STATEMENT OF ANGELINA KELLER

I, **Angelina Keller**, of Queensland Health at the Forensic and Scientific Services, 39 Kessels Road, Coopers Plains, state as follows:

Background

1. I have a Bachelor of Agricultural Science (Honours) from University of Tasmania and a Master of Science (Forensic Science) from Griffith University.

2. I am a member of the Australian and New Zealand Forensic Science Society (**ANZFSS**).

3. I am currently employed by Queensland Health at the Forensic and Scientific Services (**FSS**) as a scientist in the reporting team of Forensic DNA Analysis. I joined FSS in 2004.

4. The duties of my current role include interpretation, reporting and reviewing DNA results, as well as presenting DNA expert witness testimony in court.

5. I have worked in this role since 2010. Prior to that I was a scientist in three different teams within Forensic DNA Analysis; the Analytical team, the Volume Crime team and the Intelligence team. My duties during these roles included processing of all sample types submitted for DNA analysis (extraction, quantitation, amplification and capillary electrophoresis), evidence recovery from Volume Crime samples including presumptive testing, uploading, actioning, creating and reviewing links for the National Criminal Intelligence DNA Database (**NCIDD**). In 2006, I applied for and was selected to be trained in all aspects of bones as part of the skeletal remains project. Since this time, I have gained expertise in the triaging of remains at autopsy, evidence recovery from bones as well as other post-mortem samples, and interpretation, reporting and reviewing of DNA results for the coroner to facilitate identifications for coronial cases and disaster victim identification incidents (**DVIs**).

6. Previously from 1996 to 2004, I was employed as an Experimentalist with the Department of Primary Industries. My duties involved conducting horticultural field trials for projects.

DIFP process

7. At FSS between 2018 and 6 June 2022, samples that were quantitated between the range of 0.001 ng/µL and 0.0088 ng/µL were reported as 'DNA insufficient for further processing' (**DIFP**) and only processed further upon the request of the Queensland Police Service (**QPS**) or a reporting scientist. I was not consulted about the DIFP process.

8. I do not consider the DIFP wording in statements was correct.

9. I have never been completely comfortable with the process but was informed by Justin Howes and others in FSS that the QPS were aware of the situation, it was a routine process and there was nothing to worry about.

10. I had raised questions over the process as early as 29 May 2018, when I had a priority 1 case and obtained a DIFP sample. I sent an email to Justin asking whether to await QPS instructions to rework or allow the sample to be reported in finality as DIFP. I have no record of reply from Justin. Annexed and marked AK-01 is a copy of my email to Justin on 29 May 2018. On 27 June 2018, testing of this sample was restarted following a request from Senior Sergeant Simpfendorfer.

11. Following implementation of the 3500 Genetic Analyser instrument for the capillary electrophoresis process, I started to become more concerned about the DIFP process. I had a number of situations where we obtained usable DNA profiles from the further processing of samples originally reported as DIFP.

Greater sensitivity of the 3500 Genetic Analyser instrument

12. One example of the difference the new instrument made is in [redacted], which involved a murder from 2009. This case included a sample which was interpreted as a three contributor mixture on the 3130 capillary electrophoresis instrument, just prior to implementation of the new 3500 instrument.

13. Annexed and marked AK-02 is a copy of the interpretation page from the Forensic Register showing the sample interpreted as a three contributor mixture.

14. Annexed and marked AK-03 is a copy of page 1 of the DNA profile for the sample interpreted as a three contributor mixture with handwritten notes dated 15 February 2021.

15. Following the implementation of the 3500 capillary electrophoresis instrument, this sample was rerun through the capillary electrophoresis process and a four contributor mixture was obtained.

16. Annexed and marked AK-04 is a copy of the interpretation page from the Forensic Register showing the sample interpreted as a four contributor mixture.

17. Annexed and marked AK-05 is a copy of page 1 of the DNA profile for the sample interpreted as a four contributor mixture with handwritten notes dated 23 April 2021.

18. This sample produced two different results simply by changing the instrument performing the capillary electrophoresis process.

19. The 3500 instrument is more sensitive than the previous instrument used for this process. In my view, the DIFP process should have been reassessed due to the increased sensitivity of the new instrument.

20. In my view, any samples analysed since the implementation of the 3500 Genetic Analyser instrument and reported as DIFP should be reworked.

21. I voiced some of my concerns to Kylie Rika, my manager in a 'Reporting 2' team meeting in November 2021. At this meeting some members of Reporting 2 discussed the results we were getting from samples that were originally reported as DIFP. In my experience, by analysing samples through the 3500 instrument we were getting better results than we previously could have got, including on samples originally reported as DIFP. I got the impression from the conversation and Kylie's responses that she took this on board. From my experience, I believe Kylie would have raised this with management.

Variance in quantitation value

22. The value of a quantitation is an estimate and is variable. In my view, it is not appropriate to base a decision about whether to process a sample further on a single measure that can be so variable.

23. On a number of occasions, I have seen quantitation values vary in the same sample.

24. For example, I recently obtained a very high quantitation value of 2.222 ng/µL from a pooled and Microcon concentrated bone sample. I knew this value could not possibly be correct as the original three bone aliquots each returned three low quantitation

values prior to being pooled and Microcon concentrated. The pooled and concentrated bone sample was quantified again and returned a quantitation value of 0.003 ng/µL. I briefly mentioned this to Justin Howes while he was sitting in his office and he directed me to speak with Luke Ryan. On 31 May 2022, I sent Luke an email and received a response, which I do not completely understand. I did not follow up any further. Annexed and marked AK-06 is a copy of the email chain between me and Luke regarding the quantitation variation.

Change in the DIFP process on 6 June 2022

25. On 6 June 2022, the process for samples in the DIFP range was changed. Samples in the DIFP range were no longer to be reported as DIFP, but to be processed straight to amplification. This meant that they were not first Microcon concentrated before amplification, which I would almost always choose to do with a sample that had low quantities of DNA. For samples at the lower end of the DIFP range, I would consider a Microcon concentration to full rather than to 35 µL in the hope of obtaining a useable DNA profile.

26. In my view, processing low level samples straight to amplification is suboptimal, it wastes approximately 15 µL of DNA extract and I do not agree with it. Such samples should have been Microcon concentrated either to 35 µL or full before amplification to maximise the chance of obtaining a useable DNA profile. I do not know if there was any scientific reasoning behind proceeding straight to amplification.

27. I was not consulted about the change to the DIFP process on 6 June 2022.

Recent DIFP example involving a rework ([redacted])

28. On 2 June 2022 I was provided with a subpoena to attend court and give evidence in the matter of [redacted], which I had provided a statement in relation to. The matter involved sexual offences. After I received the subpoena, I reviewed my statement, dated 17 June 2021. I saw that both the perianal and scrotum swabs were reported as 'DNA insufficient for further processing'. I spoke to Alicia Quartermain, who had reviewed the case initially and explained that I was not happy with the results, particularly with what I had learnt since I had issued the statement about the ability to obtain profiles from samples previously reported as DIFP. Alicia agreed with my reassessment.

29. On 2 June 2022, I sent an email to Kylie Rika and said that I was concerned about these results and that I wanted to request a rework of the samples. On that same day, Kylie agreed that we should rework the samples. With Kylie's approval, I requested a Microcon concentration to full for both the perianal and scrotum swab samples. Annexed and marked AK-07 is a copy of the email chain between me and Kylie dated 2 June 2022 regarding the rework request.

30. On 6 June 2022, Sharon Johnstone sent an email to reporting scientists regarding the DIFP process and reaffirmed to staff that results that had previously been reported as DIFP should not be tested further and will remain being reported as DIFP, despite the change in process on that day. Annexed and marked AK-08 is a copy of the email from Sharon on 6 June 2022.

31. On 9 June 2022, I took leave. When I returned to work on 15 June 2022, the reworked results for the case [redacted] were available for interpretation. I was able to report a three contributor mixture supporting non-contribution for the defendant for one of the samples. I reported these results to Kylie by email on 15 June 2022. A copy of this email is included in the email chain exhibited as AK-07 above.

32. On 15 June 2022, I went to retrieve the case file for this case and ran into Sharon. I mentioned that I was about to write a replacement statement for a case as I had reworked two DIFP samples. Sharon replied with words to the effect of, '*you should not have done that unless the police requested it and the QPS know the process so the old results still stand*'. Sharon also explained that she had recently sent out an email saying not to rework samples that were marked DIFP [the 6 June 2022 email]. I said that I did what I thought was right for everyone. Sharon responded with words to the effect of '*don't you have confidence in our processes?*' and I said words to the effect of '*I had confidence at the time, but now I do not',* because of the implementation of the 3500 instrument.

33. I sent myself an email to make a contemporaneous note of what had occurred. Annexed and marked AK-09 is a copy of the email note on 15 June 2022.

34. I recall that I also read the email that Sharon had sent on 6 June 2022 about not reworking samples and confirmed that I had obtained permission to rework before that advice had been circulated. I told Sharon verbally that my request to rework was before her email on 6 June 2022.

35. Also on 15 June 2022, I emailed Lara Keller requesting to speak with her about an issue. On 17 June 2022, I met with Lara and disclosed my concerns about DIFP samples. During that conversation, I told Lara about my experience with the greater sensitivity of the 3500 Genetic Analyser instrument and Operation Hotel Fawn, the reworked DIFP samples for the case [redacted] and what happened with Sharon.

36. Lara listened to what I had to say and was very empathetic. Lara said she may follow this information up as a Public Interest Disclosure (**PID**). On 19 July 2022, I was informed by Lara that she had followed this up as a PID but there was no further outcome.

37. I prepared a replacement statement for the case [redacted] which contained the new results for the two DIFP samples that had been reworked. Annexed and marked AK-10 is a copy of the statement dated 21 June 2022.

38. This statement replaced the previous statement I had issued for the case [redacted]. Annexed and marked AK-11 is a copy of the statement dated 17 June 2021.

39. The DNA profiles of the reworked samples in the case [redacted] are annexed and marked AK-12.

40. A copy of the Exhibit Testing page from the Forensic Register for the case [redacted] dated 21 July 2022 is annexed and marked AK-13.

41. On 15 July 2022, Alicia told me that in order to clarify what the process was (in light of my conversation with Sharon) she had asked Sharon if we can rework a DIFP sample at statement stage. Alicia informed me that Sharon told her that we could, but not if a statement had already been issued. I made a note of this on a sticky note on 15 July 2022 and attached it to my email, which is exhibit AK-09 above.

Change to sample processing on 19 August 2022

42. On 19 August 2022, Forensic DNA Analysis received an email from the Acting Executive Director Helen Gregg attaching a memorandum from the Acting Director General Dr David Rosengren, advising that the Microcon concentration process was to recommence for certain samples.

43. On 22 August 2022, I sent an email to Kylie Rika forwarding the 19 August 2022 memo and requested clarification as to whether or not bone / teeth aliquots were exempt from the direction, as each bone / tooth aliquot is almost always a portion of a larger sample of bone / teeth powder and as such is not exhausted. Kylie forwarded this email to Justin Howes to seek clarification.

44. Justin responded the same day to say that bone / teeth aliquots within the range stated in the memorandum would be subject to Microcon concentration in the way described by the memorandum.

45. A copy of the email chain is annexed and marked AK-14.

46. I was not consulted about the change to sample processing on 19 August 2022.

Issue with bone / teeth aliquots (mixed DNA profiles)

47. Currently, I am seeing mixed DNA profiles (multiple contributors) in bone / teeth aliquots for a number of coronial cases. DNA profiles from bones or teeth should be single source. These affected cases tend to involve skeletal remains where the bones or teeth are not from recently deceased persons and lower levels of DNA are present. I first noticed these mixed DNA profiles for a case in November 2020. This case has since been tested by another laboratory and a single source DNA profile has been obtained.

48. This year I have worked on four coronial cases involving mixed DNA profiles. They are:

(a) [redacted] bone sample (Case Identifier [redacted]);

(b) [redacted] bone samples (Case Identifiers [redacted] and [redacted]);

(c) [redacted] bone samples (Case Identifier [redacted]); and

(d) [redacted] bone sample (Case Identifier [redacted]).

49. Copies of example DNA profiles for each of the above cases that show mixed DNA profiles are annexed and marked AK-15, AK-16, AK-17 and AK-18 respectively.

50. I have compiled results from bone samples for the past few years. Since late 2020, I have primarily obtained mixed DNA profiles from coronial bone samples (not recently

deceased persons) but not from DVI bone samples (recently deceased persons). Annexed and marked AK-19 is the spreadsheet of bone results that I have been compiling. I have noticed that we have been obtaining more mixed DNA profiles from bones since December 2020 / January 2021.

51. In my opinion, FSS should be able to confidently obtain single source DNA profiles from bones or teeth to assist the coroner with the identification of believed to be or unknown deceased persons, provided there is ample DNA in the skeletal remains to start with. This is an important service that we provide to grieving families and the wider community of Queensland. It is not acceptable to obtain mixed DNA profiles from believed to be or unknown skeletal remains.

52. I raised an OQI about the issues with bones on 29 August 2022, where I stated:

Multiple cases involving bones have generated mixed DNA profiles

53. Annexed and marked AK-20 is a copy of the OQI report dated 21 September 2022.

54. The OQI remains open at the time of signing this statement.

55. I have considered the issues with bones and can identify three processes that have changed in the laboratory that may be contributing to the number of mixed DNA profiles, namely:

(a) Change to the cleaning regime for the equipment used to sample bones / teeth;

(b) Change to the extraction method; and

(c) Implementation of the 3500 Genetic Analyser instrument

56. These three processes are outlined in paragraphs 64 to 91 below.

General bones and teeth workflow

57. In order to understand the potential significance of the three process changes, it is necessary to understand the different workflow for bones / teeth.

