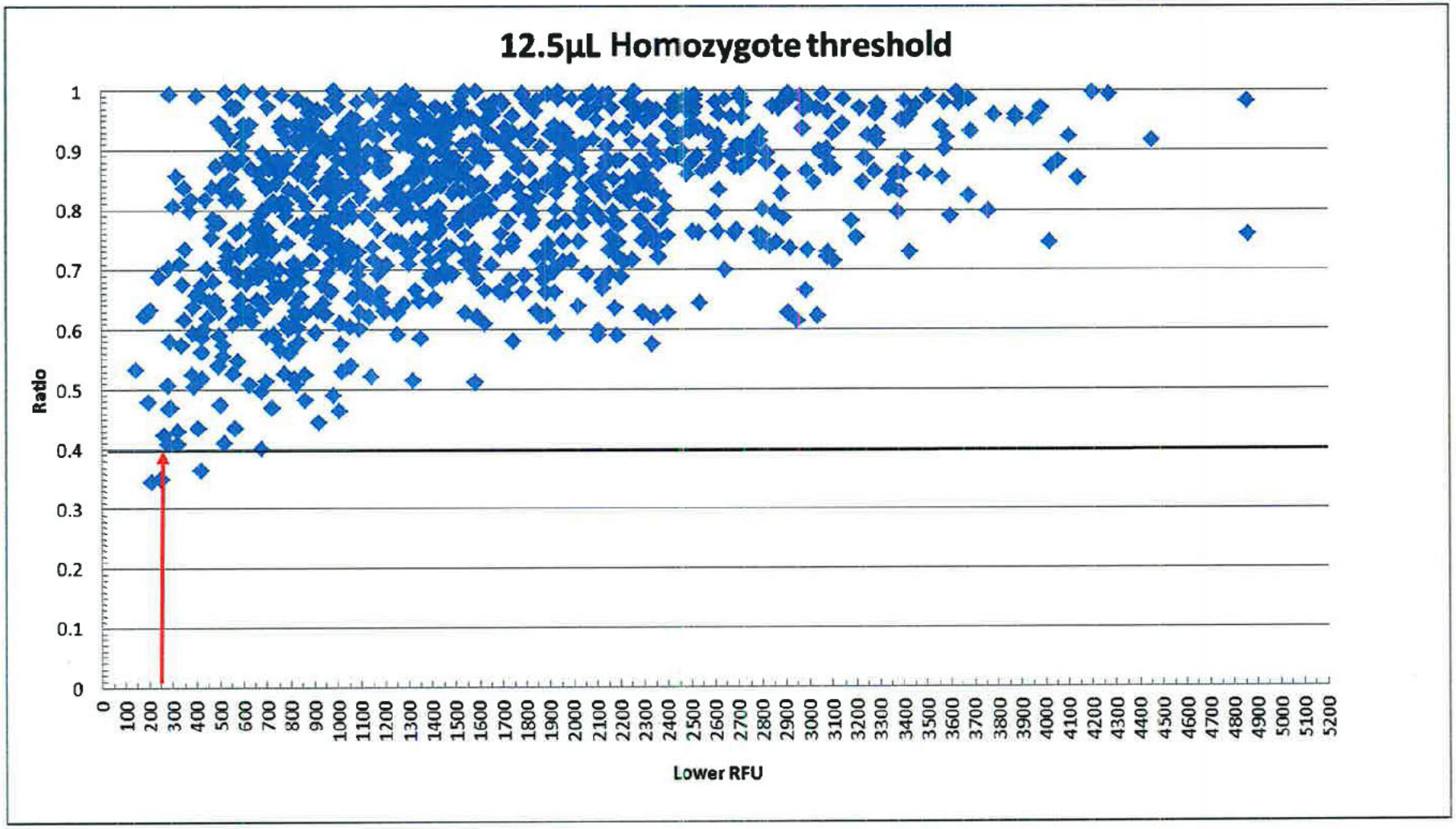


Figure 22 - Plot of the peak height ratio vs RFU of lower peak for 12.5µL. The black horizontal line is the AI_{TH}. The red vertical line is set to encompass the majority of points that fall below the AI_{TH}

6.8 Dropout Experiments

Allelic dropout is when one allele of a heterozygous pair has not appeared or has a very low peak height[44]. One cause of dropout is one allele of a heterozygous pair is preferentially amplified thus giving the false impression of a homozygous allele at a particular locus[31].

This experiment used sensitivity 1 data of the two donors from 1ng to 1pg the 4ng and 2ng data was excluded due to the excess nature of the profiles. The heat maps shown in figures 23, 24, 25 and 26 summarise the data to quickly compare the drop out events observed.

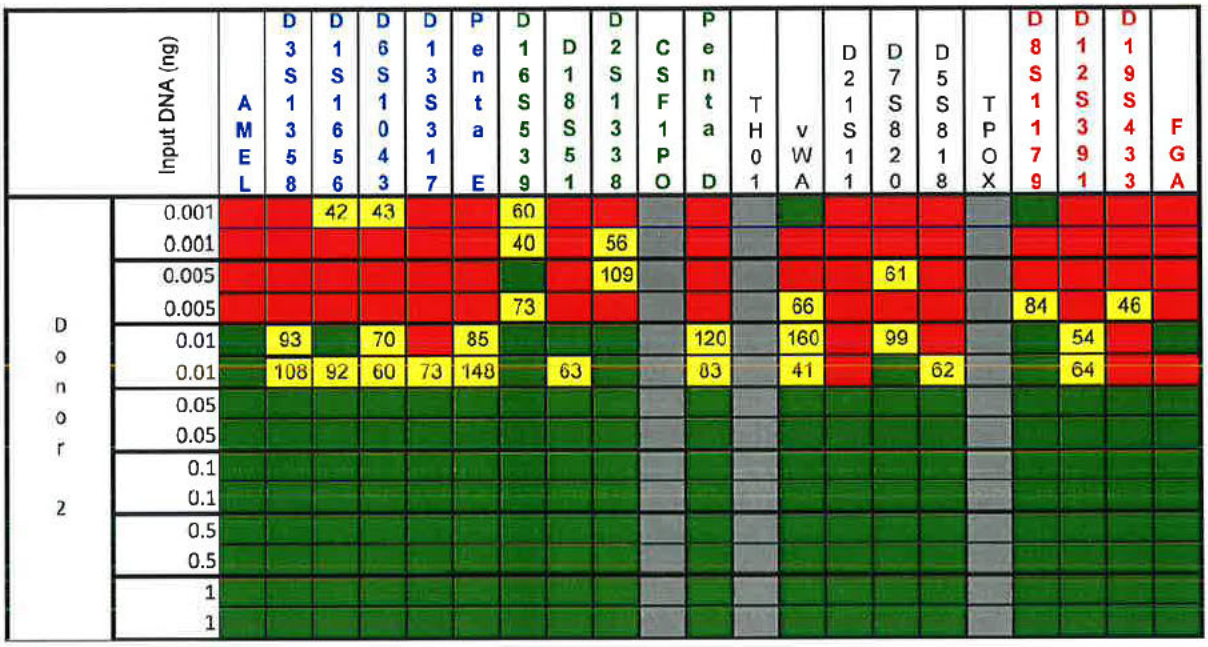
The data for the 25µL amplification shows 62 drop out events occurred across both donors from dilutions 0.001ng to 0.05ng. Figure 24 shows the highest peak height (RFU) where a heterozygous pair dropped out was at 160RFU for the 0.01ng dilution for donor 2 amplified at 25µL total PCR volume.

The data for the 12.5µL amplification shows 70 drop out events occurred across both donors from dilutions 0.001ng to 0.05ng. Figure 26 shows the highest peak height (RFU) where a heterozygous pair dropped out was at 399RFU for the 0.01ng dilution for donor 2.

Locus dropout
Allele dropout (surviving allele RFU)
Complete heterozygous locus
Homozygous locus

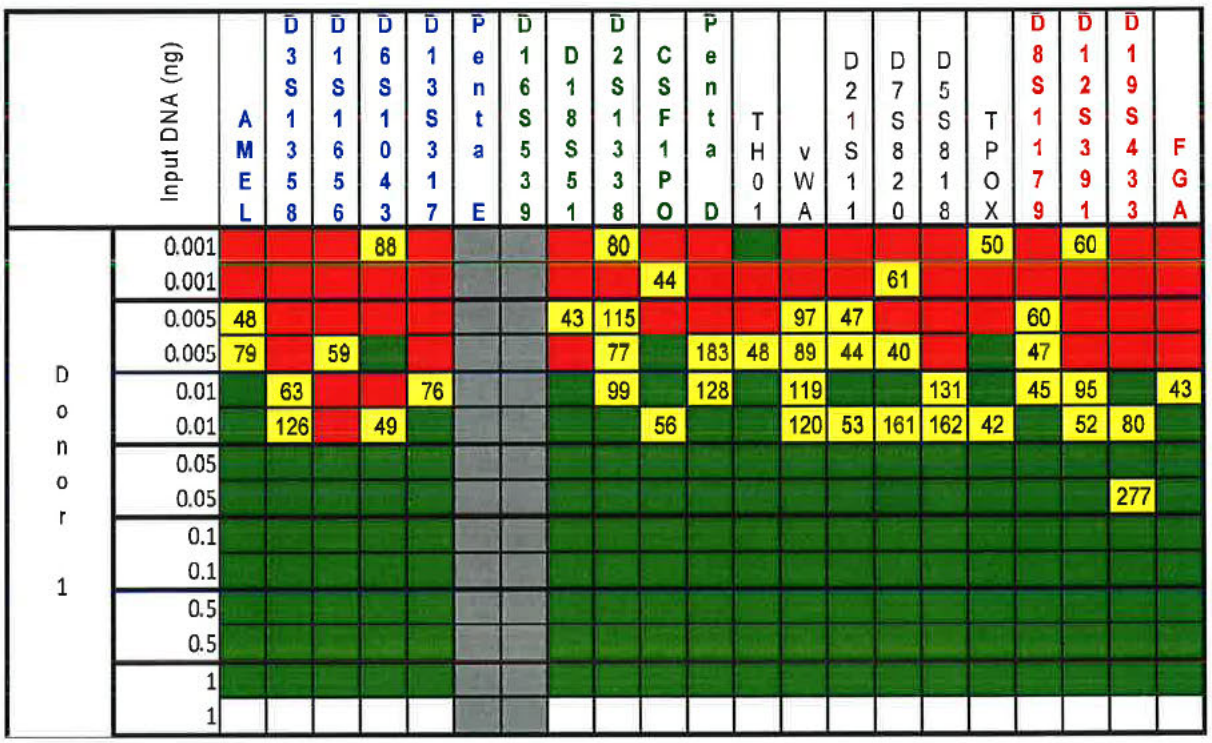
	Input DNA (ng)	AMEL	D3S1358	D1S1656	D6S1043	D13S317	Penta E	D16S539	D18S51	D2S1338	CSF1PO	Penta D	TH01	vWA	D21S11	D7S820	D5S818	TPOX	D8S1179	D12S391	D19S433	FGA
Donor 1	0.001										54											
	0.001								43													
	0.005	83												50		96					69	
	0.005	41		46		61								46				54	70			
	0.01	100	76	73						58	67	49		65	51		90	103	140			
	0.01	89								47			120		41	87	42		50	40	88	63
	0.05																131					
	0.05																					
	0.1																					
	0.1																					
	0.5																					
	0.5																					
	1																					
	1																					

Figure 23 - Heat map - Donor 1 - 25µL total PCR volume



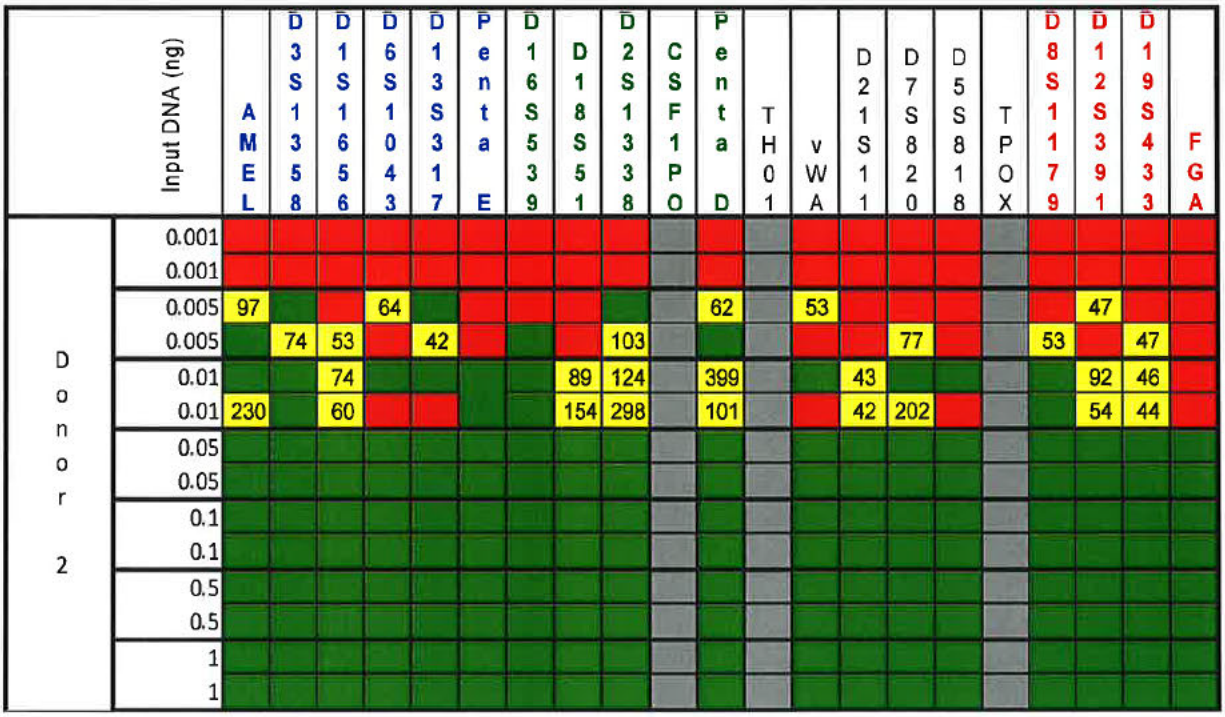
Donor 2	Input DNA (ng)	AMEL	D3S1358	D1S1656	D6S1043	D13S317	Penta E	D16S539	D18S51	D2S1338	CSF1PO	Penta D	TH01	vWA	D21S11	D7S820	D5S818	TPOX	D8S1179	D12S391	D19S433	FGA
	0.001			42	43			60														
	0.001							40		56												
	0.005									109						61						
	0.005							73						66					84		46	
	0.01		93		70		85					120		160		99				54		
	0.01		108	92	60	73	148		63			83		41			62			64		
	0.05																					
	0.05																					
	0.1																					
	0.1																					
	0.5																					
	0.5																					
	1																					
	1																					

Figure 24 - Heat map - Donor 2 - 25µL total PCR volume



Donor 1	Input DNA (ng)	AMEL	D3S1358	D1S1656	D6S1043	D13S317	Penta E	D16S539	D18S51	D2S1338	CSF1PO	Penta D	TH01	vWA	D21S11	D7S820	D5S818	TPOX	D8S1179	D12S391	D19S433	FGA
	0.001				88					80								50		60		
	0.001										44					61						
	0.005	48							43	115				97	47				60			
	0.005	79		59						77		183	48	89	44	40			47			
	0.01		63			76				99		128		119			131		45	95		43
	0.01		126		49						56			120	53	161	162	42		52	80	
	0.05																					
	0.05																				277	
	0.1																					
	0.1																					
	0.5																					
	0.5																					
	1																					
	1																					

Figure 25 - Heat map - Donor 1 - 12.5µL total PCR volume



	Input DNA (ng)	AMEL	D3S1358	D1S1656	D6S1043	D13S317	Penta E	D16S539	D18S51	D2S1338	CSF1PO	Penta D	TH01	vWA	D21S11	D7S820	D5S818	TPOX	D8S1179	D12S391	D19S433	FGA
Donor 2	0.001																					
	0.001																					
	0.005	97			64							62		53						47		
	0.005		74	53		42				103						77			53		47	
	0.01			74					89	124		399			43					92	46	
	0.01	230		60					154	298		101			42	202				54	44	
	0.05																					
	0.05																					
	0.1																					
	0.1																					
	0.5																					
	0.5																					
	1																					
	1																					

Figure 26 - Heat Map – Donor 2 - 12.5µL total PCR volume

6.8.1 Drop out 2

Analysis for drop out 2 used the data obtain from the Aboriginal dataset, 10 x10 and both sensitivity experiments for 25µL total PCR volume and the 10 x10, both sensitivity experiments and concordance for 12.5µL total PCR volume. The dropout 2 results are displayed in figures 27 and 28. Figure 27 shows the dropout events for all samples amplified at 25µL total PCR volume. Figure 28 shows the dropout events for all samples amplified at 12.5µL total PCR volume.

For both 25µL and 12.5µL total PCR volume amplifications there are more drop out events of whole loci compared with a single allele drop out event