58. The workflow for coronial cases involving bones / teeth is different to the workflow of other samples processed at Forensic DNA Analysis, although there are commonalities to both workflows. DNA profiling bones / teeth is not the core business of Forensic DNA Analysis and usually arises a few times a year at the request of a coroner.

Sampling bones / teeth involves highly specialised training as the recovery of DNA from bones / teeth is very different to other samples.

59. Communication at all stages of the bone / teeth workflow is critical as information can be convoluted and complicated partly due to the involvement of different disciplines and different organisations.

60. Firstly, it is established that there is a coronial case with bone, teeth or both that requires DNA identification. Usually this is established and communicated by a specialised staff member from the Scientific Services Liaison Unit.

61. An email is sent to staff members trained to sample bones / teeth to see who has availability to sample. There is a sampling roster to ensure staff competencies are maintained. Two scientists are chosen based on staff availability and maintaining competency. Currently, the staff members trained to sample bones / teeth are Valerie Caldwell, Janine Seymour-Murray, Abigail Ryan, Allison Lloyd and also myself (Kristina Morton is in the process of being trained). Additionally, the case is allocated to two coronial / DVI reporting scientists; a reporter and a reviewer. Currently, the staff members trained to report coronial cases / DVI incidents are Ingrid Moeller, Jacqui Wilson, Rhys Parry and also myself. This pool of trained staff is relatively small compared to the pool of staff trained in the core business of Forensic DNA Analysis.

62. The process is generally as follows:

(a) Scientists may be requested to assist pathologists with the selection of bones / teeth samples for DNA recovery to maximise the chance of obtaining a useable DNA profile as quickly as possible.

(b) The two bone / teeth sampling scientists prepare the bone for sampling. This is conducted in the bone room, which is a small laboratory separate from the main Evidence Recovery laboratory. Preparation can involve defleshing the bone. The ends of long bone sections are carefully avoided to mitigate the possibility of including biological contaminants from the mortuary environment.

(c) Bones are chiselled into smaller bone fragments using a chisel and other equipment. The smaller bone fragments and teeth are cleaned with analytical grade ethanol and placed into an enclosed cylinder that sits on a mount inside a bone crusher. This sample within the cylinder is taken to a very low

temperature using liquid nitrogen. The bone sample is then crushed into a fine power.

(d) Several aliquots of bone / teeth powder are weighed out and transferred to the Analytical team for processing by way of extraction, quantitation, amplification and capillary electrophoresis as per any other sample.

(e) The results from the bone / teeth aliquots are interpreted and reported by the allocated coronial reporting scientist to the coroner in a DNA identification statement. As with all case types, there is also an allocated coronial reviewing scientist.

63. The bone / teeth workflow is different to the general workflow as it enables reporting scientists to perform evidence recovery tasks. However, due to the loss or deskilling of reporting staff, I am currently the only reporting scientist who performs both tasks.

Three major changes in bone processes

Cleaning regime of equipment used for sampling bone / teeth

64. In approximately May 2015, Timothy Gardam commenced 'Project #148 – to optimise the cleaning protocol for bone crusher vials'. The project investigated whether changing the way vials were cleaned could optimise the process. At the time, Timothy was a subject matter expert for and the co-ordinator of bones / teeth.

65. Annexed and marked AK-21 is a copy of the final Project #148 report.

66. Previously, FSS used tergazyme to assist in the cleaning of equipment used for bone / teeth sampling. Tergazyme is an enzyme detergent that breaks down DNA. I understand that it is extremely effective, however, it is not good for the environment.

67. In around April 2016 while I was on maternity leave, the manager of Evidence Recovery Allan McNevin started to take over bone / teeth decision making from Timothy. To my knowledge, Allan had no experience in bones at this time. This change to the workflow was communicated to the affected staff in a meeting held on 21 April 2016. When I returned from maternity leave later in 2016, I was told by Timothy to refer to the meeting minutes and that Allan was in charge of bones / teeth. Although the meeting minutes indicated Allan would allocate bone / teeth sampling to maintain staff competencies, there were no other notes indicating Timothy was no longer bone / teeth co-ordinator.

68. On 5 July 2019, the process for cleaning bones was changed by Allan. The minor change management log recorded:

'Change in bone processing equipment cleaning protocol:

Cleaning of the bone crushing equipment using the dishwasher as per Proposal #148; Use bleach and / or Trigene followed by 70% ethanol (as appropriate) to clean the remaining equipment in line with other Evidence Recovery and Analytical laboratory equipment protocols'

69. Annexed and marked AK-22 is a copy of an extract of the change management log.

70. As far as I am aware, there was no validation or verification performed to confirm that bleach and / or trigene followed by 70% ethanol can effectively clean the remaining equipment used to sample bone / teeth. This remining equipment, which is unique to bone / teeth sampling, includes chisels, hammers, chisel blocks (Perspex) and sometimes includes Dremel bits, a hand saw and an electric saw. In my opinion, this equipment may not be effectively cleaned in this way.

71. On or around 26 May 2022, I noticed that the chisels used for bone sampling were rusty due to the new cleaning regime. I talked to other staff members trained to sample bones / teeth and it was agreed that the pitting on the rusty chisels could be retaining DNA and contributing to the contamination of the bone/ teeth samples.

72. On or around 26 May 2022, Allison Lloyd the current manager of Evidence Recovery purchased new chisels for bone / teeth sampling from a hardware store. These new chisels remain susceptible to rusting due to the new cleaning regime of equipment used for sampling bone / teeth. At this time, the new cleaning regime is not being reassessed outside of the OQI investigation.

Bone / teeth extraction method

73. In April 2018, the bone / teeth extraction method changed from organic (phenol chloroform) extraction to QIAGEN pre-lysis followed by QIASymphony SP extraction on the QIASymphony SP instrument.

74. Annexed and marked AK-23 is a timeline I drafted between 6 March 2018 and 14 June 2018, which identifies events that occurred during the implementation of the new bone / teeth extraction method.

75. Annexed and marked AK-24 is a copy of the Project Report #192 Validation of QIASymphony SP for Bone Extraction, dated April 2018.

76. I do not think the validation of this method was adequate, as outlined in paragraphs 77 to 88 below.

77. On 18 April 2018, Jacqui Wilson and I saw Justin Howes in his office to discuss our concerns about the change in the bone / teeth extraction method. We told Justin that we thought the new process (QIASymphony extraction) was not working as well as the old process (organic extraction). This was due to our active coronial case demonstrating a noticeable decrease in quantitation yield for bone aliquots processed after the implementation of the new process. During this discussion, Justin said something along the lines of, '*any apparent differences would be due to sample to sample variation*', and he dismissed our concerns.

78. That same day, I had a similar discussion with Kylie Rika about my concerns with the new extraction method for bones / teeth. I am aware that Kylie wrote an email to Justin regarding the extraction method for bones / teeth. I have not seen a response from Justin. A copy of Kylie's email to Justin on 18 April 2018, which I obtained from Kylie is annexed and marked AK-25.

79. On 19 April 2018, I talked to Rhys Parry about the new extraction method for bones / teeth. Rhys quickly identified an issue with the experimental design of Project #192 and went to see Paula Brisotto about his concerns.

80. On or around 24 April 2018, the laboratory reverted to the organic extraction method for bones / teeth pending further work on Project #192.

81. The organic extraction has been successfully used to isolate DNA from older more compromised bones / teeth samples for many coronial cases in our laboratory as well as other laboratories in Australia. Although this extraction method uses hazardous chemicals and requires a high level of skill from the Analytical scientists performing the manual process, it works.

82. Later, in March 2020, a 'Supplementary Repeatability and Reproducibility' report was prepared for Project Report #192 Validation of QIASymphony SP for Bone Extraction. Annexed and marked AK-26 is a copy of the supplementary report.

83. This supplementary validation appears to have partially addressed the invalid experimental design of the original validation.

84. In March 2020, again the bone / teeth extraction method changed from organic (phenol chloroform) extraction to QIAGEN pre-lysis followed by QIASymphony SP extraction on the QIASymphony SP instrument.

85. In October 2020, Project Report #192: Teeth Extraction – Verification of QIASymphony SP for Teeth Extraction was finalised. Annexed and marked AK-27 is a copy of the verification.

86. My name is listed as an author of this verification. However, I had limited involvement in this verification. The extent of my involvement was that I conducted a blind interpretation of the DNA profiles obtained from eight samples during the verification experiment.

87. A copy of an email chain between me and Luke Ryan discussing my profile interpretation for the verification is annexed and marked AK-28.

88. Since the implementation of the QIASymphony extraction, I have noticed an increase in the number of low level or no DNA profiles from bones / teeth and more recently mixed DNA profiles from bones / teeth. While I am of the view that the QIASymphony extraction works adequately for DVI incidents or litigated cases involving fresh DNA rich bone / teeth samples, I do not think this extraction method is optimal for older more compromised bone / teeth samples. I do not believe the QIASymphony extraction isolates as much DNA from older more compromised bone / teeth samples compared to the organic extraction.

Implementation of the 3500 Genetic Analyser instrument

89. The most recent change to the processing of bones has been the implementation of the 3500 Genetic Analyser instrument for the capillary electrophoresis process. It was introduced in February 2021 and has not been specifically validated for bone / teeth aliquots.

90. As mentioned previously, the new instrument has greater sensitivity.

91. The 3500 instrument may be contributing to the detection of additional low level contributors in bone / teeth aliquots that previously would not have been detected on the less sensitive 3130 instrument.

Other concerns about bone processes

Allan McNevin decision making

92. While the manager of Evidence Recovery, Allan McNevin became involved in decisions about the way bones / teeth were to be dealt with. When he first took over bones / teeth, I do not think Allan had training or experience in this specialised workflow or subject matter.

93. Allan and I have different opinions regarding tissue selection for coronial cases and DVI incidents. For a DVI incident during the early stages of the pandemic in March 2020, Allan with the support of Justin Howes made it clear that soft tissue samples for this case would be processed for DNA in preference to any other tissue. My concern was these tissue samples were affected by decomposition. I had previously told Allan verbally that soft tissue samples may not be appropriate due to the condition of remains. Further to this, the samples were submitted for DNA analysis as Priority 3 rather than Priority 2, which impacts on turnaround times as this is the lowest priority routinely assigned to samples in the laboratory. We did not obtain useable DNA profiles from most of these tissue samples and had to return to the original samples to attempt DNA recovery from bones. This resampling was successful, but it caused approximately a one week delay to the release of the final DNA identification reports to the coroner and ultimately the release of the bodies to the bereaved families for burial. I felt that my expertise in this area was not taken into account.

Reporting scientists no longer permitted to attend Coronial Identification meetings

94. Coronial ID meetings are held weekly and, historically, were attended by two reporting DNA scientists from FSS, a specialised staff member from the Scientific Services Liaison Unit, specialised staff members from Forensic Pathology, QPS officers from the Coronial Support Unit, Forensic Odontologists and bereavement counsellors. The purpose of these meetings is to minimise delays and streamline the identification process. In my view, attendance of a least one reporting DNA scientist at Coronial ID meetings is crucial as the identification process is often convoluted and complicated partly due to the involvement of many different disciplines across many different organisations. These meetings quickly highlight less than ideal communication and address any information gaps.

95. On 20 June 2019, I was advised by Justin Howes in a meeting with Jacqui Wilson that reporting scientists were not to attend Coronial ID meetings anymore, despite my attendance at the meetings for the past 12 years and my continued involvement in coronial cases. Instead, either Paula Brisotto or Allan McNevin as representatives from Evidence Recovery would attend these meetings. I raised my concerns about this with Justin at the time. Annexed and marked AK-29 is a copy of the email chain between Justin and me.

96. I understand that the direction for this change came from Cathie Allen, as this is what I was told by Justin. Although I cannot recall exactly, the reason given around this decision was the alignment of duties and tasks. I feel that I was not consulted about this decision and the reasons for the change were not fully explained to me.

97. On or around 19 May 2022, I met with Lara Keller to discuss my concerns about the Coronial ID meetings and mixtures in bones. Following the meeting, I emailed Lara and provided the 2019 correspondence between Justin and me about the Coronial ID meetings. Annexed and marked AK-30 is a copy of my email to Lara on 19 May 2022. Lara has asked me to come back to her regarding these concerns.

Reporting scientists no longer permitted to attend the mortuary

98. On 30 March 2021, FSS management directed scientists not to attend the mortuary to assist pathologists with bone / teeth and tissue selection for DNA recovery. Annexed and marked AK-31 is a copy of an email chain between Cathie Allen, Kylie Rika and me about the direction, including a copy of the original FSS direction. I was not consulted before this decision was made. Management did not explain the reasons for the decision to me.

99. In my view, assisting pathologists with sample selection for DNA recovery maximises the chance of obtaining a useable DNA profile as quickly as possible. I have attended the mortuary to assist pathologists with sample selection on many occasions over the years and I have found the experience to be very helpful to all parties involved.

100. On 31 May 2021, I met with John Doherty, then Executive Director of FSS, and his advisor Alison Slade, to discuss my concerns regarding access to the mortuary. John told me to focus on the 'where possible' wording in the document, a scientist experienced in the selection of bones / teeth samples for DNA recovery is permitted to attend the mortuary if requested by a pathologist and the viewing platform can be used

to mitigate any exposure risks (a strategy often employed by QPS officers). I understand John proceeded to discuss the topic with management. I am not sure what management John was referring to and what happened in these discussions.