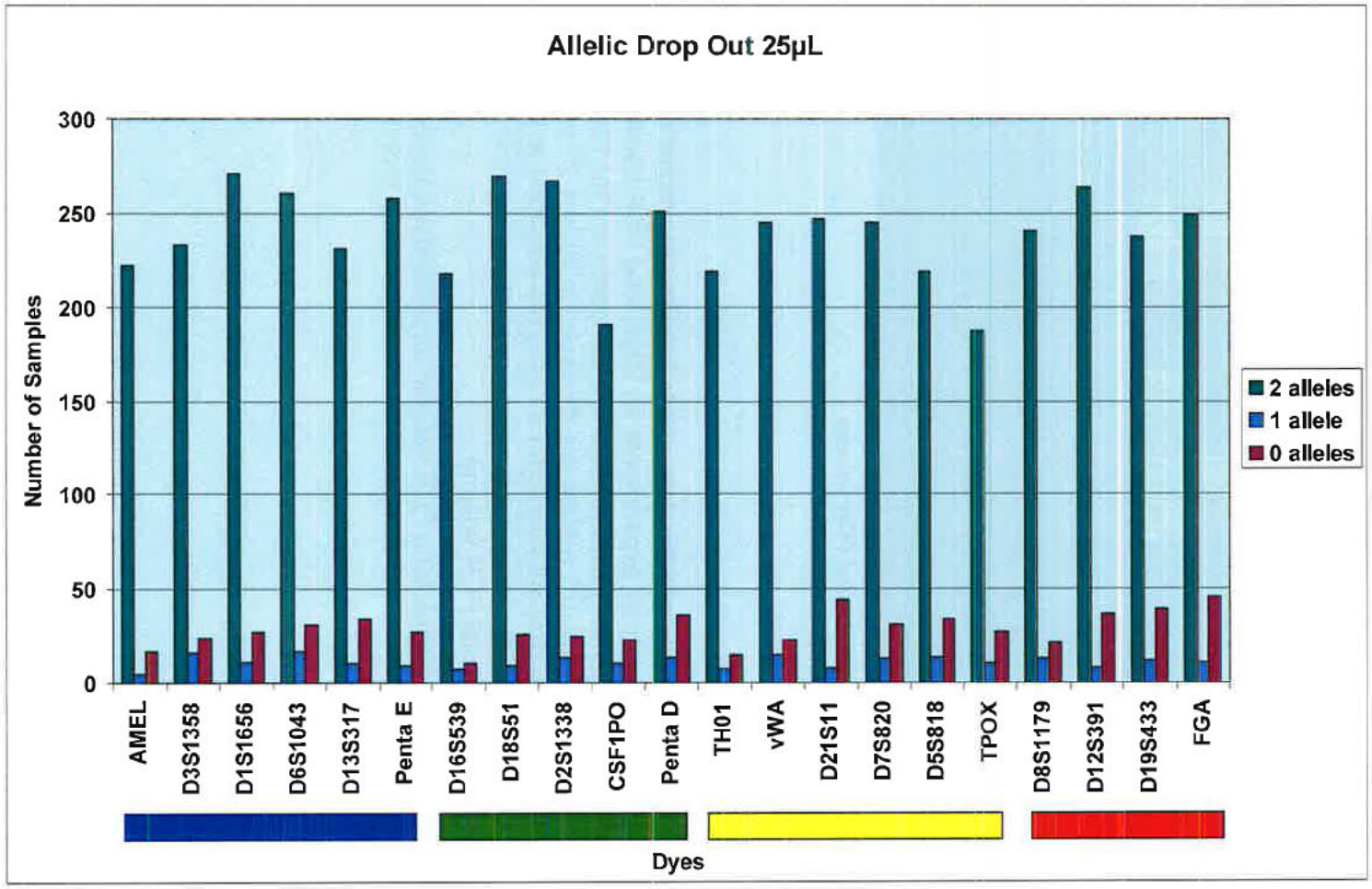


Figure 27 - Dropout events for samples amplified at 25µL

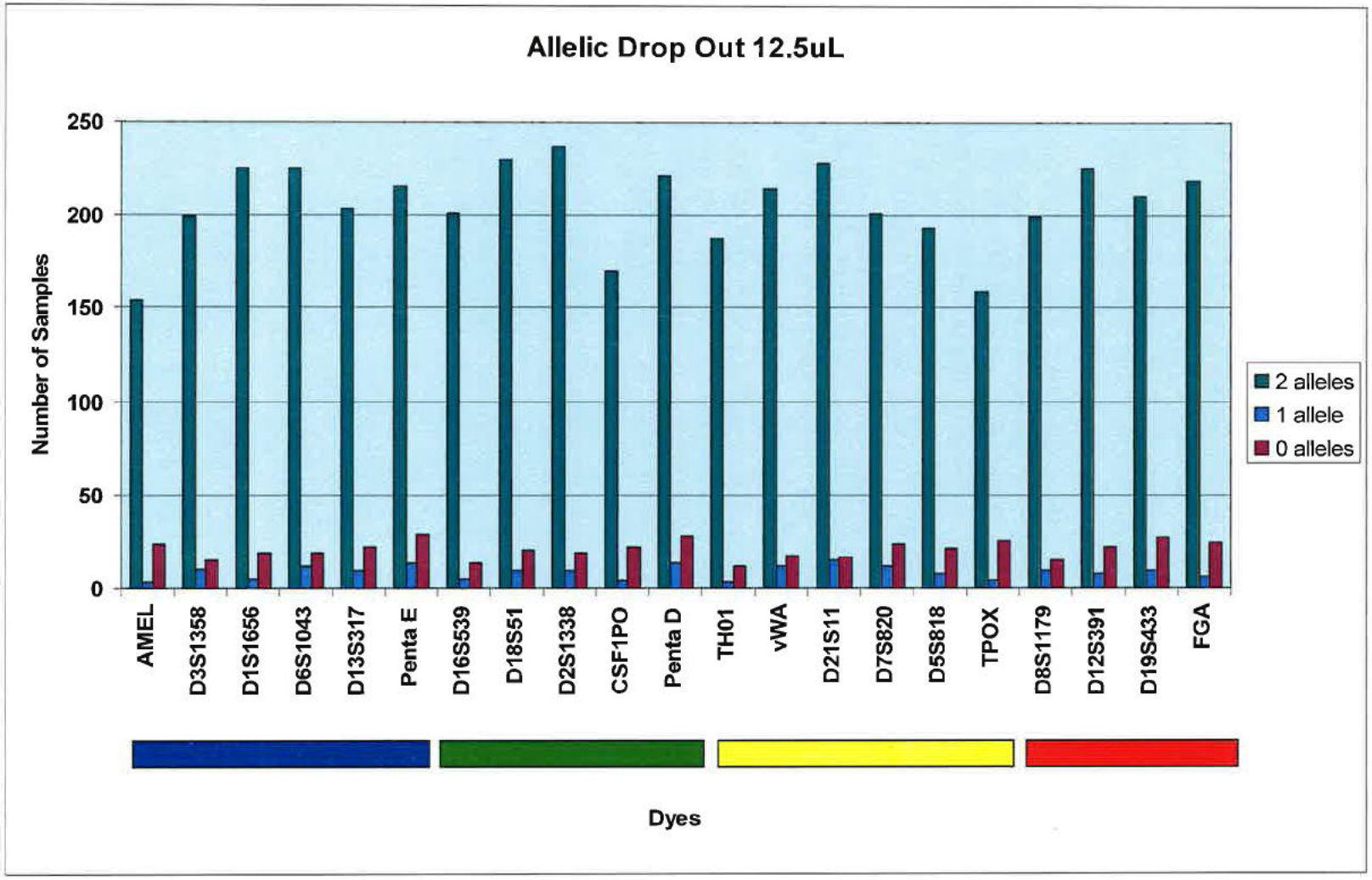


Figure 28 - Dropout events for samples amplified at 12.5µL

6.8.2 Drop out 3

Analysis for drop out 3 used the data from the baseline (10 x 10) and both sensitivity experiments at both 25µL and 12.5µL total PCR volume. There were 215 drop out events observed for the 25µL total PCR volume compared to198 drop out events observed at 12.5µL total PCR volume. Figure 29 shows the number of drop out events for a range of peak heights. This shows the majority of drop out events occur below 150RFU for 25µL total PCR volume and below 180RFU for 12.5µL total PCR volume.

Figures 30, 31 and 32 show the peak heights where one of the heterozygote pairs has dropout at each DNA template. Figure 30 shows one dropout event occurred at 226RFU for the 12.5µL total PCR volume at a DNA template of 0.131ng whereas 17 dropout events occurred at 25 µL total PCR volume at the same DNA template, however these dropout events occurred under 80RFU. The highest drop out seen for 12.5µL total PCR volume was at 234RFU at a DNA template of 0.025ng and for 25µL total PCR volume was at 106RFU. The total number of dropout events seen for the 10 x10 at 25µL total PCR volume was 68 and 30 at 12.5µL total PCR volume.

Figure 31 (Sensitivity 1) shows the highest drop out for 12.5µL total PCR volume was seen at 399RFU at a DNA template of 0.01ng and 160RFU at DNA template 0.01ng for the 25µL total PCR volume. The total number of dropout events seen for the sensitivity 1 experiment at 25µL total PCR volume was 58 and 66 at 12.5µL total PCR volume.

Figure 32 (Sensitivity 2) shows the highest drop out for 12.5µL total PCR volume was seen at 246RFU at a DNA template of 0.0094ng and 249RFU at a DNA template of 0.0375ng for the 25µL total PCR volume. The total number of dropout events seen for the sensitivity 2 experiment at 25µL total PCR volume was 89 and 102 at 12.5µL total PCR volume.

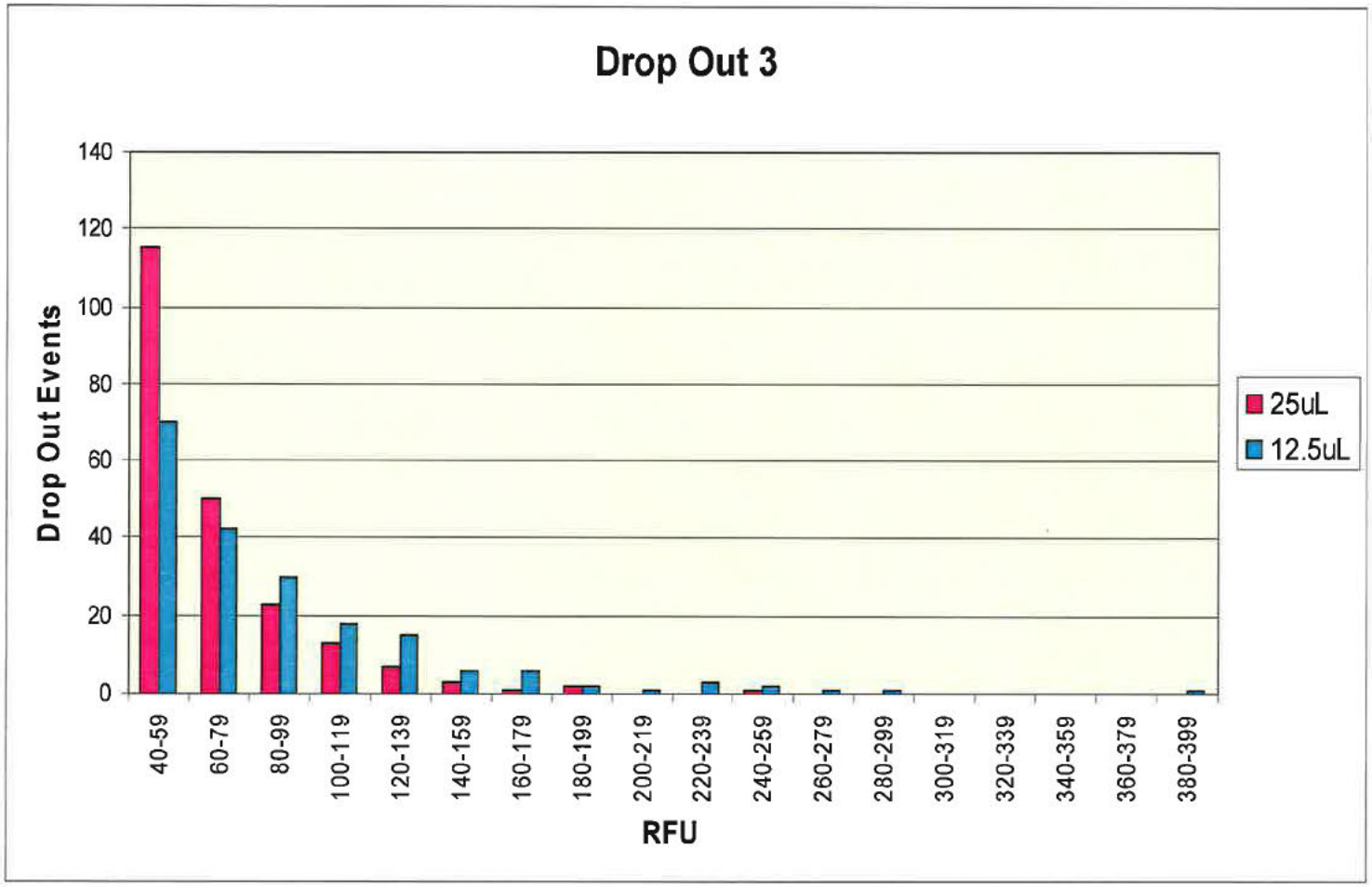


Figure 29 - Number of drop out events seen within peak height ranges at 25µL and 12.5µL amplifications

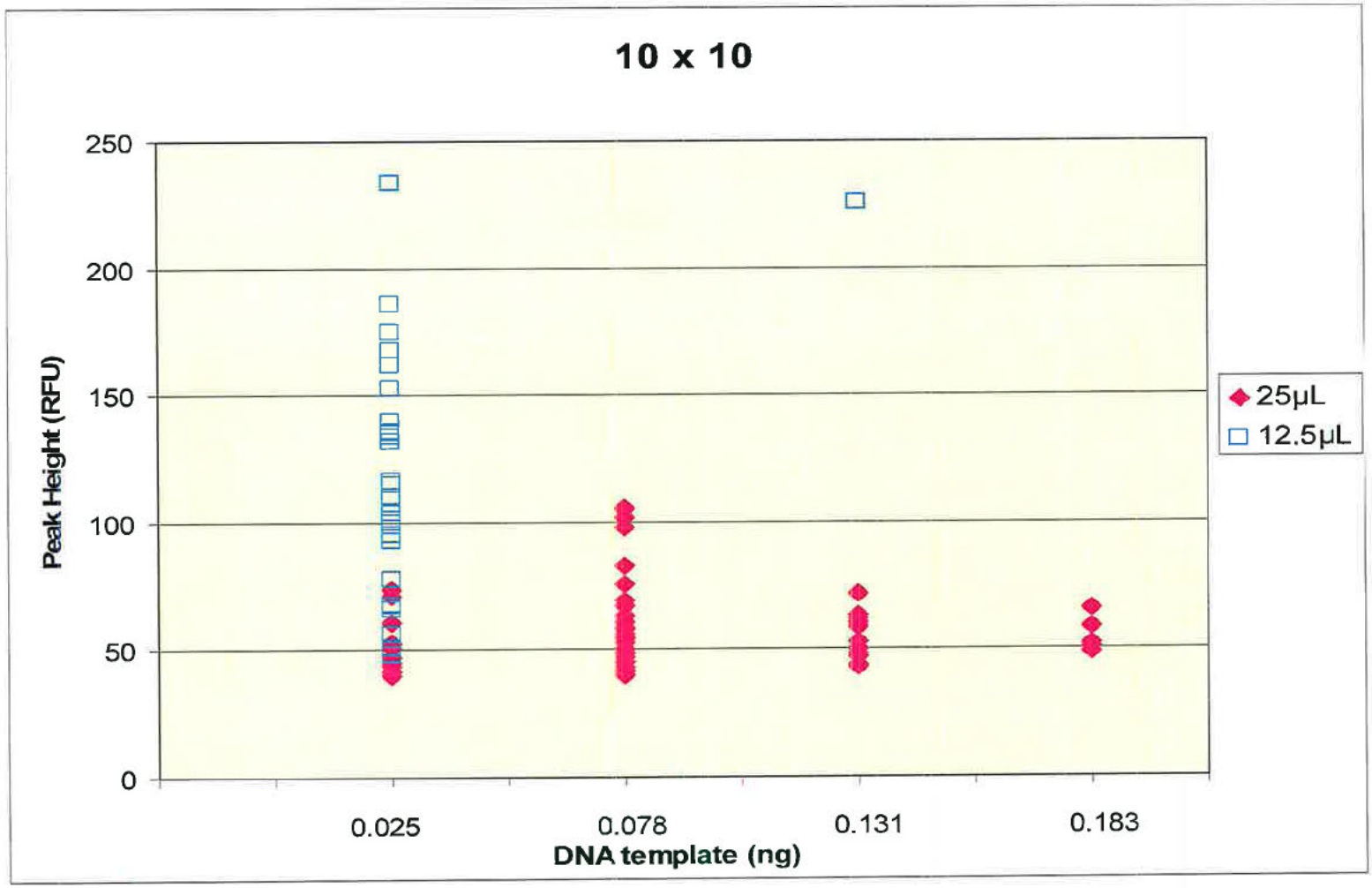


Figure 30 - Peak heights where the heterozygote pair has dropped out at different DNA templates for 12.5uL and 25uL using the baseline data (10 x10)

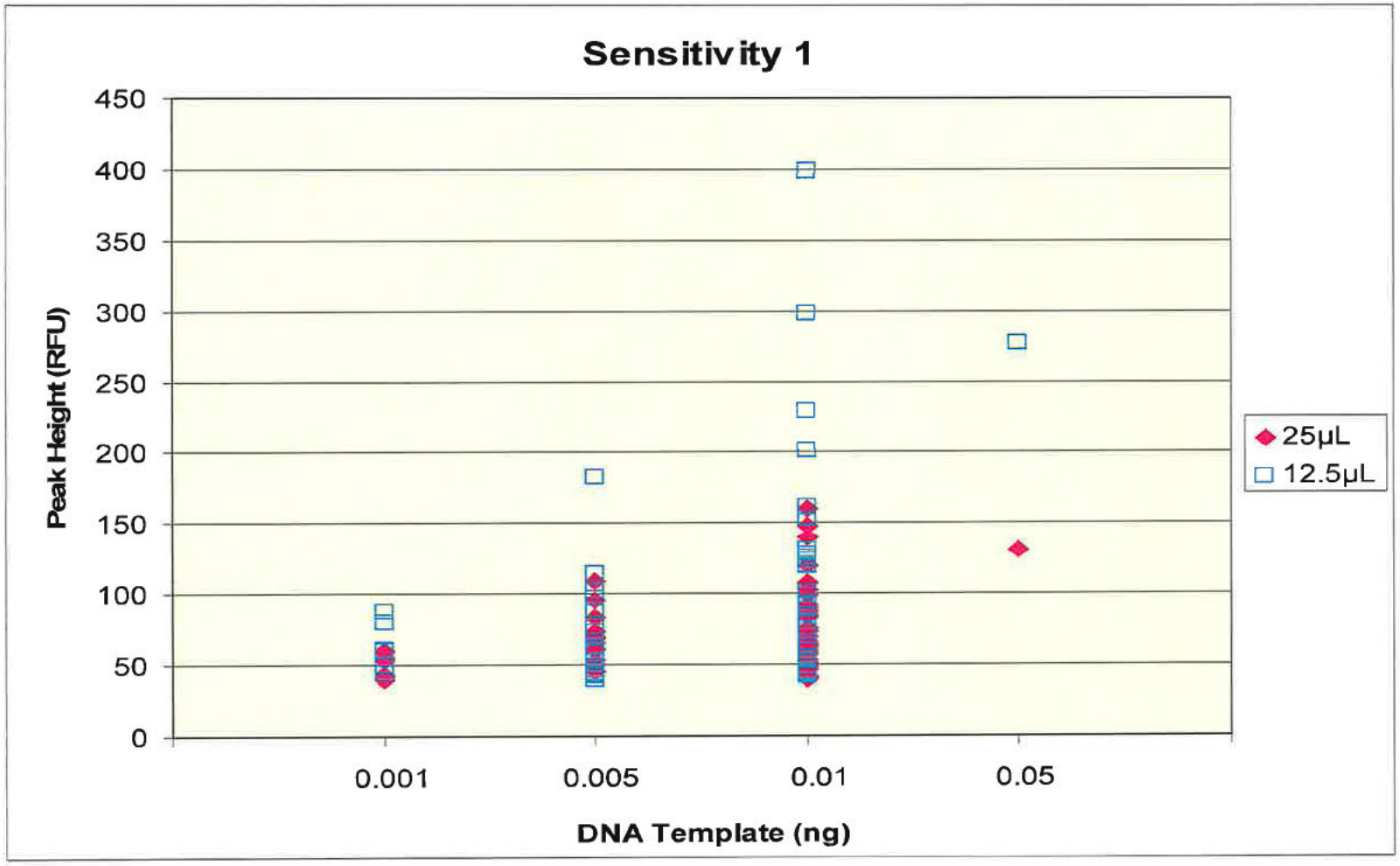


Figure 31 - Peak heights where the heterozygote pair has dropped out at different DNA templates for 12.5uL and 25uL using sensitivity 1 data

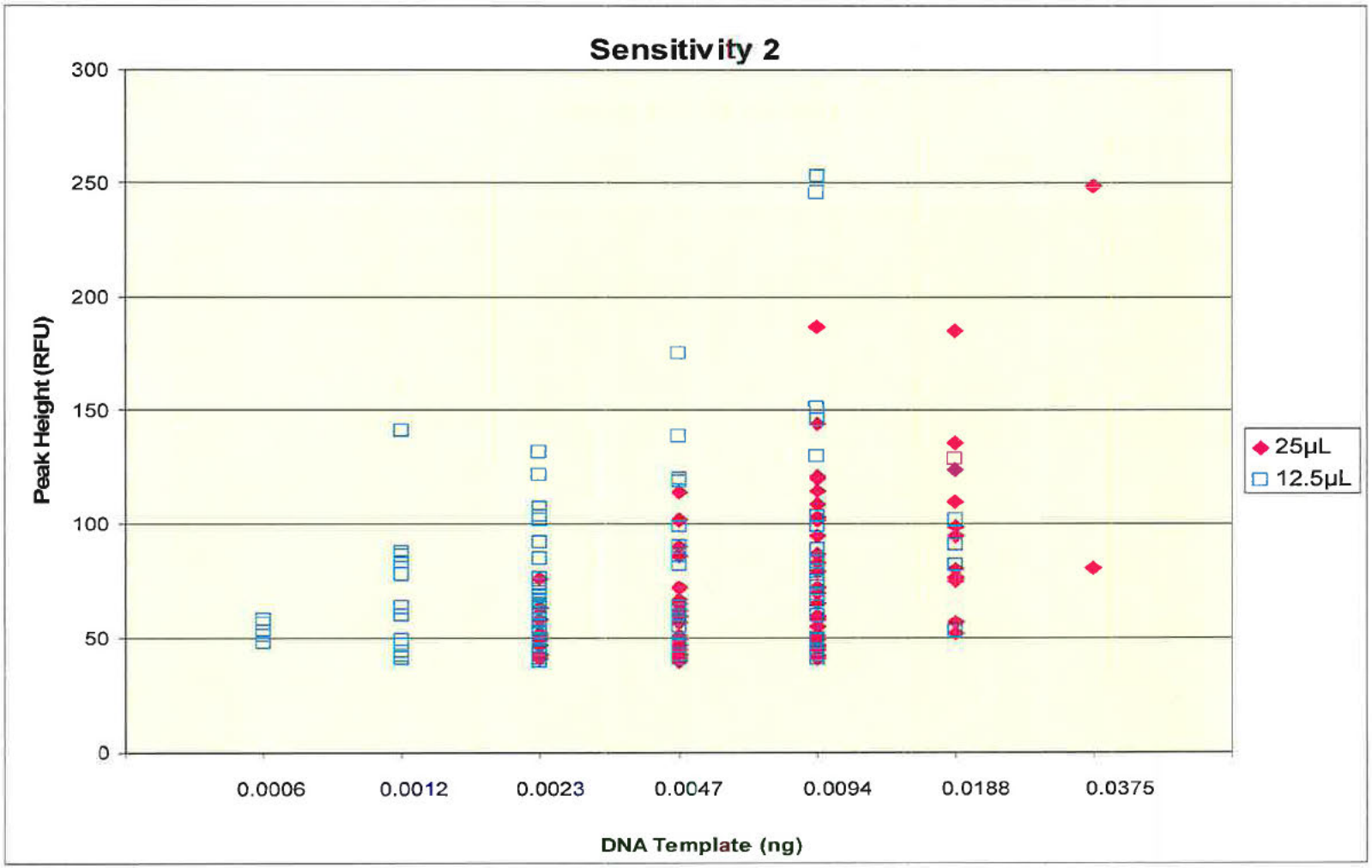


Figure 32 - Peak heights where the heterozygote pair has dropped out at different DNA templates for 12.5uL and 25uL using sensitivity 2 data

6.9 Mixture Studies

At a total input template of 0.5ng, for both 25µL and 12.5µL, all alleles were detected for the mixtures with ratios of 1:1, 2:1 and 5:1. Any allelic imbalance was observed at a level of greater than 40%.

When the template was decreased to 0.125ng for 5:1 mixtures, drop-out of the lower level contributor was observed for both 25µL and 12.5µL volumes. At this template level, allelic imbalance of down to 35% was observed for the lower level contributor at both 25µL and 12.5µL volumes, however, one of these peaks fell into the stutter position of the larger contributor.

When the template was decreased to 0.06ng for 2:1 mixtures, drop-out of the lower level contributor was observed for both 25µL and 12.5µL volume with the partner allele being as high as 562RFU. At this template level, allelic imbalance of down to 20% was observed for the lower level contributor and 23% for the higher level contributor.

For the remaining mixtures with ratios of 10:1, 20:1, 30:1 and 50:1 of varying template levels (maximum 0.5ng), the lower contributors exhibited, sometimes quite marked, stochastic variation. This included drop-out with peaks up to 392RFU and allelic imbalance as low as 20%.

The tables 16 and 17 show the approximate mixture ratio of the profile compared with the mixture ratio of the sample. For the 2 person mixtures this was averaged over all loci where there was no allele sharing between the two contributors and where the alleles did not fall into a stutter position. For the 3 person mixtures, the ratio was averaged over all loci where there was no allele sharing between the three contributors, however it was not possible to exclude loci where the alleles fell into stutter positions as there were no loci fulfilling this criteria. It was not possible to accurately calculate mixture ratios for the four person mixtures.

The data shows that the mixture ratio after DNA amplification is approximately equal to the mixture ratio of the initial sample for both 25µL and 12.5µL volumes at all ratios. The mixture ratio deviates more as the ratio increases most likely due to the stochastic effects of the lower contributor. The mixture ratios for the 25µL volume amp appear to be slightly lower than for the12.5µL volume amp.

Although mixture ratios have not been calculated for the four person mixtures, the alleles obtained are consistent with expected profiles.