101. Annexed and marked AK-32 is a copy of my email chain with John and handwritten notes from the meeting on 31 May 2021.

102. Presently, unless permission is granted by Cathie, Justin Howes or Paula Brisotto, scientists are not permitted to attend the mortuary to assist pathologists with the selection of bones / teeth samples for DNA recovery, and instead the current manager of Evidence Recovery, Allison Lloyd, is engaged. Annexed and marked AK-33 is a copy of an email chain from Paula on 9 September 2022.

103. On 31 August 2022, the Acting Mortuary Manager Ricky Truong contacted Evidence Recovery to request possible assistance from scientists with tissue selection for DVI post-mortems that were scheduled for the next day. A scientist referred Ricky to Valerie Caldwell and me to assist the Lead DVI Pathologist Nathan Milne.

104. Accordingly, on 1 September 2022, I sent an email to Kylie and Justin informing them of the Mortuary Manager's request for Valerie and my assistance.

105. In response, on the same day, Justin suggested that '*Damien Cass is ascertaining if assistance can best be provided (if ultimately required) via telephone which would be in line with any health and safety risks involved.*'. Damien Cass is the Managing Scientist of Coronial Services.

106. Annexed and marked AK-34 is a copy of my email chain with Justin.

107. In my view, providing assistance to pathologists conducting DVI post-mortems by phone is impractical, given:

(a) scientists are unable to visualise and examine the questioned remains that are often in various states of damage and prone to comingling contamination due to the circumstances of the incident; and

(b) pathologists are gowned up in personal protective equipment working in a wet mortuary environment with many biological hazards and interrupting this critical work to phone an FSS scientist for assistance is not conducive to streamlining this complicated process.

Removal from bone sampling and deskilling

108. I am concerned that, despite having a high level of expertise in all aspects of bones / teeth and approximately 16 years of experience, I will be deskilled and removed from bone / teeth sampling.

109. In around May 2022, I was advised by Kylie Rika that some members of FSS management had decided that I would not be involved at all in evidence recovery from bones / teeth and any enquiries from the mortuary should be redirected to Allison Lloyd as the manager of Evidence Recovery. I was concerned about this and felt that I was being shut out of a process that I have been heavily involved in for many years. The reason given by Cathie Allen to Kylie around this decision was along the lines of alignment of duties and tasks in preparation for the proposed Business Case for Change. I felt like there has been no transparency in the decisions and I have not been included in discussions about this change. I do not want to lose my skills and expertise in bones / teeth.

110. Currently, Allison as the manager of Evidence Recovery attends Coronial ID meetings.

111. I emailed Lara Keller with my concerns on 30 May 2022. Annexed and marked AK-35 is a copy of my email to Lara. Lara has asked me to come back to her regarding my concerns.

Reworks and changes to originally reported results

112. Reporting scientists are required to seek written permission from Cathie Allen to rework priority 2 samples that have been finalised (i.e. samples that have had a result reviewed in the Forensic Register).

113. If permission is granted, and the rework of a sample obtains a different result to the original interpretation, we are then required to provide a written update to Cathie. The line 'This sample has undergone further processing' is added to the Forensic Register followed by the new result lines.

114. In November 2019, I sought approval from Cathie to rework a sample originally interpreted as a two contributor mixture. Cathie approved the rework request, and I subsequently obtained a three contributor mixture. I provided a written update to Cathie and she decided I would additionally need to provide an Intel Report to QPS advising

them of the reasons why I had amended the result. As directed, I took time to complete Cathie's additional task.

115. Annexed and marked AK-36 is a copy of an email chain between Cathie and myself.

116. I am concerned about needing permission to order a rework as it restricts my ability to perform one of my core duties as a scientist and creates stress.

Forensic Register

117. In my experience at FSS, there have been issues with the Forensic Register. Following the implementation of a new version of the Forensic Register on 2 May 2022, the system appeared to have many 'bugs' whereby it would not operate as intended.

118. An example of a bug I experienced when accessing the Forensic Register from the generic plate reading computer is the previous user would remain logged in when another user opened up the Forensic Register, unless a new work around was used to close the Forensic Register.

119. Bugs or errors in the Forensic Register version update as of 2 May 2022 have been logged by various staff on a spreadsheet. Annexed and marked AK-37, is a spreadsheet of some errors logged, which was taken from a larger spreadsheet of all logged errors as at 23 May 2022.

Culture at FSS

120. Despite building up years of experience and trust in the workplace, staff are not easily able to access basic stationary. They are required to request administrative staff to unlock locked cupboards for access to pens. This is just one example that falls within the larger context of controlling behaviour being exhibited by management.

121. My manager is Kylie Rika, who is not included in my concerns about management. Kylie raises issues in management meetings and supports her team members.

Interaction with Commission of Inquiry

122. In July 2022, I decided to approach the Commission voluntarily to provide information. I was concerned about approaching the Commission initially, as I was afraid of reprimand from management. I met with members of the Commission in a public library on a weekend, as I did not want management at the laboratory to find out or for me to be seen at the Commission's office.

FSS Staff Surveys

123. In April 2021, an internal analysis survey was conducted within the whole of FSS. My reporting team grouped together and brainstormed a submission to the survey. Claire Gallagher drafted the response, incorporating all of the team's ideas, for each staff member to submit. I submitted the response on 30 April 2021.

124. Annexed and marked AK-38 is an email between my reporting team containing the draft submission to the survey.

125. Our Executive Director, Lara Keller, also ran a confidential workplace survey in April of this year. I understand staff provided frank responses about the difficulties they face at work. I provided a response to this effect.

Previous Director John Doherty

126. Previously the Executive Director was John Doherty. In my view, John was aware of many of the issues with the culture and different personalities at FSS. I understand members of staff who were having issues in Forensic DNA Analysis would sometimes take them directly to John. I went to John on a number of occasions to discuss the issues I was having with the culture and some scientific processes in the laboratory, particularly around bones and teeth for coronial cases and DVI incidents, and the lack of transparency and inclusion in decision making.

127. Issues I raised with John between May 2019 and July 2021 include reporters attendance at Coronial ID meetings and scientists attendance at the mortuary to assist pathologists with tissue selection, bone sampling processes (including permissions), deskilling, incorrect results, working from home decisions, structure of DNA and suboptimal science.

128. Before finishing up his role at FSS, on 3 September 2021, John responded to one of my catch up request emails and apologised for not 'seeing everything through for me'. I understand 'everything' in this context to mean all of the issues we had discussed. Annexed and marked AK-39 is a copy of the email from John dated 3 September 2021.

Livingstones investigation into Project 181

129. On or around 16 January 2017, I received correspondence regarding an independent investigation Livingstones was conducting into the Sensitivity of Spermatozoa

Microscopy project, commonly known as Project #181. Annexed and marked AK-40 is a copy of the letter dated 16 January 2017.

130. Following receipt of the letter, I was invited to attend an interview with Mark Brady from Livingstones. I attended the interview with Mark Brady. During the interview, I was not asked anything about Project #181, but rather, I was quizzed about whether I preferred Allan McNevin or Amanda Reeves and their different personalities.

131. I recall signing a contemporaneous document prepared by Mark Brady that summarised our discussion at the conclusion of my interview. I left the interview feeling very uneasy about the whole process.

132. I do not know what the purpose or the outcome of the interview was.

Workplace Edge consultation 2017

133. On 1 December 2017, I understand an email was distributed to some FSS staff stating that specialist consultants Workplace Edge had been appointed to provide guidance and support to improve FSS and support the effective delivery of services. I was not initially included in the email, which became apparent after I spoke with a colleague who discussed the email. Consequently, I followed up with Kylie Rika about not receiving the email.

134. Later that day, on 1 December 2017, I received an email from Cathie Allen apologising for not including me in the email correspondence and stating that it was '*an oversight*' on her behalf. Annexed and marked AK-41 is a copy of the email from Cathie dated 1 December 2017.

135. Prior to the interview, I prepared notes. On 5 December 2017 at 10:30 am, I attended my assigned Workplace Edge interview with Alan Holz and Anekah Russon as my support person. During the interview, I covered a range of points including the culture, the science, my love for the work we do and coronial cases / DVI incidents.

136. Around 9 February 2018, all reporters attended a meeting with Cathie, which summarised the information gathered during the Workplace Edge interviews. This feedback presentation included no information that I had provided during my interview and did not represent my views. I left this meeting feeling confused. I felt like management did not take into consideration my feedback and views or include me in decisions.

Training and professional development

137. There is no formal professional development plan for scientists at FSS. Management seems focused on 'numbers' and turnaround times for the QPS. Scientists are celebrated for producing high quantity rather than high quality results. I feel like this is demoralising to our profession.

138. I am subject to performance reviews with my manager. I understand that these are supposed to occur yearly, however, in my experience, the reviews I have attended have been less frequent than yearly. I have performance reviews approximately every few years. During the most recent performance reviews, Kylie Rika has demonstrated leadership and as much support and understanding as she possibly could.

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

TAKEN AND DECLARED before me at Brisbane in the State of Queensland this 6th day of October 2022.

[redacted] .....

Angelina Keller

[redacted] .....

Witness [redacted]

[redacted] .....

Angelina Keller

[redacted] .....

Witness [redacted]

CERTIFICATE OF EXHIBIT

Exhibit No.	Description	Date
AK-01	Email from Keller to Howes about rework of a DIFP P1 sample	29.05.2018
AK-02	Case example – Forensic Register showing sample identified as 3-contributor mixture	26.02.2021
AK-03	Case example – electropherograms of sample interpreted as 3-contributor mixture on 3130	15.02.2021
AK-04	Case example – Forensic Register showing rerun sample identified as 4-contributor mixture	30.04.2021
AK-05	Case example – electropherograms of rerun sample interpreted as 4-contributor mixture on 3500	23.04.2021
AK-06	Email chain between Keller and Ryan about quantitation variation in a sample	30.05.2022 – 31.05.2022
AK-07	Email chain between Keller and Rika about reworking DIFP results	02.06.2022 – 15.06.2022
AK-08	Email from Johnstone to reporting scientists about DIFP reporting	06.06.2022
AK-09	Contemporaneous email file note about discussion with Johnstone regarding DIFP rework	15.06.2022
AK-10	Replacement statement for case example containing two reworked DIFP results	21.06.2022
AK-11	Original statement for case example prior to reworked DIFP results	17.06.2022
AK-12	Electropherograms of reworked DIFP samples	10.06.2022
AK-13	Exhibit testing page from the Forensic Register	21.07.2022
AK-14	Email chain between Rosengren, Gregg, Rika, Howes and Keller about 19 August 2022 decision and bones	19.08.2022 – 22.08.2022
AK-15	Electropherogram for coronial case example involving mixed DNA profile	02.06.2022
AK-16	Electropherogram for coronial case example involving mixed DNA profile	29.04.2022
AK-17	Electropherogram for coronial case example involving mixed DNA profile	27.06.2022
AK-18	Electropherogram for coronial case example involving mixed DNA profile	01.07.2022
AK-19	Spreadsheet of bone sample results between 2019 – 2022	2019 - 2022
AK-20	OQI Report 56724 Mixtures in Bones	17.06.2022

[redacted]

Angelina Keller

Witness [redacted]

AK-21	Project #148 – to optimise the cleaning protocol for bone crusher vials	05.2015
AK-22	Extract of the change management log regarding change in bone processing	05.07.2019
AK-23	Timeline drafted by Keller identifying events during implementation of new bone extraction method	06.03.2018 – 14.06.2018
AK-24	Project Report #192 – Validation of QIAsymphony SP for Bone Extraction	04.2018
AK-25	Email from Rika to Howes about Project 192 and bone extraction	18.04.2018
AK-26	Project Report #192 – Validation of QIAsymphony SP for Bone Extraction Supplementary Repeatability and Reproducibility	03.2020
AK-27	Project Report #192 – Validation of QIAsymphony SP for Teeth Extraction	10.2020
AK-28	Email chain between Ryan and Keller about Project Report #192 for Teeth Extraction	11.08.2020 – 28.08.2020
AK-29	Email chain between Howes and Keller about coronial meetings	20.06.2019
AK-30	Email from Angelina Keller to Lara Keller about coronial meetings	19.05.2022
AK-31	Email chain between Allen, Rika and Keller regarding direction from management advising that scientists are not to attend the mortuary	30.03.2021
AK-32	Email chain between Keller and Doherty and handwritten file note by Keller about the meeting	17.05.2021 – 31.05.2021
AK-33	Email chain between Brisotto, Keller and mortuary staff about mortuary attendance by FSS scientists	08.09.2022 – 09.09.2022
AK-34	Email chain between Keller, Rika and Howes about attendance to mortuary to assist pathologist	01.09.2022
AK-35	Email from Angelina Keller to Lara Keller about Angelina's removal from bone sampling procedures	30.05.2022
AK-36	Email chain between Keller and Allen requesting permission to rework a final result	14.11.2019 – 28.11.2019
AK-37	Spreadsheet of errors identified in the Forensic Register	23.05.2022
AK-38	Email chain between Keller scientists in Reporting 2 about response to FSS Staff Survey	29.04.2021
AK-39	Email chain between Keller and Doherty about issues identified by Keller	03.09.2021

AK-40	Letter from Health Support Queensland to Keller about Livingstones investigation	16.01.2017
AK-41	Email from Allen to Keller about Workplace Edge consultation	01.12.2017

AK-01

Angelina Keller

15/07/2022
No Reply
? Vet Dog

From: Angelina Keller
Sent: Tuesday, 29 May 2018 9:34 AM
To: Justin Howes
Cc: Matthew Hunt
Subject: FW: P1 request

Hi Justin,

FYI – 5 x of the below P1s are No DNA ([redacted] The last is Insufficient [redacted], Quant 0.00112).

For Debs P1 that I am reviewing, Ewen requested the 2 x Insufficients were processed further and the outcome after M'cons were SS profiles (initial quants were 0.00651 and 0.008).

Did you want to wait for QPS or just allow the last P1 to be Insufficient?

Thanks,
Ange

From: Matthew Hunt
Sent: Monday, 28 May 2018 9:01 AM
To: Angelina Keller
Subject: RE: P1 request

Hi,

Just to let you know I'm on standby for court in Ipswich on Tuesday, and will be out of the office on Wed morning (in case the P1s are ready and need to find another reviewer).

Thanks,
Matt

From: Justin Howes
Sent: Friday, 25 May 2018 2:24 PM
To: Angelina Keller; Matthew Hunt
Subject: FW: P1 request

HI, please see FR comms on this. P1 request allocated to you both.

Thanks
Justin

Justin Howes
Team Leader – Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Forensic & Scientific Services,
Health Support Queensland, **Department of Health**

p | [redacted] m | [redacted]
a | 39 Kessels Road, Coopers Plains, QLD 4108
w | Queensland Health e | [redacted]

AK-02

Forensic Services

Worklist | Batch | Sample | Administration

Exhibit Detail

Exhibit Detail

Barcode No: [redacted] Forensic No: [redacted] QPRIME No: [redacted]

Offence: MURDER Complainant: [redacted] Subject: [redacted] 29/05/76

Category Swab Loc D DNA rear middle sash belt; Re-extract spin basket from [redacted]

Batch No

Case Scientist: 440112 KELLER.A Review Scientist: 440190 VALENZIA.A **Auslab Sent to Peer Review** Status: P2 26/02/2021 08:11 NCIDD

Profile Analysis

440112 KELLER.A

Barcode	DNA Extraction & Post Ext.	µL	DNA Quantification	ng/µL	STR Amplification	SV1	TV1	SV2	TV2	Capillary Electrophoresis	Include
[redacted]											
		!	CDNAQUA20201203-01	0.028	CSTRAMP20210119-01 B04	15.0	0.0	0.0	0.0	CCE20210122-01 B04	☑

Profile Interpretation

Contributors: ○1 ○2 ◎3 ○4 ○5 Profile: ○CX ○NP ○PU ☐ST STRmix™ Notes

	D3	D1	D6	D13	PentE	D16	D18	D2	CSF	PentD	TH01	vWA	D21	D7	D5	TPOX	D8	D12	D19	FGA
1	16,18	13,15	12,19	9,12	10,18	12,0	17,17	25,25	11,12	11,11	6,8	18,0	31.2,31.2	11,11	12,12	8,8	15,0	18.3,19	14,14	24,27
2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

Profile Record

Amel	D3	D1	D6	D13	PentE	D16	D18	D2	CSF	PentD	TH01	vWA	D21	D7	D5	TPOX	D8	D12	D19	FGA
X,X	16,18	13,15	12,19	9,12	10,18	12,0	17,17	25,25	11,12	11,11	6,8	18,0	31.2,31.2	11,11	12,12	8,8	15,0	18.3,19	14,14	24,27

[redacted] UKF1 ☑ + CPT ☑ + NCIDD -intel1 ☐

Case Profiles

Barcode	Name	Association	Category	STRmix™	H1	H2	AC	LR	Reported LR	Employee	Reviewer	Include
[redacted]	[redacted] 22/10/1956		VLP ☐		☐	☑	☐	1.7714E-1	6	440112		☐
			VLP ☐		☐	☑	☐	6.4832E-1	2	440112		☐
UKF1			CASE ☐ △		☐	☐	☐			440112		☐

Sample Notes

[redacted] Batch audit entry noted. Result reportable. AK 19/01/2021
440112 25/02/2021 09:20 [redacted] UKF1 [+NCIDD]

AK-03

appliedbiosystems

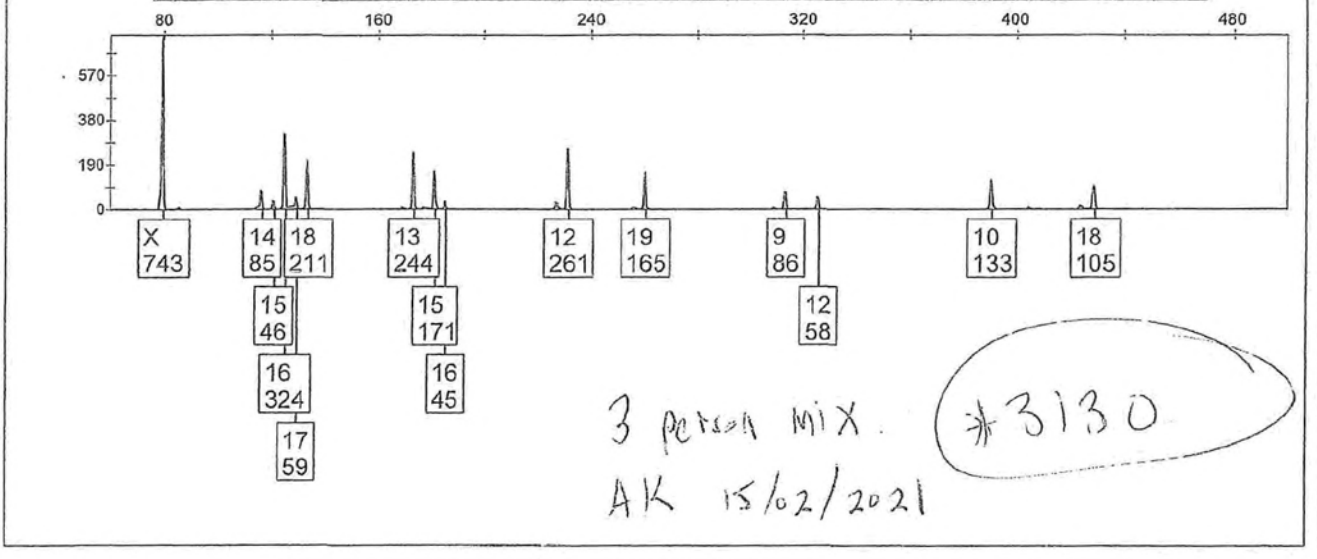
GeneMapper™ ID-X 1.6

Re-extract spin basket from [redacted]

Project: [redacted]

Swab; Loc D DNA rear middle sash belt

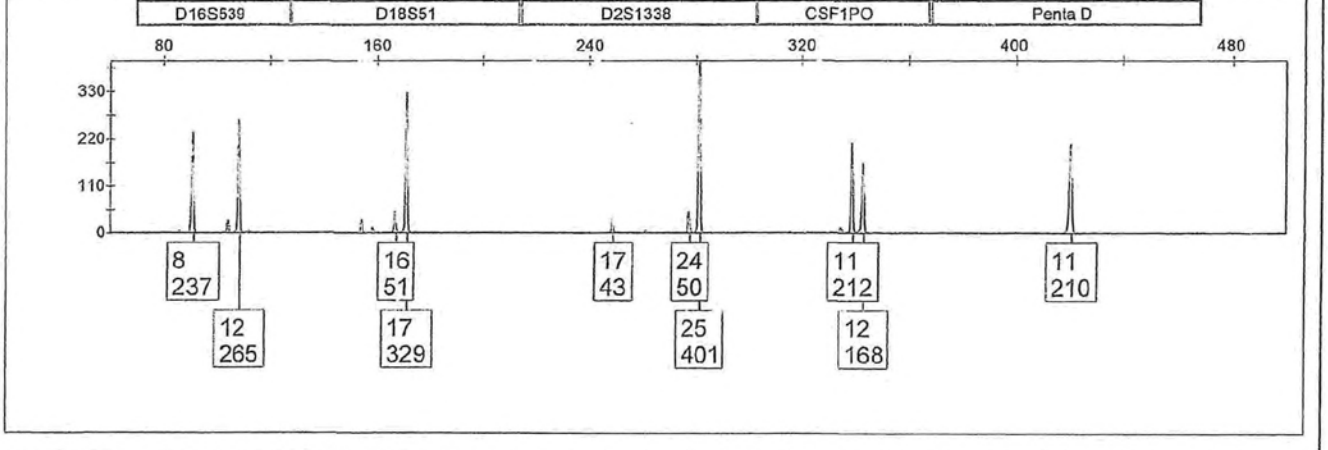
Sample Name	Panel
[redacted]	PowerPlex 21 IDX v3.3



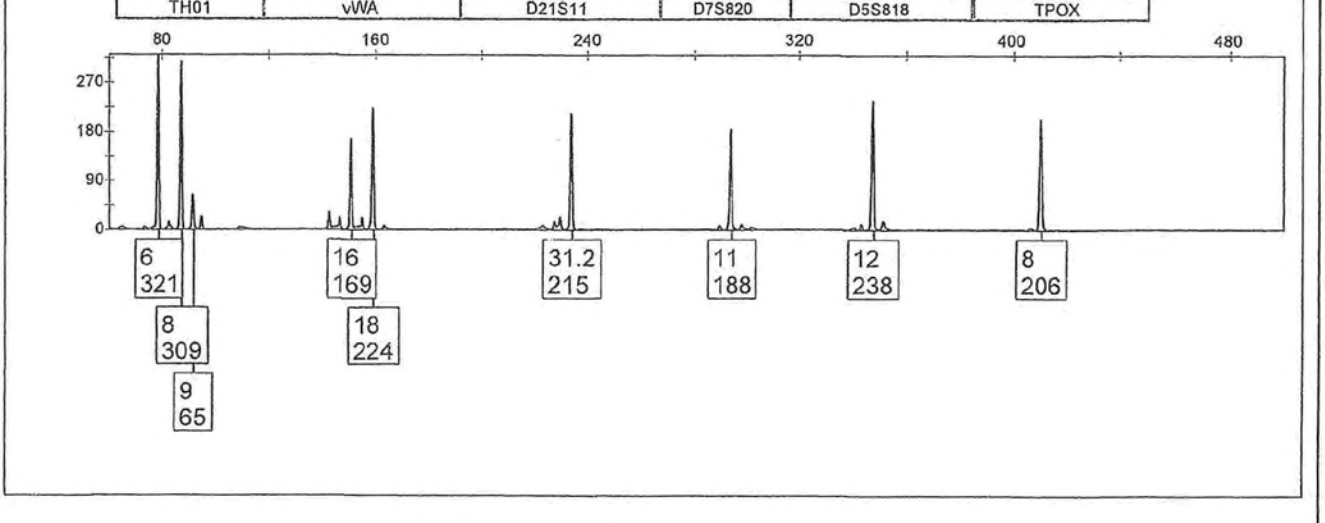
3 person MIX. *3130

AK 15/02/2021

[redacted] PowerPlex 21 IDX v3.3



[redacted] PowerPlex 21 IDX v3.3



AK-04

6/21/22, 9:38 AM [redacted]

Profile Analytical Detail

Exhibit Detail

Barcode No: [redacted] Forensic No: [redacted] QPRIME No: [redacted]

Operation: [redacted]

Offence: Murder (Inc. attempts) Complainant: [redacted]

Batch No: Subject: [redacted] 29/05/76

Category Swab Loc D DNA rear middle sash belt; Re-extract spin basket from [redacted]

Case Scientist: 440112 KELLER.A Review Scientist: 440190 VALENZIA.A Auslab Sent to Peer Review Status: P2 21/06/2022 09:32 Profile Review

Profile Analysis

440112 KELLER.A

Barcode	DNA Extraction & Post Ext.	µL	DNA Quantification	ng/µL	DNA Amplification	SV1	TV1	SV2	TV2	Capillary Electrophoresis	Include
[redacted]	[redacted] A		CDNAQUA20201203-01	0.028	CSTRAMP20210119-01 B04	15.0	0.0	0.0	0.0	CCE20210122-01 B04	☐ STRmix™ B.
										CCE20210316-01 B02	☑ STRmix™ B.