Table 18 - 12.5µL total PCR volume mixture studies

Mixture Ratio of Sample	Total Input Template (ng)	Approximate Mixture Ratio of Profile
2 Person Mixtures		
1:1	0.500	1.2:1
2:1	0.500	2.2:1
	0.060	2.9:1
5:1	0.500	6.1:1
	0.125	6.1:1
10:1	0.500	12:1
	0.125	11:1
20:1	0.500	24:1
	0.250	16:1
	0.125	19:1
30:1	0.500	21:1
50:1	0.500	35:1
	0.250	49:1
	0.125	Unable to calculate
3 Person Mixtures		
5:2:1	0.500	4.2:1.3:1
	0.125	Unable to calculate
10:5:1	0.500	13:9.1:1
20:10:1	0.500	10:5.7:1
	0.125	Unable to calculate
4 Person Mixtures		
5:3:2:1	0.500	Unable to calculate
	0.125	Unable to calculate

Table 19 - 25µL total PCR mixture studies

Mixture Ratio of Sample	Total Input Template (ng)	Approximate Mixture Ratio of Profile
2 Person Mixtures		
1:1	0.500	1.2:1
2:1	0.500	1.8:1
	0.060	1.7:1
5:1	0.500	4.1:1
	0.125	4.8:1
10:1	0.500	8.5:1
	0.125	6.3:1
20:1	0.500	22:1
	0.250	17:1
	0.125	10:1
30:1	0.500	15:1
50:1	0.500	26:1
	0.250	9.2:1
	0.125	6.7:1
3 Person Mixtures		
5:2:1	0.500	2.9:1.5:1
	0.125	2.7:1.1:1
10:5:1	0.500	7.4:5.4:1
20:10:1	0.500	10:6.4:1
	0.125	10:4.7:1
4 Person Mixtures		
5:3:2:1	0.500	Unable to calculate
	0.125	Unable to calculate

7 Conclusion

The results from this validation support that Promega's PowerPlex®21 System is suitable for analysis of STRs.

Despite slight differences observed between the two 3130xl analysers, the use of single LOD and LOR of 16RFU and 40RFU is more practical for use in DNA Analysis.

The PowerPlex21® System displays full concordance with all alleles observed in testing being concordant.

The three national population datasets (Caucasian, Aboriginal and SE Asian) created collaboratively within Australia, have been externally validated and will be implemented in conjunction with STRmix™ for statistical interpretation.

12.5µL total PCR volumes gave higher peak heights than their 25µL counterparts at the same DNA template.

The PowerPlex®21 system is a very sensitive amplification kit when used at either the standard amplification volume (25µL) or reduced volume amplification (12.5µL); however the increased sensitivity does not necessarily result in more reliable information.

The two sensitivity experiments explored the range on DNA template inputs from very large inputs (4ng) to very small inputs (0.00059ng). Within this validation complete PowerPlex® 21 DNA profiles were obtained with as little as 0.01875ng of template DNA. However, the PHR data indicate that as the amount of template DNA decreases the μ_{PHR} decreases and σ_{PHR} increases. The risk of type 2 errors is greatly increased from template DNA amounts of less than 0.132ng for both 25µL and 12.5µL total PCR volumes, which is supported by the experimental drop out data.

The data presented within this report indicates that input templates less than 0.132ng total DNA (concentrations 0.0176ng/µL if using 12.5µL total PCR volume or 0.0088ng/µL for 25µL total PCR volume) may result in increased stochastic effects.

As previously documented in DNA Analysis[45, 46], the Quantifiler™ Human DNA Quantification kit gives an estimate of the DNA concentration. Careful consideration of the DNA profile is required before reporting because the precision within a quantification method and between different quantification methods may vary.

For the range of DNA templates specified above, significant differences between 12.5µL and 25µL total PCR volumes was not observed. The use of 12.5µL total amplification volume as the default protocol with DNA Analysis is indicated. The disadvantage of the 12.5µL total PCR volume are the physical constraints of the process i.e. a maximum of 7.5µL of sample can be used compared with 15µL for the 25µL total PCR volume. However, higher peak heights and the cost savings associated with reduced volume amplifications even with additional processes to increase the sample concentration, mitigate the disadvantage.

The implementation of PowerPlex® 21 for amplification of DNA extracts will coincide with the implementation of STRmix™. The combination of the two processes will apply a continuous biological model rather than a binary model to DNA interpretation. STRmix™ models stutter, drop out, heterozygote balance and homozygote threshold for case work samples.

The rate of drop in events has been calculated for both total PCR volumes and will be implemented in conjunction with STRmix™.

At a total input template of 0.5ng, for 25µL and 12.5µL total PCR volumes, all alleles were detected for the mixtures with ratios of 1:1, 2:1 and 5:1.

For the remaining mixtures with ratios of 10:1, 20:1, 30:1 and 50:1 of varying template levels (maximum 0.5ng), the lower contributors exhibited, sometimes quite marked, stochastic variation.

Mixture interpretation is beyond the scope of this validation and will be dealt with in the STRmix™ validation report.

8 Recommendations

1. A common LOD/LOR (16RFU/40RFU) will be used for both 3130xl instruments as outline in section 6.4.
2. The default total PCR volume will be 12.5µL. Samples can also be amplified at 25µL total PCR volume.
3. Initially samples with concentrations below 0.01ng/µL will not be routinely processed in the first instance. If necessary, these samples may undergo post extraction concentration via centrifugal filter concentration procedure to increase the concentration or re-amplify at 25µL total PCR volume.
4. Initially samples with concentrations between 0.01ng/µL and 0.0176ng/µL will not be routinely amplified. These samples are considered as candidates for post extraction concentration via centrifugal filter concentration procedure to increase the concentration to the point that stochastic effects are minimized.
5. Initially samples with concentrations between 0.0176ng/µL and 0.0244ng/µL will be amplified and assessed for stochastic effects during case management to ensure the suitability of these DNA profiles for reporting.
6. Samples with concentrations above 0.0244ng/µL will be routinely amplified.
7. AI_{TH} to be set at 40% and Hom_{TH} 250RFU for extracted reference, environmental and quality control samples amplified at 12.5µL total PCR volume.
8. AI_{TH} to be set at 45% and Hom_{TH} 200RFU for extracted reference, environmental and quality control samples amplified at 25µL total PCR volume.

9. Adoption of the national Caucasian, Asian and Aboriginal sub-population datasets that DNA Analysis contributed to as part of this validation for use within statistical calculations.
10. Adoption of the locus specific stutter filter as per results section.
11. Thresholds listed in 7 and 8 are to be used as a guidelines when assessing the number of contributors in a mixture.
12. A post implementation review should be performed to review the appropriateness of points 3 – 8. The review will at minimum examine the outcomes of samples amplified within 0.0176ng/µL and 0.0244ng/µL concentration range. Similarly, all of the extracted reference samples will be reviewed with regards to the AI_{TH} and homozygote threshold.

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PB151

Fletcher, Caitlin 67002

From: Kirsten Scott <[redacted]
Sent: Friday, 9 September 2022 10:25 AM
To: Thomas Nurthen; Paula Brisotto
Subject: FW: Minor Change - Comparison of NIST Standards 2372 and 2372a
Attachments: Proposal for Comparison of NIST Standards 2372 and 2372a.pdf

Tom,

Complete but not yet signed off.
Welcome to send feedback if you think it is needed

Kirsten

From: Tara Prowse <[redacted]
Sent: Friday, 9 September 2022 6:43 AM
To: Paula Brisotto <[redacted] Luke Ryan <[redacted] Kirsten Scott <[redacted]
Cc: Chelsea Savage <[redacted]
Subject: Minor Change - Comparison of NIST Standards 2372 and 2372a

Hi All,

Attached is the PDF version of the Proposal for Minor Change - Comparison of NIST Standards 2372 and 2372a ready for signing. It can be found:

I:\Change Management\Minor Change Forms - completed\NIST Standard\Proposal for Comparison of NIST Standards2372 and 2372a

Regards,
Tara

Tara Prowse
Scientist – Analytical Team

Forensic DNA Analysis, Forensic and Scientific Services
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CLEAN HANDS
SAVE LIVES
Wash your hands regularly
to stop the spread of germs.

Minor Process Change

Stage 2

		Project #:	
Proposed by :	Tara Prowse and Chelsea Savage	**Date:**	13/07/2022
Title:	Comparison of NIST Standards 2732 and 2732a		
Comment to be added to SOP:	☐ Yes QIS#_____ ☐ No	**Completed date:**	
Email communication sent:	☐ Yes Team/s_____ ☐ No	**Completed date:**	
Add to minor change register	☐ Yes	**Completed date:**	

Outline of Minor Change:

During the "Yfiler™ Plus Validation and implementation" (Project #206) it was found that the NIST standards (NIST SRM 2372) used in the "Validation of the two QuantStudio™ 5 Real-Time PCR Systems" (Project #185) were used after the expiration of the Certificate of Analysis. The majority of the labwork for Project #185 was performed in March 2018. The Certificate of Analysis provides apparent absorbance values for the NIST Standards within specified uncertainty and was valid until its expiry on 31 December 2017. These findings are outlined more extensively in OQI# 56218 – Use of NIST standard in Project #185. The current NIST standards (NIST SRM 2372a) have not been opened and have an expiration date of 13 February 2023. As the NIST SRM 2372 standards used in the 2018 validation still had volume remaining, we were able to test and assess their current accuracy against the original Certificate of Analysis. Similarly we could test the accuracy of NIST SRM 2372a standards against the Certificate of Analysis.

This document has been written to propose that NIST SRM 2372 and NIST SRM 2372a be compared to their relevant Certificates of Analysis to provide more detailed information for OQI# 56218. The two NIST standards were diluted to four known concentrations for each set of NIST standards and were Quantified in triplicate. The DNA concentrations of NIST SRM 2372 and NIST SRM 2372a were compared to their Certificates of Analysis.

Experimental Design

Serial dilutions were calculated and prepared for using NIST SRM 2372a in Project #206 (I:\Change Management\Proposal#206 - Y Filer Plus\2.0_Project Planning\3.13 Mixture studies). These calculations were used for the serial dilutions for the NIST SRM 2372. All Standards were diluted to a normalised 10 ng/µL (using concentrations from the Certificate of Analysis) and serial dilutions performed. Table 1 outlines the serial dilutions for NIST SRM 2372. The samples highlighted in green were the proposed concentrations to be used for this experiment. Two are in the high concentration range, one mid range and one low range. The same concentrations were used for NIST SRM 2372a.