Profile Interpretation

Contributers: ○1 ○2 ○3 ◉4 ○5+ Profile: ○CX ○NP ○PU ☐ST STRmix™: ☑ (v2.8.0) AAQ rev Notes

C	D3S1358	D1S1656	D6S1043	D13S317	PentaE	D16S539	D18S51	D2S1338	CSF1PO	PentaD	TH01	vWA	D21S11	D7S820	D5S818	TPOX	D8S1179	D12S391	D19S433	FGA
1	16,18	13,15	12,19	9,12	10,18	8,12	17,17	25,25	11,12	11,11	6,8	16,18	31.2,31.2	11,11	12,12	8,8	12,15	18.3,19	14,14	24,27
2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

Profile Record

○ GlobalFiler ◉ PowerPlex21 ○ Profiler Plus ○ VeriFiler Plus

MEL	D3S1358	D1S1656	D6S1043	D13S317	PentaE	D16S539	D18S51	D2S1338	CSF1PO	PentaD	TH01	vWA	D21S11	D7S820	D5S818	TPOX	D8S1179	D12S391	D19S433	FGA
X	16,18	13,15	12,19	9,12	10,18	12,0	17,17	25,25	11,12	11,11	6,8	16,0	31.2,31.2	11,11	12,12	8,8	15.0	18.3,19	14,14	24,27
[redacted] UKF1															☐ + CPT	☐ + NCIDD			-intell	
X,X	16,18	13,15	12,19	9,12	10,18	8,12	17,17	25,25	11,12	11,11	6,8	16,18	31.2,31.2	11,11	12,12	8,8	12,15	18.3,19	14,14	24,27
[redacted] UKF1															☐ + CPT	☑ + NCIDD			-mix1	

Case Profiles

[Show All]

Barcode	Name	Association	Category	STRmix™	H1	H2	AC	LR	Reported LR	Report	Employee	Reviewer	Include
[redacted]	[redacted]			☑ (v2.8.0)	☐	☑	☐	6.7827E-1	2	[redacted]	440112		☐ B.
			VL	☑ (v2.8.0)	☐	☑	☐	1.9080E-3	530		440112		☐ B.
			VL	☑ (v2.8.0)	☑	☑	☐	1.2409E0	1		440112		☐ B.
		UKM6	SU	☑ (v2.8.0)	☐	☑	☐	4.1574E-2	25		440112		☑ B.
			SU	☑ (v2.8.0)	☐	☑	☐	2.3914E-3	420		440112		☑ B.
			SU	☑ (v2.8.0)	☐	☑	☐	1.7822E-3	570		440112		☑ B.
	UKF1		CASE		☐	☐	☐				440112		☐ B.
	UKM2		CASE A		☐	☐	☐				440112		☐ B.
	UKM4		CASE A		☐	☐	☐				440112		☐ B.
	UKM5		CASE A		☐	☐	☐				440112		☐ B.
	UKM6	[redacted]	CASE A		☐	☐	☐				440190		☐ B.

Sample Notes

[redacted]: Batch audit entry noted. Result reportable. AK 19/01/2021
440112 25/02/2021 09:20 576093756 UKF1 [+NCIDD]
440112 30/04/2021 13:31 576093756 (37,0) UKF1 [+NCIDD [Modify 576093756-intell]]

TONE 09:29 AM 21/06/2022 440112 (Australia/Brisbane) 2022-06-21 09:38 164.112.251.224

AK-05

applied biosystems

GeneMapper™ ID-X 1.6

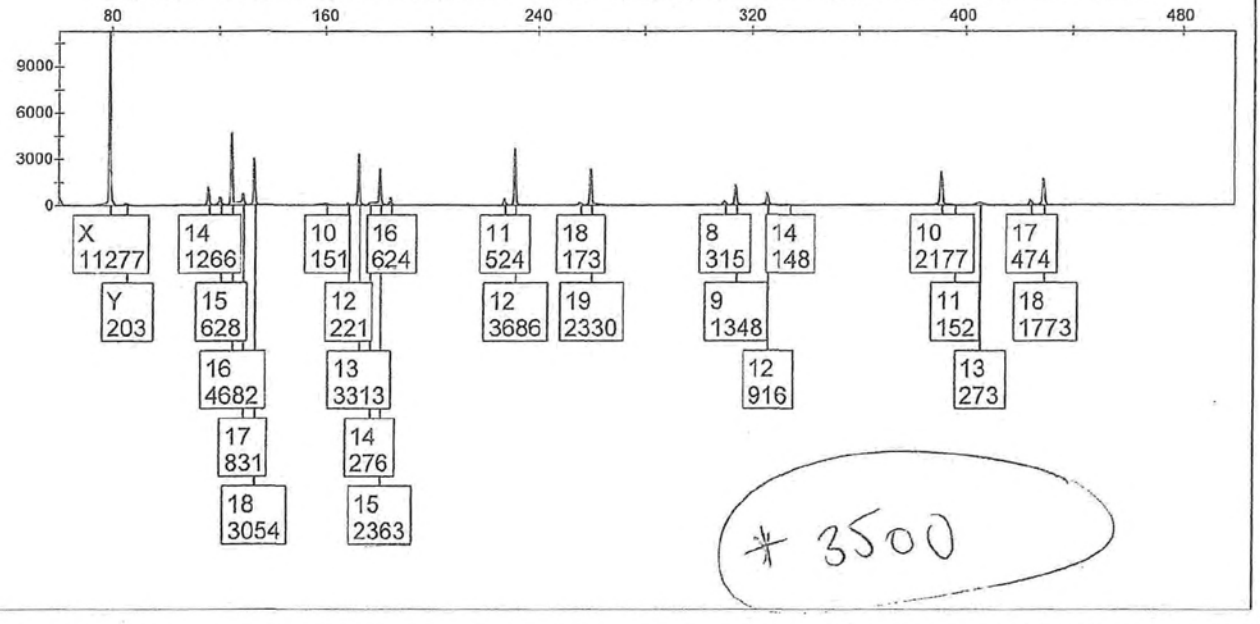
Re-extract spin basket from [redacted]

Project: [redacted]

Swab; Loc D DNA rear middle sash belt

Sample File	Sample Name	Panel
[redacted]05_2021-03-16-12-24-52_3500 Instrument.hid	[redacted]	PowerPlex_21_IDX_v3.3

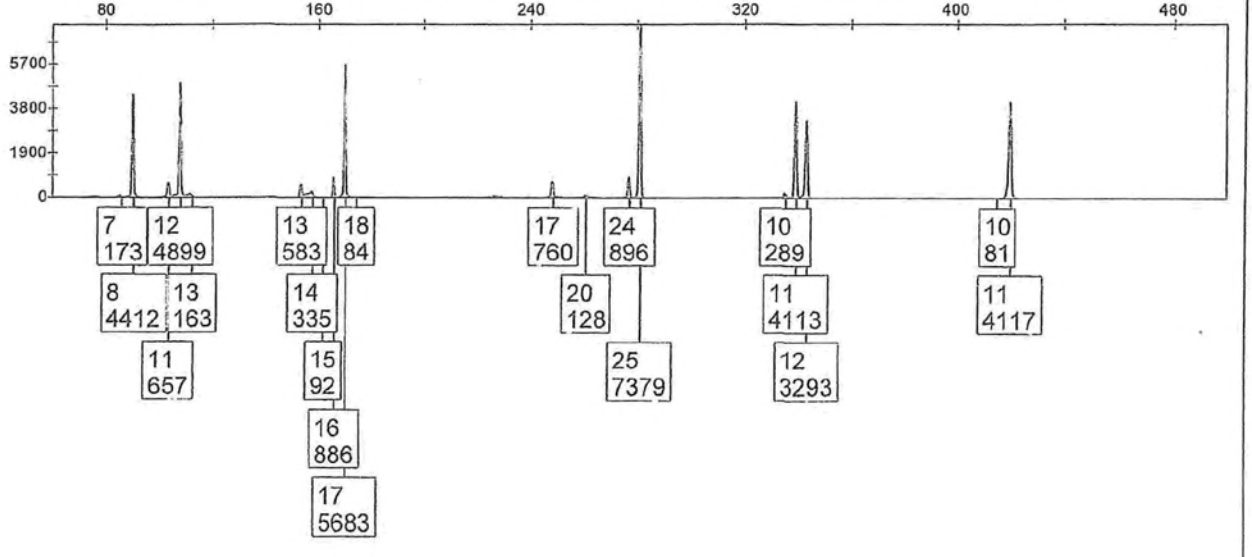
AMEL	D3S1358	D1S1656	D6S1043	D13S317	Penta E



* 3500

[redacted] 2021-03-16-12-24-52_3500 Instrument.hid [redacted] PowerPlex_21_IDX_v3.3

D16S539	D18S51	D2S1338	CSF1PO	Penta D



4 person Mix.

AK 23/04/2021

AK-06

Angelina Keller

From: Luke Ryan
Sent: Tuesday, 31 May 2022 6:29 AM
To: Angelina Keller
Subject: RE: Quant variation for sample [redacted]

Follow Up Flag: Follow up
Flag Status: Completed

Hi Angelina
Looking at the results, the second one is accurate. When you get variations like this, have a look at the LAT and Y targets (if there is male DNA). For both replicates the Y target is similar to the second SAT. The first and second LAT are both undetermined.

The IPCCT for the first replicate is on the low side - 25.8 which may indicate a bubble, or some spike in the amp plot early on in the quant reaction (hard to know exactly without looking at the amp plot). The IPCCT for the second quant 27.5 is in the range we usually see.

Does this help?

Thanks
Luke

From: Angelina Keller [redacted]
Sent: Monday, 30 May 2022 4:02 PM
To: Luke Ryan [redacted]
Subject: Quant variation for sample [redacted]

Hi Luke,

The quant for this sample has gone from 2.222 to 0.003 ng/uL. I just saw Justin and he suggested talking to you (I was popping over to Paula as Tom mentioned this happened a while ago and Paula knew about it?). There does not appear to be anything obviously wrong with the quants. Can you see anything that may have contributed to the high initial quant? Thanks.

Kind regards,
Ange

AK-07

Angelina Keller

From: Angelina Keller
Sent: Wednesday, 15 June 2022 2:36 PM
To: Kylie Rika
Cc: Alicia Quartermain
Subject: FW: Matter of [redacted]

Hi ladies,

FYI (provided Alicia agrees) – I obtained:

[redacted] - complex unsuitable; and
- 3p mix cond on the complainant, supporting non-contribution for the defendant

Kind regards,
Angelina

From: Angelina Keller
Sent: Thursday, 2 June 2022 3:41 PM
To: Kylie Rika [redacted]
Cc: Alicia Quartermain [redacted]
Subject: RE: Matter of [redacted]

Thank you so much Kylie. I will let you know what the new results are.

Ange

From: Kylie Rika [redacted]
Sent: Thursday, 2 June 2022 3:38 PM
To: Angelina Keller [redacted]
Cc: Alicia Quartermain [redacted]
Subject: RE: Matter of [redacted]

Hi Ange

Absolutely further process these two. Justin advised me recently that we don't need to seek RW authorisation from Cathie on these ones as the DIFP result is like an interim result.

Thanks
Kylie

From: Angelina Keller [redacted]
Sent: Thursday, 2 June 2022 3:31 PM
To: Kylie Rika [redacted]
Cc: Alicia Quartermain [redacted]
Subject: RE: Matter of [redacted]

Hi Kylie,

[redacted] (scrotum) and [redacted] (perianal).

Thanks,

Ange

From: Kylie Rika <[redacted]
Sent: Thursday, 2 June 2022 3:24 PM
To: Angelina Keller <[redacted]>
Subject: RE: Matter of [redacted]

Hi Ange

What are the barcodes of the 2 samples?

Thanks
Kylie

From: Angelina Keller <[redacted]
Sent: Thursday, 2 June 2022 3:04 PM
To: Kylie Rika <[redacted]>
Cc: Alicia Quartermain <[redacted]
Subject: FW: Matter of [redacted]

Hi both,

I have double checked this case and I am concerned about two results contained within given all the information available to me at this point in time. This is a child SA case and the two insufficient samples are intimate samples (both insufficients are in the timeframe since the implementation of the 3500). Can I please rework these two samples and issue a replacement statement?

Kind regards,
Angelina

From: forensics <[redacted]
Sent: Thursday, 2 June 2022 2:40 PM
To: Angelina Keller <[redacted]>
Subject: FW: Matter of [redacted]

Hi Angelina,

Could you please let me know if you will be available between 15.08.22 – 19.08.22 to give evidence for this matter?

Regards,

Alex Skocic
Liaison Officer, Scientific Services Liaison Unit
Client Services
Forensic and Scientific Services
Prevention Division, Queensland Health

p [redacted]
a 39 Kessels Road, Coopers Plains, 4108
[redacted] w www.health.qld.gov.au/healthsupport

From: Nolan.JasonL[SER] <[redacted]
Sent: Thursday, 2 June 2022 2:11 PM
To: forensics <[redacted]
Subject: Matter of [redacted]

Fellow colleagues.

Can I request that the attached statement be forwarded to Angelina KELLER. Further can I request that acknowledgment be sent through via reply e-mail.

Thankyou

Jason NOLAN
Plain Clothes Senior Constable [redacted]
Logan District CPIU
Logan Central Police Station
[redacted]

AK-08

Angelina Keller

From: Sharon Johnstone
Sent: Monday, 6 June 2022 3:13 PM
To: Adrian Pippia; Alicia Quartermain; Angela Adamson; Anne Finch; Cassandra James; Emma Caunt; Jacqui Wilson; Josie Entwistle; Kerry-Anne Lancaster; Rhys Parry; Allan McNevin; Angelina Keller; Claire Gallagher; Deborah Nicoletti; Ingrid Moeller; Matthew Hunt; Penelope Taylor; Tegan Dwyer; Thomas Nurthen
Cc: Kylie Rika; Allison Lloyd; Luke Ryan
Subject: FW: DNA Insufficient - Quant transition to Amp

Importance: High

Follow Up Flag: Follow up
Flag Status: Completed

Hi all,
Please see below instructions stemming from today's announcements. These have been agreed to by QPS.
Please also note that any sample that has already been DNA insufficient is to be continued to be reported as such at statement stage. These results are known to the QPS. If it is their wish to have them restarted they will let us know.

Regards,
Sharon

Sharon Johnstone
Senior Scientist – Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream
Prevention Division, Queensland Health

Please note that I may be working from a different location during the COVID-19 pandemic. The best contact method is via email.

p [redacted]
a 39 Kessels Road, Coopers Plains, QLD 4108
e [redacted] w www.health.qld.gov.au/fss

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

From: Justin Howes <[redacted]
Sent: Monday, 6 June 2022 1:55 PM
To: Kylie Rika [redacted]; Sharon Johnstone <[redacted]

Cc: Paula Brisotto <[redacted]>
Subject: FW: DNA Insufficient - Quant transition to Amp
Importance: High

Hi
Please note the DIFP process is currently suspended (the range correction to below is 0.001-0.0088ng/uL). Any new samples in this range will go directly for amp.

Previously reported DIFP that are requested for a restart, will go to microcon as per current process.

P3 samples will continue to be case managed in the same way as always – without rework unless not amped at max (of which the samples in the pertinent range will be amped at max).

Regards
Justin

Justin Howes
Team Leader - Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream, Forensic & Scientific Services
Prevention Division, Queensland Health

p [redacted] m [redacted]
a 39 Kessels Road, Coopers Plains, QLD 4108
e [redacted] w www.health.qld.gov.au/fss

Please note that I may be working from a different location during the COVID-19 Pandemic. The best contact method is via email.

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.

From: Paula Brisotto <[redacted]>
Sent: Monday, 6 June 2022 1:23 PM
To: Justin Howes <[redacted]>
Subject: FW: DNA Insufficient - Quant transition to Amp
Importance: High

FYI

From: Luke Ryan <[redacted]>
Sent: Monday, 6 June 2022 1:20 PM
To: Adam Kaity <[redacted]>; Alanna Darmanin <[redacted]>; Amy Cheng <[redacted]>; Belinda Andersen <[redacted]>; Biljana Micic <[redacted]>; Generosa Lundie <[redacted]>; Lai-Wan Le <[redacted]>; Lisa Farrelly <[redacted]>; Maria Aguilera <[redacted]>; Melissa Cipollone <[redacted]>; Nicole Roselt

<[redacted]>; Pierre Acedo <[redacted]>; Sharelle Nydam
<[redacted]>; Tara Prowse <[redacted]>
Cc: Paula Brisotto <[redacted]>; Cathie Allen <[redacted]>
Subject: DNA Insufficient - Quant transition to Amp
Importance: High

Afternoon All
The premier has requested we test (amp) all samples in the current DNA Insufficient Range (i.e. above 0.001 – 0.088 ng/µL).

When transitioning Quant batches, please ensure all samples in the DNA Insufficient range are transitioned to the Amp WL. We are not reporting DNA Insufficient result lines as of now.

Please also ensure when reviewing No DNA Detected samples, look for samples with the DNA Insufficient result which have not been transitioned to the Amp WL. Please reallocate these to the Amp WL. I will go through the No DNA review list now and allocate these to the Amp WL.

There is no change to rules for No DNA Detected samples.

FR will be modified so that these rules are incorporated into the Quant transition page, but this will be a manual process until these changes are made.

Thanks
Luke

Luke Ryan
Senior Scientist – Analytical Team

Forensic DNA Analysis, Forensic and Scientific Services
Prevention Division, Queensland Health

p [redacted] m [redacted]
a 39 Kessels Rd, Coopers Plains, QLD 4108
e [redacted] w www.health.qld.gov.au/healthsupport/businesses/forensic-and-scientific-services

AK-09

Angelina Keller

From:	Angelina Keller
Sent:	Wednesday, 15 June 2022 11:47 AM
To:	Angelina Keller
Subject:	Discussion about two insufficient reworks for a replacement statement

At approximately 11 am on 15 June I was returning from Block 3 to Block 6 and Sharon was also returning to Block 6. I was retrieving a case file for a case I have been subpoenaed to give evidence for in August. I told Sharon I had reworked two insufficient samples from a child SAIK where there was sperm (there actually wasn't – that was my mistake from my memory) but insufficient and a statement had already been issued. She said I shouldn't have unless the police had requested this (they knew what we did and the old results still stand). I said I had reworked as I had doubt given all the information I had at this point in time and I wanted to do what I thought was right for everyone. I had confidence at the time but I didn't have confidence now and it was in the post-implementation of the 3500. There was an e-mail send on the 6 June saying don't rework insufficients. I let her know I had permission from my line manager and reviewer to rework prior to this e-mail.

15/07/2022
Alicia asked
Shaton verbally
if we can re-
work at statement
stage and she
said yes, but
not after a
statement has
been issued.

AK-10

Queensland Government

Forensic and Scientific Services
HealthSupport Queensland

STATEMENT OF WITNESS

Peer Reviewed ..... (Yes) No
Case Analyst... [redacted]
Peer Analyst... [redacted]
Date Issued... 21 June 2022

Client Reference : [redacted]
Report Number : [redacted]

QUEENSLAND)
TO WIT)

I, Angelina KELLER, of Brisbane in the State of Queensland, do solemnly and sincerely declare that:-

1. I am employed by Queensland Health Forensic and Scientific Services (QHFSS) at Coopers Plains, Brisbane.

2. I hold the position of scientist in the Forensic DNA Analysis laboratory of QHFSS.

3. I was awarded a Bachelor of Agricultural Science (Honours) from University of Tasmania.
   I was awarded a Master of Science (Forensic Science) from Griffith University.

4. I am a member of the Australian and New Zealand Forensic Science Society.

5. This is my replacement Statement of Witness in relation to the alleged offence that Occurrence Number [redacted] refers. The defendant in this matter is [redacted]. The complainant in this matter is [redacted].

   This replacement Statement of Witness has been issued following the further processing of two samples. This Statement of Witness replaces the Statement of Witness issued by me on 17 June 2021.

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Phone [redacted]
Fax
Email

6. Laboratory records show that on 1 March 2021, S/CONST JASON LEIGH NOLAN delivered the following item:

   [redacted]

7. Laboratory records show that on 9 March 2021, Mandy KAKKAR delivered the following reference sample:

   [redacted] 07/08/1997

8. Laboratory records show that on 23 March 2021, S/CONST PAUL JAMES MCPHEE delivered the following reference sample:

   [redacted] 09/10/2012

9. The results of the scientific examinations conducted in the laboratory are as follows:

   Reference Samples

   [redacted]

   A DNA profile was obtained from each of these reference samples. These reference DNA profiles are different to each other.

   Item Received on 1 March 2021

   Examined by Forensic DNA Analysis

   [redacted] **– A; Sexual Assault Investigation Kit (SAIK);** [redacted]

   This SAIK contained the following items:

   1 x penis dorsum swab
   1 x scrotum swab
   1 x perianal swab
   1 x cheeks swab
   1 x right forearm swab
   1 x left arm swab
   1 x dropsheet

   The penis dorsum, cheeks, right forearm and left arm swabs each tested positive for the possible presence of saliva. These four swabs were submitted separately for DNA analysis.

   The scrotum and perianal swabs each tested negative for the presence of saliva. These two swabs were submitted separately for DNA analysis.

   The dropsheet appeared unused. No further testing was conducted on this item.

   DNA analysis results
   Penis dorsum swab

   Due to the complex and partial nature of this DNA profile, including uncertainty as to the number of contributors, in my opinion this DNA profile is unsuitable for further interpretation.

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STATEMENT OF WITNESS Client Reference : [redacted]

Scrotum swab

Due to the complex and partial nature of this DNA profile, including uncertainty as to the number of contributors, in my opinion this DNA profile is unsuitable for further interpretation.

Perianal swab

The mixed DNA profile obtained from this sample indicates the presence of DNA from three contributors, one of whom could be [redacted]. Since this sample is said to have been collected from [redacted] it would not be unexpected to find DNA which could have come from him. To interpret this mixed DNA profile an assumption of DNA from three contributors, one of whom is [redacted], has been made for statistical analysis.

The reference DNA profile of [redacted] has been compared to this mixed DNA profile, to assess whether or not he may have contributed DNA, along with [redacted]

Based on statistical analysis, it is estimated that the mixed DNA profile obtained is approximately 83 times more likely to have occurred if there has not been a contribution of DNA from [redacted] rather than if there has.

Cheeks swab

The DNA profile obtained from this sample indicates the presence of DNA from a single contributor and matches the reference DNA profile of [redacted].

Since this sample is said to have been collected from [redacted], it would not be unexpected to find DNA which could have come from him, and therefore statistical analysis has not been conducted.

Right forearm swab

The mixed DNA profile obtained from this sample indicates the presence of DNA from three contributors, one of whom could be [redacted] Since this sample is said to have been collected from [redacted] it would not be unexpected to find DNA which could have come from him. To interpret this mixed DNA profile an assumption of DNA from three contributors, one of whom is [redacted] has been made for statistical analysis.

The reference DNA profile of [redacted] has been compared to this mixed DNA profile, to assess whether or not he may have contributed DNA, along with [redacted]

Based on statistical analysis, it is estimated that the mixed DNA profile obtained is approximately 2,000 times more likely to have occurred if there has not been a contribution of DNA from [redacted] rather than if there has.

Left arm swab

Due to the complex nature of this DNA profile, including uncertainty as to the number of contributors, in my opinion this DNA profile is unsuitable for further interpretation.

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STATEMENT OF WITNESS Client Reference : [redacted]

APPENDIX

Procedural and technical overview of DNA profiling at Forensic DNA Analysis, Forensic and Scientific Services, Health Support Queensland

Forensic Biologist

It is a forensic biologist's role to:

1. Report on the examination of items submitted in relation to a case for the presence of possible biological material. If identified, a sample of the biological material is analysed in an attempt to obtain a DNA profile.

2. Report on the DNA profiles obtained from samples submitted by the Queensland Police Service (QPS) in relation to a case.

Any DNA profiles that are obtained from these samples are compared with the DNA profile obtained from an individual's reference sample to assess whether or not the individual may be a contributor of DNA. Where multiple reference DNA profiles are analysed for a case, they are all genetically different to each other unless otherwise specified. That is, they all possess differing allelic designations – see the DNA Profiling section below.

The signed Statement is the report issued by Forensic and Scientific Services. Any other attachments or information provided is not considered to be the issued report.

Examinations

Unless otherwise stated, the examinations of items for biological material were conducted by staff within the QPS. Sub-samples from these items were forwarded to Forensic and Scientific Services (FSS), Health Support Queensland, for the purposes of conducting DNA analysis.

The descriptions of items and/or samples submitted to Forensic DNA Analysis by the QPS, and listed within this Statement, are derived from the descriptions entered by the QPS into the Forensic Register.

Receipt details are listed for items reported in this statement, including reference samples where relevant. The period of testing on these items is from the date of the first submission, to the date of this Statement. Details of specific dates of testing are retained within the Laboratory Information Management System (LIMS). Where relevant, any items that are still in progress at the time of issuing this Statement are identified and final results will be reported in an addendum Statement.

Forensic DNA Analysis operates under the agreement that the QPS are responsible for item prioritisation, sample selection, selection of screening/sampling methods, anti-contamination procedures and the application of Standard Operating Procedures (SOPs) on work undertaken on the items/samples prior to submission to the Forensic DNA Analysis laboratory. As such, forensic biologists may not be able to provide information or opinion on possible biological origin of DNA profiles that may be obtained from these samples.

At the discretion of the QPS, some items may be submitted to this laboratory for the purposes of both examination and DNA profiling. These examinations are performed in accordance with the SOPs of this laboratory. For these items, contemporaneous notes are made during the examination by the examining scientist and these notes form part of the casefile.

Forensic DNA Analysis casefiles and any samples remaining are available for independent examination and/or testing upon request.

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As a representative of the laboratory, I am only able to comment on the processes performed within Forensic DNA Analysis.

Chain of Custody

All Forensic DNA Analysis case files and exhibits are electronically tracked, monitored, and securely stored to ensure that appropriate chain of custody and continuity measures are maintained. The QPS case number and sample submission information is provided from the QPS via an electronic interface to FSS, and this information is cross-checked against labelling on the exhibit packaging prior to processing.

Entry into Forensic DNA Analysis is restricted to authorised persons only, via electronic proximity access cards. Forensic DNA Analysis forms part of a Health Support Queensland campus site wherein access is controlled and monitored by a security team. Records of visitors to Forensic DNA Analysis are retained.

Accreditation

Forensic DNA Analysis first achieved accreditation by the National Association of Testing Authorities (NATA) to conduct forensic DNA analyses in 1998, and has continuously maintained NATA accreditation since this date. NATA ensures continued compliance with the accreditation requirements through routine reassessment (every 3 years) and an intermediate surveillance visit (at approximately 18 months between reassessment surveys).

NATA accredited facilities are assessed against best international practices based on the ISO/IEC 17025 standard. Laboratories are deemed able to competently perform testing and analysis activities if the laboratory demonstrates that it meets compliance with the standard within the scope of their accreditation.

The parameters assessed during accreditation include:

- Organisation and management
- Quality management system
- Personnel
- Evidence management
- Methods and procedures
- Quality control and Proficiency Testing
- Equipment
- Reporting of results
- Procurement of services and supplies
- Accommodation and safety
- Security and access

For details of the current ISO/IEC 17025 Standard, refer to Standards Australia.

http://www.nata.com.au

DNA Profiling

Deoxyribonucleic Acid (DNA) is a complex chemical found in almost all cells of the human body. It carries genetic information that determines the physical and chemical characteristics of a person. Forensic DNA Analysis uses two main systems for the generation of DNA profiling results. In this case, the PowerPlex® 21 System was used, which examines 21 regions (loci) of DNA, 20 of which contain highly variable short tandem repeats (STRs). The 21st region gives an indication as to the genotypic sex of the donor (for details see Table 1). The generation of a DNA profile involves a method known as the polymerase chain reaction

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(PCR), which is used to produce numerous copies of these specific regions of the DNA. In this way, minimal amounts of DNA isolated from small or degraded samples can be increased to a level where the DNA can be detected, profiled, and compared with DNA profiles from other samples.