Table 1 Dilution Calculations (2372)

Sample	Desc	Conc ng/µL	T Vol µL	R vol µL	V req µL	D req µL	Quant µL
Neat	NIST A	57	55	37	18	82	0
1	NIST A_1	10	100	67	33	67	6
2	NIST A_2	3.333333	100	67	33	67	6
3	NIST A_3	1.111111	100	61	33	67	0
4	NIST A_4	0.37037	100	61	33	67	6
5	NIST A_5	0.123457	100	61	33	67	0
6	NIST A_6	0.041152	100	67	33	67	6
7	NIST A_7	0.013717	100	61	33	67	0
8	NIST A_8	0.004572	100	67	33	67	0
9	NIST A_9	0.001524	100	94	0	0	0
Neat	NIST B	61	55	39	16	84	0
1	NIST B _1	10	100	67	33	67	6
2	NIST B _2	3.333333	100	67	33	67	6
3	NIST B _3	1.111111	100	61	33	67	0
4	NIST B _4	0.37037	100	61	33	67	6
5	NIST B _5	0.123457	100	61	33	67	0
6	NIST B _6	0.041152	100	67	33	67	6
7	NIST B _7	0.013717	100	61	33	67	0
8	NIST B _8	0.004572	100	67	33	67	0
9	NIST B _9	0.001524	100	94	0	0	0
Neat	NIST C	59	55	38	17	83	0
1	NIST C_1	10	100	67	33	67	6
2	NIST C_2	3.333333	100	67	33	67	6
3	NIST C_3	1.111111	100	61	33	67	0
4	NIST C_4	0.37037	100	61	33	67	6
5	NIST C_5	0.123457	100	61	33	67	0
6	NIST C_6	0.041152	100	67	33	67	6
7	NIST C_7	0.013717	100	61	33	67	0
8	NIST C_8	0.004572	100	67	33	67	0
9	NIST C_9	0.001524	100	94	0	0	0

T Vol = Total volume of extract

R Vol = Remaining volume after dilution and quant

V req = Sample required for dilution

D req = Diluent required

Quant = amount of volume used for quant

These samples were prepared for Quantification in triplicate using the Hamilton Microlab® STARlet. These were Quantified using the Applied Biosystems™ QuantStudio 5 Real-Time PCR system. Results from both NIST SRM 2372 and NIST SRM 2372a were assessed to determine if they fell within the certified range. Following this a risk assessment will be undertaken to address the issues raised in OQI# 56218.

Results

Serial Dilutions for both NIST SRM 2372 and NIST SRM 2372a were prepared in triplicate as described in the experimental design. The samples were quantified using the Applied Biosystems™ QuantStudio 5 Real-Time PCR system. The results of the quantification processes are outlined in Appendix 1. The expected Quantification concentration results were compared to the actual Quantification results obtained and the percent change calculated. Figure 1 shows the inaccuracy of the quantification results obtained for NIST SRM 2372 compared with the inaccuracy of the quantification results obtained for NIST SRM 2372a.

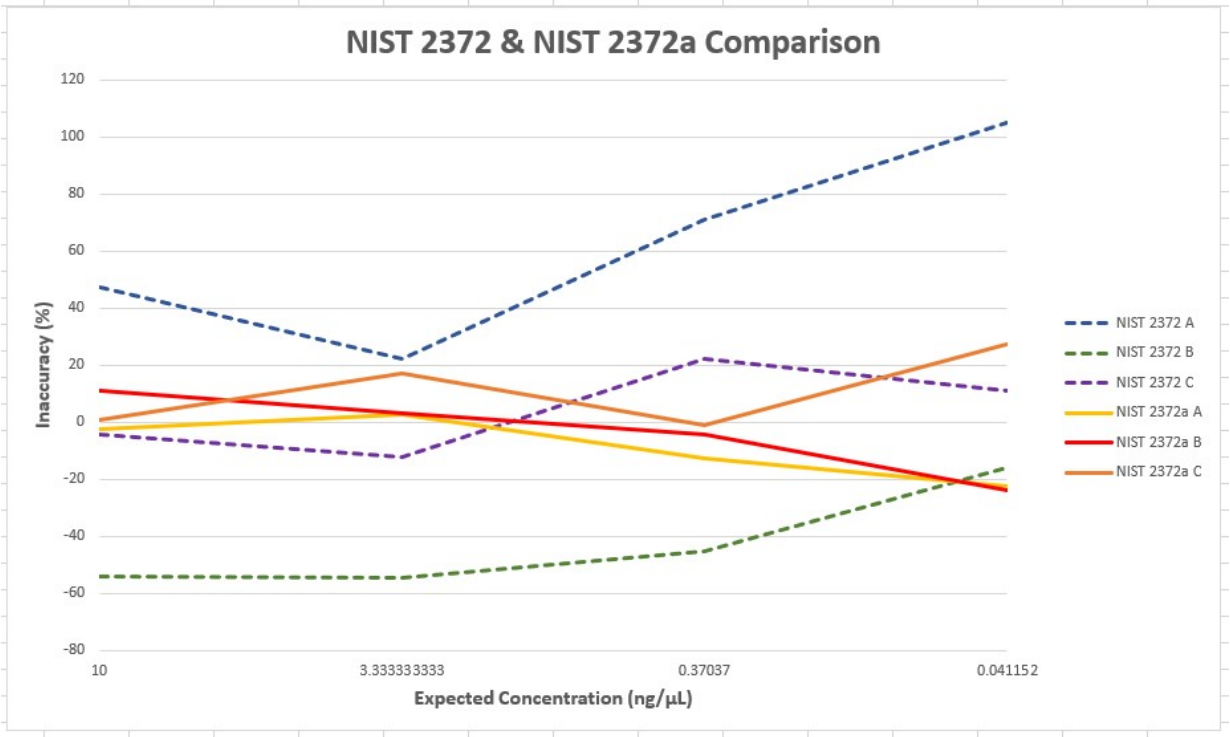

Figure 1 Inaccuracy NIST 2372 & NIST 2372a (%)

NIST SRM 2372a was opened immediately prior to performing the serial dilutions. The Certificate of Analysis for NIST SRM 2372a was issued on 13 March 2018 and is valid until 13 February 2023. The variation between the expected quantification values and the actual quantification values for all three standards (A, B and C) obtained were within +/- 30%. This range is similar to the variation seen in previous internal validations.

The Certificate of Analysis for NIST SRM 2372 was issued on 08 January 2013 and was valid until 31 December 2017. NIST SRM 2372 was used for this experiment approximately 4.5 years outside of the Certificate of Analysis validity. The inaccuracy of the quantification values obtained was greater than that of NIST SRM 2372a.

The quantification values obtained for NIST SRM 2372 (A) were higher than expected, obtaining values that ranged from 22% - 105% more than the expected quantification values. NIST SRM 2372 (B) returned quantification values 16% - 55% lower than the expected quantification values. NIST SRM 2732 (C) performed the best out of the triplicates and was within the +/- 30% variation range observed with NIST SRM 2372a.

A subset of results from dilutions tested in Project #185 (closest in concentration to those tested in this minor change) have been analysed and the inaccuracy calculated. These are outlined in Appendix 2 with the subset analysed highlighted in yellow.

Discussion

NIST SRM 2372a obtained quantification values which were within +/-30% of the expected quantification value. DNA quantification is only an estimation of the DNA concentration and therefore variation is expected. Furthermore, DNA quantification uses real time PCR which has run to run variation. In addition, preparation of the serial dilution introduces variation due to pipetting error, which is compounded with each successive step. It is therefore not unexpected to see variation with quantification results, as seen for NIST SRM 2372a.

When tested for this Minor Change NIST SRM 2372 (A) in this experiment gave higher quantification values than expected (up to 105% higher for the lowest dilution), indicating the concentration of NIST SRM 2372 (A) has increased over time. It was noted prior to the commencement of testing that there was just enough of standard (A) remaining to use for the serial dilutions. The low remaining volume of NIST 2372 (A) may have contributed to the higher than expected results in this experiment, due to inadequate mixing during previous use and/or evaporation during extended storage. It should be noted that these explanations for the observed increased concentration could not be confirmed. The quantification results for NIST SRM 2372 (A) obtained in Project #185 produced an inaccuracy of between -48% to +9% (refer to Table 8), which is closer to the variation in quantification results obtained for NIST SRM 2372a in this experiment. The variation seen for NIST SRM 2372 (A) in project #185 may be explained by the expected variation discussed above, or by the theory that this standard was not mixed adequately before being used for this project, resulting in lower than expected quantification values when used for project #185, and higher than expected quantification values in this minor change.

When tested in this Minor Change, NIST SRM 2372 (B) gave quantification results that were lower than expected, with results 15% - 50% less than the expected quantification results. When tested in Project #185, quantification results for serial dilutions of similar concentrations were 8% - 57% lower than expected (refer to Table 9). The inaccuracy observed for NIST SRM 2372 (B) in this Minor Change and Project #185 were comparable, indicating that NIST SRM 2372 (B) has not significantly decreased in concentration since it was used in Project #185.

When tested in this Minor Change NIST SRM 2372 (C) gave inaccuracy of -4% to +22%, and NIST SRM 2372a C gave inaccuracy of -1 to 27%. In Project# 185, NIST SRM 2372 (C) gave inaccuracy of 9% - 35%. The results obtained for NIST SRM 2372 (C) in this minor change and in project #185 are consistent, and show the same variation produced by NIST SRM 2372a and similar variation seen in previous internal validations. These results indicate that NIST SRM 2372 (C) has not significantly decreased in concentration since it was used in Project #185.

The results of this Minor Change can be used to inform the investigation in OQI# 56218 – Use of NIST standard in Project #185. Please refer to Audit QIS# 29759 for more detail regarding the risk of using NIST SRM 2372 outside of the Expiration of Certification for Project #185.

Appendix 1

NIST 2372

Table 2 NIST 2372 (A)

NIST A (Single male)	2372					
Sample	Concentration (ng/uL)	Quant Value	Average Quant	% Change	STD DEV	CV (%)
[redacted]	10	14.773	14.72	47.2	0.78	5.30
	10	13.915				
	10	15.472				
	3.333333333	4.326	4.068333333	22.05	0.34	8.26
	3.333333333	3.688				
	3.333333333	4.191				
	0.37037	0.616	0.633	70.91017091	0.04	5.64
	0.37037	0.609				
	0.37037	0.674				
	0.041152	0.09	0.084333333	104.9313116	0.01	10.63
	0.041152	0.074				
	0.041152	0.089				

Table 3 NIST 2372 (B)

NIST B (Multiple female)	2372					
Sample	Concentration (ng/uL)	Quant Value	Average Quant	% Change	STD DEV	CV (%)
[redacted]	10	4.831	4.612	-53.88	0.22	4.80
	10	4.617				
	10	4.388				
	3.333333333	1.544	1.516	-54.52	0.09	6.08
	3.333333333	1.591				
	3.333333333	1.413				
	0.37037	0.207	0.202	-45.45994546	0.02	8.92
	0.37037	0.217				
	0.37037	0.182				
	0.041152	0.028	0.034666667	-15.75946086	0.01	20.26
	0.041152	0.034				
	0.041152	0.042				

Table 4 NIST 2372 (C)

NIST C (Multiple male and female)	2372					
Sample	Concentration (ng/uL)	Quant Value	Average Quant	% Change	STD DEV	CV (%)
[redacted]	10	9.427	9.572666667	-4.273333333	0.88	9.23
	10	8.771				
	10	10.52				
	3.333333333	3.021	2.929666667	-12.11	0.26	8.76
	3.333333333	2.64				
	3.333333333	3.128				
	0.37037	0.487	0.452	22.04012204	0.05	10.33
	0.37037	0.399				
	0.37037	0.47				
	0.041152	0.04	0.045666667	10.97071021	0.01	12.06
	0.041152	0.046				
	0.041152	0.051				

Table 5 NIST 2372a (A)

NIST A (Single male)	2372a					
Sample	Concentration (ng/uL)	Quant Value	Average Quant	% Change	STD DEV	CV (%)
[redacted]	10	8.81	9.742333333	-2.576666667	0.86	8.79
	10	10.493				
	10	9.924				
	3.333333333	3.079	3.426666667	2.8	0.32	9.21
	3.333333333	3.506				
	3.333333333	3.695				
	0.37037	0.322	0.323333333	-12.6999127	0.00	0.47
	0.37037	0.325				
	0.37037	0.323				
	0.041152	0.03	0.032	-22.23950233	0.00	6.25
	0.041152	0.032				
	0.041152	0.034				

Table 6 NIST 2372a (B)

NIST B (Multiple female)	2372a					
Sample	Concentration (ng/uL)	Quant Value	Average Quant	% Change	STD DEV	CV (%)
[redacted]	10	11.078	11.11233333	11.12333333	0.74	6.70
	10	10.386				
	10	11.873				
	3.333333333	3.481	3.433666667	3.01	0.27	7.75
	3.333333333	3.147				
	3.333333333	3.673				
	0.37037	0.326	0.353666667	-4.50990451	0.03	8.73
	0.37037	0.348				
	0.37037	0.387				
	0.041152	0.037	0.031333333	-23.8595127	0.00	15.74
	0.041152	0.028				
	0.041152	0.029				

Table 7 NIST 2372a (C)

NIST C (Multiple male and female)	2372a					
Sample	Concentration (ng/uL)	Quant Value	Average Quant	% Change	STD DEV	CV (%)
[redacted]	10	9.185	10.06533333	0.653333333	0.78	7.73
	10	10.35				
	10	10.661				
	3.333333333	3.392	3.905	17.15	0.73	18.78
	3.333333333	4.745				
	3.333333333	3.578				
	0.37037	0.335	0.366333333	-1.08990109	0.05	14.11
	0.37037	0.426				
	0.37037	0.338				
	0.041152	0.043	0.052333333	27.17081389	0.01	24.57
	0.041152	0.047				
	0.041152	0.067				

Appendix 2

Table 8 Project #185 - NIST 2372 (A)

NIST A (Single male)			
Sample	SAT (ng/ul)	Average Quant	% Diff
Dilution #1 (5 ng/µL)	5.682607651	5.514417887	9.328598182
Dilution #1 (5 ng/µL)	5.346228123		
Dilution #2 (1 ng/µL)	0.924901009	0.902277887	-10.83060048
Dilution #2 (1 ng/µL)	0.879654765		
Dilution #3 (0.5 ng/µL)	0.361483485	0.340923667	-46.660396
Dilution #3 (0.5 ng/µL)	0.320363849		
Dilution #4 (0.1 ng/µL)	0.077558234	0.087002698	-14.93896387
Dilution #4 (0.1 ng/µL)	0.096447162		
Dilution #5 (0.09 ng/µL)	0.041029278	0.065881466	-36.60898093
Dilution #5 (0.09 ng/µL)	0.090733655		
Dilution #6 (0.07 ng/µL)	0.065472864	0.071041241	1.465685663
Dilution #6 (0.07 ng/µL)	0.076609619		
Dilution #7 (0.05 ng/µL)	0.037703525	0.033626165	-48.69373192
Dilution #7 (0.05 ng/µL)	0.029548805		
Dilution #8 (0.03 ng/µL)	0.024915397	0.026327913	-13.94750639
Dilution #8 (0.03 ng/µL)	0.027740428		

Table 9 Project #185 NIST 2372 (B)

NIST B (Multiple female)			
Sample	SAT (ng/ul)	Average Quant	% Diff
Dilution #1 (5 ng/µL)	4.914865017	4.569596529	-9.41885062
Dilution #1 (5 ng/µL)	4.224328041		
Dilution #2 (1 ng/µL)	0.684210658	0.700813681	-42.69127833
Dilution #2 (1 ng/µL)	0.717416704		
Dilution #3 (0.5 ng/µL)	0.289112657	0.317624852	-57.41841256
Dilution #3 (0.5 ng/µL)	0.346137047		
Dilution #4 (0.1 ng/µL)	0.090883873	0.091955028	-8.748810869
Dilution #4 (0.1 ng/µL)	0.093026184		
Dilution #5 (0.09 ng/µL)	0.070036992	0.062982963	-42.89578506
Dilution #5 (0.09 ng/µL)	0.055928934		
Dilution #6 (0.07 ng/µL)	0.062150575	0.075302295	7.041346296
Dilution #6 (0.07 ng/µL)	0.088454016		
Dilution #7 (0.05 ng/µL)	0.035086077	0.043606566	-14.66163076
Dilution #7 (0.05 ng/µL)	0.052127056		
Dilution #8 (0.03 ng/µL)	0.017214114	0.016776706	-78.81936916
Dilution #8 (0.03 ng/µL)	0.016339298		

Table 10 Project #185 NIST 2372 (C)

NIST C (Multiple male and female)			
Sample	SAT (ng/ul)	Average Quant	% Diff
Dilution #1 (5 ng/µL)	9.154876709	7.713918447	35.18209929
Dilution #1 (5 ng/µL)	6.272960186		
Dilution #2 (1 ng/µL)	1.138574004	1.174975812	14.89186506
Dilution #2 (1 ng/µL)	1.211377621		
Dilution #3 (0.5 ng/µL)	0.551242232	0.553751379	9.706771173
Dilution #3 (0.5 ng/µL)	0.556260526		
Dilution #4 (0.1 ng/µL)	0.125467464	0.107362572	6.857671123
Dilution #4 (0.1 ng/µL)	0.08925768		
Dilution #5 (0.09 ng/µL)	0.103902243	0.100897275	10.80036594
Dilution #5 (0.09 ng/µL)	0.097892307		
Dilution #6 (0.07 ng/µL)	0.07812082	0.086683925	19.2468502
Dilution #6 (0.07 ng/µL)	0.09524703		
Dilution #7 (0.05 ng/µL)	0.063319393	0.059030121	15.29748035
Dilution #7 (0.05 ng/µL)	0.05474085		
Dilution #8 (0.03 ng/µL)	0.027936369	0.028054655	-6.93412708
Dilution #8 (0.03 ng/µL)	0.02817294		

Line Manager Signature:		**Comments:**
Quality & Projects Signature:		**Comments:**

Please convert to PDF, e-sign and lock document on completion.

HealthSupport
Forensic and Scientific Services

Health and Safety Risk Assessment Form

Refer to QIS 29106: Risk Management Guideline

RISK ASSESSMENT (provide name / title of risk assessment.)	Risk assessment – Use of expired NIST SRM 2372 in Project #185 'Validation of two Quantstudio 5 Real-Time PCR Systems'	**PURPOSE / SCOPE** (for consistency the assessment team can use template wording or devise their own purpose and scope)	To identify any risks involved with using the expired NIST SRM 2372 in project #185
LOCATION (include work unit as identified in Organisational structure)	Forensic DNA Analysis	**MANAGER / SUPERVISOR** (include first and last name rather than initials)	Luke Ryan
WORK AREA (include relevant name and room numbers)	Block 3	**QIS AUDIT NO.**	QIS 29759
CONTACT PERSON(s) (include first and last name rather than initials)	Luke Ryan	**QIS OQI NO.**	N/A
ASSESSMENT TEAM (include first and last names rather than initials)	Chelsea Savage		
ASSESSMENT DATES (include preparation dates, when conducted, to time Audit/OQI created)	15/08/2022 – 22/08/2022	**REVIEW DATE** (subject to risks identified – should a review, at this time, be conducted in 6 months, 1 year, 2 years, 3 years, 4 years, 5 years)	N/A

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STEP 1. Provide insight as to the work task or process, item or samples, work space, environment. (outline below)	
• Project #185 was conducted in the first quarter of 2018 to validate DNA quantification using the Quantfiler Trio kit on the QS5 instrument. • The purpose of this validation was to compare the 7500 and QS5 to assess whether the QS5 performs the same as or better than the 7500 and is therefore a suitable replacement. The validation was not a validation of the quantifiler trio kit. • The NIST SRM 2372 is a human DNA quantification standard, which includes three component genomic DNA materials labelled A, B and C. The certificate of Analysis was issued for this NIST standard on 08/01/2013, which provides apparent absorbance values (e.g. DNA concentrations) for NIST SRM 2372 components A, B and C within specified uncertainty. This certificate was valid until expiry on 31 December 2017 after which the relative absorbance values are not guaranteed • Project #185 used the NIST SRM 2372 for Experiment 1: Sensitivity, limit of Detection and Inaccuracy, and Experiment 2: Comparison of QS5s and 7500. These experiments were conducted in Q1 2018, after the certificate of analysis had expired.	
(Note: do not re-iterate the entire SOP / method but provide informative context, and be descriptive, as to the work activity, work space, other activities occurring at the same time, complexity of process which may inform of various hazards – so a reviewer can gain a better understanding / appreciation)	
Provide associated QIS numbers (e.g. SOPs, name of document)	QIS 34045 V5: Quantification of Extracted DNA using the Quantifiler Trio DNA Quantification kit Minor change: Comparison of NIST Standards 2732 and 2372a
Frequency and complexity of task: (conducted daily, weekly, monthly and size / number of batches, samples, items etc)	N/A – this is a risk assessment of the NIST standards used in project #185
Duration of task: (takes minutes, hours, split over days, weeks etc. Provide details if this will assist a reviewer.)	N/A – this is a risk assessment of the NIST standards used in project #185
Number of workers exposed: (in immediate work area and subject to hazards and risks in the broader work areas, where applicable)	No risk to staff, this risk assessment is assessing the risk to samples.

STEP 2. Identify the hazards

- ☐ Animals / insects (specify < >)
- ☐ Asbestos
- ☐ Biological (e.g. Bacteria, viruses, blood, body fluids, if sewage – specify biological hazards)
- ☐ Chemical (e.g. Chemicals, cleaning agents, fuels, oils, LPG, pesticides, glues, particulates / dusts, lead)
- ☐ Confined Space (e.g. Storage tanks, pits, tunnels, boilers, pipes)
- ☐ Electrical (e.g. Electrical equipment (specify < >), power cords, power boards, double adaptors, live wires)
- ☐ Ergonomics (e.g. Repetitive tasks, fixed posture, workstation setup)
- ☐ Fatigue (try to keep separate from Ergonomics)
- ☐ Gases (specify gas and mixtures)
- ☐ Manual Handling (e.g. Lifting, pushing, pulling, twisting, bending, carrying)
- ☐ Noise (provide descriptions: loudness, humming, drumming, constant, background etc)
- ☐ Physical (e.g. slips / trips / falls, sharp objects, moving objects)
- ☐ Plant / equipment / machinery (e.g. laboratory equipment, tools, forklifts)
- ☐ Psychological (e.g. critical incident, work pressures, workload, harassment, aggressive behaviour, specify clinical environment where appropriate)
- ☐ Radiation (e.g. ionizing, ultraviolet, infrared, laser, radiofrequency, electromagnetic)
- ☐ Security
- ☐ Thermal (e.g. hot / cold environments, hot/cold surfaces, heavy work, steam, hot liquids, freezers)
- ☐ Vehicles / driving
- ☐ Vibration (provide descriptions: e.g. buzzing, repetitiveness / frequency)
- ☐ Work environment (e.g. workplace design and space allocation for tasks, lighting, ventilation)
- ☐ Working alone (specify risk and reasons for working alone)
- ☐ Working at heights (e.g. roofs, scaffolding, ladders, elevating platforms, cherry pickers)
- ☒ Other (Please specify): Risk to samples