The individual components (alleles) of a DNA profile are represented by a series of peaks on a graph that are measured and given a designation, using standard sizing ladders. A person will have two alleles (represented graphically as peaks) for each locus, one inherited from the biological mother and one inherited from the biological father. However, if the same allele is inherited from both parents, only one peak will be graphically represented at that locus.

A DNA profile obtained from biological material such as blood, semen, saliva, hair or cellular material (eg. touch DNA) can be compared with the DNA profile obtained from the reference sample from any person.

Table 1: PowerPlex® 21 system, list of loci

Abbreviated Name	Scientific Name	Chromosomal Name
Amel	AMELOGENIN	Sex (X and Y)
D3	D3S1358	3
D1	D1S1656	1
D6	D6S1043	6
D13	D13S317	13
Penta E	Penta E	15
D16	D16S539	16
D18	D18S51	18
D2	D2S1338	2
CSF	CSF1PO	5
Penta D	Penta D	21
TH01	TH01	11
vWA	HUMVWAFA31/A	12
D21	D21S11	21
D7	D7S820	7
D5	D5S818	5
TPOX	TPOX	2
D8	D8S1179	8
D12	D12S391	12
D19	D19S433	19
FGA	HUMFIBRA	4

Statistical Analysis of DNA profiles

STRmix™ is an expert statistical DNA profile analysis system developed and validated in Australia and New Zealand. Forensic DNA Analysis uses the STRmix™ software to assist in the interpretation of DNA profiles and the calculation of likelihood ratios for DNA profiles generated using the PowerPlex® 21 system.

DNA profiles are initially assessed to determine the number of contributors. This value will be the minimum number of people that are required to reasonably explain the observed profile, however, it is noted that there is always the possibility that the profile is a result of a different number of contributors.

As such, if there is no indication of a contribution by more than one person, then a DNA profile is described as being from a "single contributor". If less than 40 alleles present in a DNA profile, this is referred to as a

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"partial" or "incomplete" DNA profile. If there are indications of two or more contributors, then a DNA profile is described as being a "mixed" DNA profile.

DNA Profiles Assumed To Originate From One Person (Single Source)

A person can be excluded as a possible source of the biological material if the alleles of the crime-scene DNA profile are different from the corresponding alleles of the person's reference DNA profile. If the corresponding alleles of the crime-scene DNA profile contain the same information, then that person, along with any other person who has the same corresponding alleles as the crime-scene DNA profile, can be considered as a potential contributor of the DNA.

The evidential significance of such a match is assessed by considering two competing propositions:

Proposition 1: The crime-scene DNA originated from the person of interest.

Proposition 2: The crime-scene DNA originated from someone other than, and unrelated to, the person of interest.

The resultant figure (termed the 'Likelihood Ratio') compares the two opposing propositions. The likelihood ratio describes how likely the DNA profile obtained from the biological material is to have occurred if Proposition 1 were true (the DNA originated from the person of interest) rather than if Proposition 2 were true (the DNA originated from someone other than, and unrelated to, the person of interest).

The likelihood ratio is calculated by taking into account the characteristics of the DNA profile and the frequency of occurrence of the individual alleles that make up the DNA profile.

If less than the 20 STR regions of DNA are observed in a DNA profile, the likelihood ratio will be smaller than the likelihood ratio calculated for a full DNA profile obtained from the same individual. In other words, the more incomplete a DNA profile is, the greater the likelihood that the obtained DNA profile could have come from someone other than, and unrelated to the person of interest.

DNA Profiles Assumed To Originate From More Than One Person (Mixed DNA Profiles)

In order to assess whether a person may or may not have contributed to a mixed DNA profile, a set of competing propositions (similar to the single source DNA profile example) are considered. For example, for a two-person mixture:

Proposition 1: The crime-scene DNA originated from the person of interest and an unknown person, unrelated to the person of interest.

Proposition 2: The crime-scene DNA originated from two unknown people, both unrelated to the person of interest.

The likelihood ratio provides a statistical assessment of the possibility that the DNA profile obtained was the result of a DNA contribution by the person of interest.

The likelihood ratio will not always favour Proposition 1 (the DNA originated from the person of interest and an unknown person unrelated to the person of interest). The likelihood ratio could favour Proposition 2 (the DNA originated from two unknown people unrelated to the person of interest).

In certain circumstances, if the ownership of an item is established or if the sample was collected from an identified person's body, then it is possible to make the reasonable assumption that the identified person has contributed DNA to the resultant mixed DNA profile. In these cases, a mixed DNA profile can be 'conditioned' on the DNA profile of the assumed contributor, such that the presence of the alleles corresponding with the assumed contributor's reference DNA profile can be factored into the statistical interpretation. This may

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facilitate a more meaningful statistical analysis of potential second and/or third contributors to the DNA profile. In this situation, the likelihood ratio is based on the following propositions:

Proposition 1: the DNA has originated from the assumed contributor and the person of interest;

Proposition 2: the DNA has originated from the assumed contributor and an unknown individual unrelated to the person of interest.

DNA profiles with an uncertain number of contributors

Forensic DNA Analysis is currently validated for the interpretation of DNA profiles with 1-4 contributors. Once the assessment of the number of contributors has been made, this information is used in the STRmix™ analysis.

When a DNA profile is deemed unsuitable for interpretation it may be described as 'complex'. This can occur under the following circumstances:

- When a DNA profile could have a large number of contributors and therefore it is difficult to exclude individuals and/or determine whether a person could be a potential contributor to the DNA profile
- When there is very limited information available and/or the quality of the profile is poor.

It is important to note that should further information be received that renders the assumptions used in an analysis invalid, the DNA profile will require additional statistical interpretation.

Datasets Used in Statistical Analyses

Three validated datasets consisting of DNA profiles obtained from individuals of the Australian Caucasian, Aboriginal, and South-East Asian populations are used to calculate the likelihood ratio. A population correction factor, θ (theta), is applied to all likelihood ratio calculations in order to correct for the common genetic ancestry of people within a particular population (sharing of DNA components inherited from a common ancestor). In Forensic DNA Analysis, a likelihood ratio that represents a stratified population statistic is reported irrespective of whether the DNA profile of interest is single source or a mixed DNA profile.

The likelihood ratio is calculated using all three datasets and the relative proportions of each ethnic grouping in the overall Australian population based on data sourced from the Australian Bureau of Statistics. This calculation, which is known as stratification, allows for the possibility that a random unknown person, unrelated to the person of interest, from anywhere in Australia could have contributed to the DNA profile obtained. Therefore, the likelihood ratio is not dependent on the ethnicity of the person of interest, but it is representative of the relative proportion of the person of interest's ethnic population within the larger Australian population.

Theta cannot account for close blood relatives. Closely related people, such as siblings, will have a greater chance of sharing similar components within their DNA profiles. However, due to the nature of parental DNA recombination during conception, the probability that two siblings would share the same 40 alleles would be very small. As this relationship becomes more distant, the probability of two relatives having the same DNA profile becomes smaller still. If it were thought that a close blood relative might have been a contributor of DNA, the most meaningful approach to interpretation would be to submit the reference sample from the relative in question for DNA analysis and subsequent direct comparison to the crime-scene DNA profile.

Often the calculated likelihood ratio produces numbers of thousands (1000s) or even millions (1000,000s) of billions. To avoid the use of potentially confusing mathematical terminology, a "ceiling figure" for the likelihood ratio of 100 billion is used (this is called "truncation"). For example, a calculated likelihood ratio of "150 000 billion times more likely", would be reported as "greater than 100 billion times more likely". The actual calculated figure can be provided upon request.

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STATEMENT OF WITNESS Client Reference : [redacted]

Touch DNA / Transfer of DNA

When a person touches a surface, it is possible for their DNA to be transferred onto that surface. This transferred DNA can often be recovered (sampled) by a swab, tape lift, or excision depending upon the nature of the surface in question, and the sample can then be subjected to DNA profiling.

This transferred DNA can originate from cells or cellular material within sweat and/or oils on the skin. The generation of a DNA profile will depend on many factors. These include the amount of DNA transferred, the nature of the surface being touched, and the amount of genetic material available for transfer. The persistence of any transferred genetic material to a surface will depend largely upon the nature of the surface and the conditions the surface has been subjected to in the time between deposition and recovery of the DNA. For example, DNA could be lost from the surface by washing or mechanical action such as abrasion.

It therefore follows that the inability to obtain a DNA profile from a touched surface does not necessarily mean that a person has not had contact with the surface. It is possible for a person to contact a surface without a detectable amount of their DNA being transferred or subsequently recovered for analysis.

Saliva

A presumptive chemical test (Phadebas) may be used to detect the possible presence of saliva. This test exploits the enzyme activity of a constituent of saliva called amylase. Amylase is usually present at a relatively high concentration in saliva, though this can vary considerably between individuals. Amylase may also be detected in other body fluids such as sweat, vaginal fluid and anal secretions, although usually at much lower concentration than that found in saliva.

The presence of saliva on a surface may be the result of spitting or direct oral contact. Saliva may subsequently be transferred onto other items such as clothing or other areas of the body. Possible saliva stains may then be detected on skin swabs or items of clothing by the Phadebas test, as long as the clothing or skin has not been washed. Cellular material within the saliva, if present in sufficient quantities, can be used to obtain a DNA profile.

JUSTICES ACT 1886

I acknowledge by virtue of Section 110A (6C) (c) of the Justices Act 1886 that:

(i) This written statement by me dated 21 June 2022 and contained in the pages numbered 1 to 9 is true to the best of my knowledge and belief; and

(ii) I make it knowing that, I would be liable to prosecution for stating anything that I know is false.

[redacted].....

Angelina KELLER

Signed at BRISBANE on 21 June 2022

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AK-11

Queensland Government

Forensic and Scientific Services
HealthSupport Queensland

STATEMENT OF WITNESS

Peer Reviewed.....(Yes) No

Case Analyst. [redacted]

Peer Analyst. [redacted]

Date Issued. 17 June 2021

Client Reference : [redacted]
Report Number :

QUEENSLAND)
TO WIT)

I, Angelina KELLER, of Brisbane in the State of Queensland, do solemnly and sincerely declare that:-

1. I am employed by Queensland Health Forensic and Scientific Services (QHFSS) at Coopers Plains, Brisbane.
2. I hold the position of scientist in the Forensic DNA Analysis laboratory of QHFSS.
3. I was awarded a Bachelor of Agricultural Science (Honours) from University of Tasmania. I was awarded a Master of Science (Forensic Science) from Griffith University.
4. I am a member of the Australian and New Zealand Forensic Science Society.
5. This is my statement in relation to the alleged offence that Occurrence Number [redacted] refers. The defendant in this matter is [redacted]. The complainant in this matter is [redacted].

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Email [redacted]

STATEMENT OF WITNESS Client Reference : [redacted]

6. Laboratory records show that on 1 March 2021, S/CONST JASON LEIGH NOLAN delivered the following item:

   [redacted]

7. Laboratory records show that on 9 March 2021, Mandy KAKKAR delivered the following reference sample:

   [redacted] 07/08/1997

8. Laboratory records show that on 23 March 2021, S/CONST PAUL JAMES MCPHEE delivered the following reference sample:

   [redacted] 09/10/2012

9. The results of the scientific examinations conducted in the laboratory are as follows:

Reference Samples

[redacted]

A DNA profile was obtained from each of these reference samples. These reference DNA profiles are different to each other.

Item Received on 1 March 2021

Examined by Forensic DNA Analysis

[redacted]**– A; Sexual Assault Investigation Kit (SAIK);** [redacted]

This SAIK contained the following items:

1 x penis dorsum swab
1 x scrotum swab
1 x perianal swab
1 x cheeks swab
1 x right forearm swab
1 x left arm swab
1 x dropsheet

The penis dorsum, cheeks, right forearm and left arm swabs each tested positive for the possible presence of saliva. These four swabs were submitted separately for DNA analysis.

The scrotum and perianal swabs each tested negative for the presence of saliva. These two swabs were submitted separately for DNA analysis.

The dropsheet appeared unused. No further testing was conducted on this item.

DNA analysis results
Penis dorsum swab

Due to the complex nature of this DNA profile, including uncertainty as to the number of contributors, in my opinion this DNA profile is unsuitable for further interpretation.

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Scrotum swab

This sample contained insufficient DNA for analysis and therefore, it was not processed further.

Perianal swab

This sample contained insufficient DNA for analysis and therefore, it was not processed further.

Cheeks swab

The DNA profile obtained from this sample indicates the presence of DNA from a single contributor and matches the reference DNA profile of [redacted]

Since this sample is said to have been collected from [redacted], it would not be unexpected to find DNA which could have come from him, and therefore statistical analysis has not been conducted.

Right forearm swab

The mixed DNA profile obtained from this sample indicates the presence of DNA from three contributors, one of whom could be [redacted] Since this sample is said to have been collected from [redacted] it would not be unexpected to find DNA which could have come from him. To interpret this mixed DNA profile an assumption of DNA from three contributors, one of whom is [redacted], has been made for statistical analysis.

The reference DNA profile of [redacted] has been compared to this mixed DNA profile, to assess whether or not he may have contributed DNA, along with [redacted]

Based on statistical analysis, it is estimated that the mixed DNA profile obtained is approximately 2,000 times more likely to have occurred if there has not been a contribution of DNA from [redacted] rather than if there has.

Left arm swab

Due to the complex nature of this DNA profile, including uncertainty as to the number of contributors, in my opinion this DNA profile is unsuitable for further interpretation.