STEP 3. Assess the risk & recommend controls										
No.	Hazard (use / follow order of hazards in Step 2)	Risk (What could happen in context of work environment and process?)	Risk Rating *without* controls			Risk controls currently in place and Risk controls required (assess if currently existing controls are appropriate and consistently employed)	Risk Rating *with* controls			
			Consequence	Likelihood	Risk Rating (High, Medium, Low and number rating)		Consequence	Likelihood	Risk Rating (High, Medium, Low and number rating)	

1	Risk to samples	Incorrect results from project due to expired NIST standards used.	N/A	N/A	N/A	This project compared the QS5 to the 7500, and the same NIST SRM 2372 was used to evaluate each instrument. The NIST SRM 2372 starting concentration and the concentration of each serial dilution was not the critical element of this experiment. The critical element was the use of the same serial dilutions to test the instruments (7500-A, QS5-A and QS5-B). The quantitative values obtained during this project were not compared to the absorbance values cited on the Certificate of Analysis. This experiment was comparing the ability of the instruments to produce quantification results, irrespective of the starting concentration value. The use of the expired NIST SRM 2372 therefore does not affect the validity of this experiment, as the results from this experiment demonstrated comparable performance between the three instruments. A minor change was conducted to compare the quantification results from NIST SRM 2372 and NIST SRM 2372a. The certificate of analysis for NIST 2372a was issued on 13 March 2018 and expires on 13 February 2023. NIST SRM 2372a produced quantification results which were within +/- 30% of the expected quantification value. NIST SRM 2372 (C) produced results consistent with NIST SRM	N/A	N/A	N/A

STEP 3. Assess the risk & recommend controls										
No.	**Hazard** (use / follow order of hazards in Step 2)	**Risk** (What could happen in context of work environment and process?)	**Risk Rating *without* controls**			**Risk controls currently in place** and **Risk controls required** (assess if currently existing controls are appropriate and consistently employed)	**Risk Rating *with* controls**		
			Consequence	**Likelihood**	**Risk Rating (High, Medium, Low and number rating)**		**Consequence**	**Likelihood**	**Risk Rating (High, Medium, Low and number rating)**
						2372a. NIST SRM (A) produced quantification results which were significantly greater than the expected quantification results, while NIST SRM (B) produced results which were lower than expected. See the minor change for a discussion into these results. The results obtained from NIST SRM (C) during this minor change show that the quantification values obtained during project #185 would have been within +/- 30% of the expected quantification value. The variation observed in NIST SRM (B) was not unexpected due to the standard being 4.5 years post expiry. The variation observed in NIST SRM (A) may have been observed due to a combination of ineffective mixing during use and/or evaporation.			

2	Risk to samples	Incorrect limit of detection calculated due to the use of expired NIST standards	N/A	N/A	N/A	The QS5 validation limit of detection experiment was conducted to compare the instruments (7500, QS5A and QS5B) when analysing samples with concentrations above and below the LOD (which was calculated in a previous internal validation). Multiple concentrations above, and one concentration below, the LOD were assessed by the instruments, and therefore the accuracy of the concentration of each serial dilution is not the critical element. The critical element is the use of the same serial dilution to test the instruments to enable a comparison of the performance at these reducing concentrations. The LOD for the 7500 was 0.001 ng/µl, as implemented from previous internal validations. The results from the experiment showed that below the LOD, both the 7500 and QS5 were not able to estimate the quantity of DNA present accurately or reliably in the sample, and therefore recommended the LOD reman at 0.001 ng/µl.	N/A	N/A	N/A
3	Risk to samples	Ongoing risk to samples with the use of the QS5	N/A	N/A	N/A	As can be seen above, the accuracy of the concentration of the NIST standards was not the critical factor in the experiments. Given that the same expired NIST SRM 2372 was used for all three instruments the comparative assessment holds.	N/A	N/A	N/A

STEP 3. Assess the risk & recommend controls										
No.	Hazard (use / follow order of hazards in Step 2)	Risk (What could happen in context of work environment and process?)	Risk Rating *without* controls			Risk controls currently in place and Risk controls required (assess if currently existing controls are appropriate and consistently employed)	Risk Rating *with* controls			
			Consequence	Likelihood	Risk Rating (High, Medium, Low and number rating)		Consequence	Likelihood	Risk Rating (High, Medium, Low and number rating)	
						Furthermore, the minor change comparing NIST SRM 2372 and NIST 2372a detailed the use of NIST SRM 2372 4 months post expiry posed minimal risk to the validity of the results obtained from Project #185. Therefore, the use of NIST SRM 2372 in project #185 is not considered to be a retrospective risk to samples already reported or an ongoing risk to samples on the QS5.				

STEP 4. Implement recommended controls (*Refer to Appendix 3: Hierarchy of Control*)				
IMPLEMENTATION PLAN				
Controls Required (be descriptive if not already outlined in Step 3)	**Action(s) Required** (actions should be included in the associated OQI)	**Person(s) Responsible** (use first and last names)	**Action By Date(s)**	**Date Completed** or **Date follow up is required by**
Short term controls (< ~ 30 days to implement)				
1. Nil	Nil	Nil	Nil	Nil
Long term controls (> 30 days to implement)				
1. Nil	Nil	Nil	Nil	Nil

Note: It is the responsibility of the assessment team to ensure that the risk assessment is presented, explained / discussed with the manager / supervisor (this includes handing over the hardcopy of the risk assessment and advising location of electronic copy in file path: I:/Quality e. Projects/Risk Assessments/Risk assessments in QIS.

Name: Chelsea Savage
(Assessment team member 1)

Signature: [redacted]

Date: 09/09/2022
dd / mm / yyyy

Name:
(Assessment team member 2)

Signature:

Date: / / 20
dd / mm / yyyy

FOLLOW UP CHECKLIST (for Assessment team and Manager / Supervisor)	YES	NO	Relevant numbers for reference (e.g. QIS number, Order number etc) and Comments (where appropriate)
QIS OQIs generated		x	
CMMS Order generated		x	
Other – please specify (e.g. Escalate issue to higher level – HSQ Safety, HR, EDFSS, FSS Manager)	Nil		
Team Leader / Manager / Supervisor (Person taking responsibility / accountability for risk assessment and risk mitigation strategies)	Name: Luke Ryan	Signature: [redacted]	
	Date: 09/04/2022 (dd/mm/yyyy)		

STEP 5. Monitor and review	YES	NO	Further action(s) taken (provide details of additional actions and make comment where applicable)
Have new control measures been implemented?			N/A
Control measures were implemented in what timeframe? (provide dates where applicable)			N/A
Are new control measures minimising risk? Comment.			N/A
Are further control measures required to minimise risk? Comment			N/A
Do new control measures introduce any new risk? Comment			N/A
Has a copy of this risk assessment been provided to all relevant	x		Risk assessment referenced in the NIST standard minor change

STEP 5. Monitor and review			
	YES	**NO**	**Further action(s) taken** **(provide details of additional actions and make comment where applicable)**
personnel? How was this achieved?			
Have details of the risk assessment been fully documented and referenced in relevant procedures, methods etc? (Specify QIS numbers etc where appropriate)			N/A
The signature below is an acknowledgement to verify that all identified short term control measures have been implemented to control and mitigate identified risks – and that long-term controls have commenced.			
Team Leader / Manager	**Name:** Luke Ryan **Signature** [redacted] **Date:** 09/04/2020 (dd/mm/yyyy)		
Risk Assessment review date:	N/A		
Reason / comment for review date period. (Provide mechanism to ensure risk assessment is reviewed / followed up. Comment)	Review not necessary due to scope of risk assessment.		

Instructions

Step 1: Describe the task / activity (include frequency, duration and number of workers).

Step 2: Identify the hazards.

Step 3: Assess the risk and recommend controls.
- Determine what the most likely outcome would be – 'Consequences' (*Refer to Appendix 2, Table 1*)
- Determine how likely the consequences are – 'Likelihood' (*Refer to Appendix 2, Table 2*)
- Calculate the risk rating (Refer to *Appendix 2, Table 3*)
- Recommend controls using the HIERARCHY OF CONTROLS (*Refer to Appendix 3*): (Elimination, Substitution, Isolation, Engineering, Administrative, PPE)

Step 4: Implement recommended controls.
- Determine action/s required, person/s responsible, 'action by' date and completed date
- Complete follow-up checklist

Step 5: Monitor and review.
- Determine that all actions are completed
- Sign off by Team Leader / Manager

Record / Documentation requirements:

- All sections of the form must be completed and signed off by the Team Leader / Manager.
- Details of the risk assessment are to be recorded on QIS in the QIS Audit Module.
- Details of any OQIs generated are to be recorded on QIS in the QIS OQI Module.
- Details of the risk assessment are to be fully documented and referenced in relevant procedures, methods etc.

Table 1: Consequence

	Negligible	Minor	Moderate	Major	Extreme
Work Health and Safety	No injury. First aid treatment only. No time lost	Medical treatment injury. A full shift/workday has not been lost	Lost time injury or serious injury or illness without permanent impairment (as defined by *S36 Work Health & Safety Act (QLD) 2011)*	Serious injury or illness with permanent impairment (as defined by *S36 Work Health & Safety Act (QLD) 2011)*	Reportable fatality (as defined by *S35 Work Health & Safety Act (QLD) 2011)*

Table 2: Likelihood

Likelihood	Description	Probability
Almost Certain	The risk/event will likely occur in most circumstances.	**>90%**
Likely	The risk/event will probably occur at least once.	**60-90%**
Possible	The risk/event could be expected to occur at some time.	**30-60%**
Unlikely	The risk/event could occur at some time but is not expected.	**5-30%**
Rare	The risk/event may occur only in exceptional circumstances.	**<5%**

Table 3: Risk Rating

Likelihood	Consequence: Negligible	Minor	Moderate	Major	Extreme
Almost Certain	Medium (7)	Medium (11)	High (17)	Very High (23)	Very High (25)
Likely	Medium (6)	Medium (10)	High (16)	High (20)	Very High (24)
Possible	Low (3)	Medium (9)	High (15)	High (18)	High (22)
Unlikely	Low (2)	Medium (8)	Medium (12)	Medium (14)	High (21)
Rare	Low (1)	Low (4)	Low (5)	Medium (13)	High (19)

Table 4: Hierarchy of Control

Control mechanism	***Details***	
Elimination	The preferred and most effective control measure, which involves removing the hazard from the workplace (e.g. introducing automation to eliminate manual handling / ergonomics hazards.	**Most preferred**
Substitution	Involves replacing a hazard with one that presents a lower and more manageable hazard (e.g. using a less toxic chemical)	
Isolation	Use of barriers to separate or isolate a hazard. Examples include installing screens or barriers around hazardous areas or guarding around machinery.	
Engineering / Redesign	Designing and installing equipment to min mise hazards, for example exhaust systems to extract fumes / dusts etc.	
Administrative Controls	Involves minimising exposure to risk through a range of controls such as procedures, training, job rotation, signage, permit to work systems, exclusion and supervision.	
Personal Protective Equipment (PPE)	This is the least preferred method and should be used in combination with higher order control measures. Included are items such as safety glasses, boots, gloves, masks, ear plugs.	**Least preferred**