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APPENDIX

Procedural and technical overview of DNA profiling at Forensic DNA Analysis, Forensic and Scientific Services, Health Support Queensland

Forensic Biologist

It is a forensic biologist's role to:

1. Report on the examination of items submitted in relation to a case for the presence of possible biological material. If identified, a sample of the biological material is analysed in an attempt to obtain a DNA profile.

2. Report on the DNA profiles obtained from samples submitted by the Queensland Police Service (QPS) in relation to a case.

Any DNA profiles that are obtained from these samples are compared with the DNA profile obtained from an individual's reference sample to assess whether or not the individual may be a contributor of DNA. Where multiple reference DNA profiles are analysed for a case, they are all genetically different to each other unless otherwise specified. That is, they all possess differing allelic designations – see the DNA Profiling section below.

Examinations

Unless otherwise stated, the examinations of items for biological material were conducted by staff within the QPS. Sub-samples from these items were forwarded to Forensic and Scientific Services (FSS), Health Support Queensland, for the purposes of conducting DNA analysis.

The descriptions of items and/or samples submitted to Forensic DNA Analysis by the QPS, and listed within this Statement, are derived from the descriptions entered by the QPS into the Forensic Register.

Receipt details are listed for items reported in this statement, including reference samples where relevant. The period of testing on these items is from the date of the first submission, to the date of this Statement. Details of specific dates of testing are retained within the Laboratory Information Management System (LIMS). Where relevant, any items that are still in progress at the time of issuing this Statement are identified and final results will be reported in an addendum Statement.

Forensic DNA Analysis operates under the agreement that the QPS are responsible for item prioritisation, sample selection, selection of screening/sampling methods, anti-contamination procedures and the application of Standard Operating Procedures (SOPs) on work undertaken on the items/samples prior to submission to the Forensic DNA Analysis laboratory. As such, forensic biologists may not be able to provide information or opinion on possible biological origin of DNA profiles that may be obtained from these samples.

At the discretion of the QPS, some items may be submitted to this laboratory for the purposes of both examination and DNA profiling. These examinations are performed in accordance with the SOPs of this laboratory. For these items, contemporaneous notes are made during the examination by the examining scientist and these notes form part of the casefile.

Forensic DNA Analysis casefiles and any samples remaining are available for independent examination and/or testing upon request.

As a representative of the laboratory, I am only able to comment on the processes performed within Forensic DNA Analysis.

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Chain of Custody

All Forensic DNA Analysis case files and exhibits are electronically tracked, monitored, and securely stored to ensure that appropriate chain of custody and continuity measures are maintained. The QPS case number and sample submission information is provided from the QPS via an electronic interface to FSS, and this information is cross-checked against labelling on the exhibit packaging prior to processing.

Entry into Forensic DNA Analysis is restricted to authorised persons only, via electronic proximity access cards. Forensic DNA Analysis forms part of a Health Support Queensland campus site wherein access is controlled and monitored by a security team. Records of visitors to Forensic DNA Analysis are retained.

Accreditation

Forensic DNA Analysis first achieved accreditation by the National Association of Testing Authorities (NATA) to conduct forensic DNA analyses in 1998, and has continuously maintained NATA accreditation since this date. NATA ensures continued compliance with the accreditation requirements through routine reassessment (every 3 years) and an intermediate surveillance visit (at approximately 18 months between reassessment surveys).

NATA accredited facilities are assessed against best international practices based on the ISO/IEC 17025 standard. Laboratories are deemed able to competently perform testing and analysis activities if the laboratory demonstrates that it meets compliance with the standard within the scope of their accreditation.

The parameters assessed during accreditation include:

- Organisation and management
- Quality management system
- Personnel
- Evidence management
- Methods and procedures
- Quality control and Proficiency Testing
- Equipment
- Reporting of results
- Procurement of services and supplies
- Accommodation and safety
- Security and access

For details of the current ISO/IEC 17025 Standard, refer to Standards Australia.

http://www.nata.com.au

DNA Profiling

Deoxyribonucleic Acid (DNA) is a complex chemical found in almost all cells of the human body. It carries genetic information that determines the physical and chemical characteristics of a person. Forensic DNA Analysis uses two main systems for the generation of DNA profiling results. In this case, the PowerPlex® 21 System was used, which examines 21 regions (loci) of DNA, 20 of which contain highly variable short tandem repeats (STRs). The 21st region gives an indication as to the genotypic sex of the donor (for details see Table 1). The generation of a DNA profile involves a method known as the polymerase chain reaction (PCR), which is used to produce numerous copies of these specific regions of the DNA. In this way, minimal amounts of DNA isolated from small or degraded samples can be increased to a level where the DNA can be detected, profiled, and compared with DNA profiles from other samples.

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The individual components (alleles) of a DNA profile are represented by a series of peaks on a graph that are measured and given a designation, using standard sizing ladders. A person will have two alleles (represented graphically as peaks) for each locus, one inherited from the biological mother and one inherited from the biological father. However, if the same allele is inherited from both parents, only one peak will be graphically represented at that locus.

A DNA profile obtained from biological material such as blood, semen, saliva, hair or cellular material (eg. touch DNA) can be compared with the DNA profile obtained from the reference sample from any person.

Table 1: PowerPlex® 21 system, list of loci

Abbreviated Name	Scientific Name	Chromosomal Name
Amel	AMELOGENIN	Sex (X and Y)
D3	D3S1358	3
D1	D1S1656	1
D6	D6S1043	6
D13	D13S317	13
Penta E	Penta E	15
D16	D16S539	16
D18	D18S51	18
D2	D2S1338	2
CSF	CSF1PO	5
Penta D	Penta D	21
TH01	TH01	11
vWA	HUMVWAFA31/A	12
D21	D21S11	21
D7	D7S820	7
D5	D5S818	5
TPOX	TPOX	2
D8	D8S1179	8
D12	D12S391	12
D19	D19S433	19
FGA	HUMFIBRA	4

Statistical Analysis of DNA profiles

STRmix™ is an expert statistical DNA profile analysis system developed and validated in Australia and New Zealand. Forensic DNA Analysis uses the STRmix™ software to assist in the interpretation of DNA profiles and the calculation of likelihood ratios for DNA profiles generated using the PowerPlex® 21 system.

DNA profiles are initially assessed to determine the number of contributors. This value will be the minimum number of people that are required to reasonably explain the observed profile, however, it is noted that there is always the possibility that the profile is a result of a different number of contributors.

As such, if there is no indication of a contribution by more than one person, then a DNA profile is described as being from a "single contributor". If less than 40 alleles present in a DNA profile, this is referred to as a "partial" or "incomplete" DNA profile. If there are indications of two or more contributors, then a DNA profile is described as being a "mixed" DNA profile.

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DNA Profiles Assumed To Originate From One Person (Single Source)

A person can be excluded as a possible source of the biological material if the alleles of the crime-scene DNA profile are different from the corresponding alleles of the person's reference DNA profile. If the corresponding alleles of the crime-scene DNA profile contain the same information, then that person, along with any other person who has the same corresponding alleles as the crime-scene DNA profile, can be considered as a potential contributor of the DNA.

The evidential significance of such a match is assessed by considering two competing propositions:

Proposition 1: The crime-scene DNA originated from the person of interest.

Proposition 2: The crime-scene DNA originated from someone other than, and unrelated to, the person of interest.

The resultant figure (termed the 'Likelihood Ratio') compares the two opposing propositions. The likelihood ratio describes how likely the DNA profile obtained from the biological material is to have occurred if Proposition 1 were true (the DNA originated from the person of interest) rather than if Proposition 2 were true (the DNA originated from someone other than, and unrelated to, the person of interest).

The likelihood ratio is calculated by taking into account the characteristics of the DNA profile and the frequency of occurrence of the individual alleles that make up the DNA profile.

If less than the 20 STR regions of DNA are observed in a DNA profile, the likelihood ratio will be smaller than the likelihood ratio calculated for a full DNA profile obtained from the same individual. In other words, the more incomplete a DNA profile is, the greater the likelihood that the obtained DNA profile could have come from someone other than, and unrelated to the person of interest.

DNA Profiles Assumed To Originate From More Than One Person (Mixed DNA Profiles)

In order to assess whether a person may or may not have contributed to a mixed DNA profile, a set of competing propositions (similar to the single source DNA profile example) are considered. For example, for a two-person mixture:

Proposition 1: The crime-scene DNA originated from the person of interest and an unknown person, unrelated to the person of interest.

Proposition 2: The crime-scene DNA originated from two unknown people, both unrelated to the person of interest.

The likelihood ratio provides a statistical assessment of the possibility that the DNA profile obtained was the result of a DNA contribution by the person of interest.

The likelihood ratio will not always favour Proposition 1 (the DNA originated from the person of interest and an unknown person unrelated to the person of interest). The likelihood ratio could favour Proposition 2 (the DNA originated from two unknown people unrelated to the person of interest).

In certain circumstances, if the ownership of an item is established or if the sample was collected from an identified person's body, then it is possible to make the reasonable assumption that the identified person has contributed DNA to the resultant mixed DNA profile. In these cases, a mixed DNA profile can be 'conditioned' on the DNA profile of the assumed contributor, such that the presence of the alleles corresponding with the assumed contributor's reference DNA profile can be factored into the statistical interpretation. This may facilitate a more meaningful statistical analysis of potential second and/or third contributors to the DNA profile. In this situation, the likelihood ratio is based on the following propositions:

Proposition 1: the DNA has originated from the assumed contributor and the person of interest;

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Proposition 2: the DNA has originated from the assumed contributor and an unknown individual unrelated to the person of interest.

DNA profiles with an uncertain number of contributors

Forensic DNA Analysis is currently validated for the interpretation of DNA profiles with 1-4 contributors. Once the assessment of the number of contributors has been made, this information is used in the STRmix™ analysis.

When a DNA profile is deemed unsuitable for interpretation it may be described as 'complex'. This can occur under the following circumstances:

- When a DNA profile could have a large number of contributors and therefore it is difficult to exclude individuals and/or determine whether a person could be a potential contributor to the DNA profile
- When there is very limited information available and/or the quality of the profile is poor.

It is important to note that should further information be received that renders the assumptions used in an analysis invalid, the DNA profile will require additional statistical interpretation.

Datasets Used in Statistical Analyses

Three validated datasets consisting of DNA profiles obtained from individuals of the Australian Caucasian, Aboriginal, and South-East Asian populations are used to calculate the likelihood ratio. A population correction factor, θ (theta), is applied to all likelihood ratio calculations in order to correct for the common genetic ancestry of people within a particular population (sharing of DNA components inherited from a common ancestor). In Forensic DNA Analysis, a likelihood ratio that represents a stratified population statistic is reported irrespective of whether the DNA profile of interest is single source or a mixed DNA profile.

The likelihood ratio is calculated using all three datasets and the relative proportions of each ethnic grouping in the overall Australian population based on data sourced from the Australian Bureau of Statistics. This calculation, which is known as stratification, allows for the possibility that a random unknown person, unrelated to the person of interest, from anywhere in Australia could have contributed to the DNA profile obtained. Therefore, the likelihood ratio is not dependent on the ethnicity of the person of interest, but it is representative of the relative proportion of the person of interest's ethnic population within the larger Australian population.

Theta cannot account for close blood relatives. Closely related people, such as siblings, will have a greater chance of sharing similar components within their DNA profiles. However, due to the nature of parental DNA recombination during conception, the probability that two siblings would share the same 40 alleles would be very small. As this relationship becomes more distant, the probability of two relatives having the same DNA profile becomes smaller still. If it were thought that a close blood relative might have been a contributor of DNA, the most meaningful approach to interpretation would be to submit the reference sample from the relative in question for DNA analysis and subsequent direct comparison to the crime-scene DNA profile.

Often the calculated likelihood ratio produces numbers of thousands (1000s) or even millions (1000,000s) of billions. To avoid the use of potentially confusing mathematical terminology, a "ceiling figure" for the likelihood ratio of 100 billion is used (this is called "truncation"). For example, a calculated likelihood ratio of "150 000 billion times more likely", would be reported as "greater than 100 billion times more likely". The actual calculated figure can be provided upon request.

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Touch DNA / Transfer of DNA

When a person touches a surface, it is possible for their DNA to be transferred onto that surface. This transferred DNA can often be recovered (sampled) by a swab, tape lift, or excision depending upon the nature of the surface in question, and the sample can then be subjected to DNA profiling.

This transferred DNA can originate from cells or cellular material within sweat and/or oils on the skin. The generation of a DNA profile will depend on many factors. These include the amount of DNA transferred, the nature of the surface being touched, and the amount of genetic material available for transfer. The persistence of any transferred genetic material to a surface will depend largely upon the nature of the surface and the conditions the surface has been subjected to in the time between deposition and recovery of the DNA. For example, DNA could be lost from the surface by washing or mechanical action such as abrasion.

It therefore follows that the inability to obtain a DNA profile from a touched surface does not necessarily mean that a person has not had contact with the surface. It is possible for a person to contact a surface without a detectable amount of their DNA being transferred or subsequently recovered for analysis.

Saliva

A presumptive chemical test (Phadebas) may be used to detect the possible presence of saliva. This test exploits the enzyme activity of a constituent of saliva called amylase. Amylase is usually present at a relatively high concentration in saliva, though this can vary considerably between individuals. Amylase may also be detected in other body fluids such as sweat, vaginal fluid and anal secretions, although usually at much lower concentration than that found in saliva.

The presence of saliva on a surface may be the result of spitting or direct oral contact. Saliva may subsequently be transferred onto other items such as clothing or other areas of the body. Possible saliva stains may then be detected on skin swabs or items of clothing by the Phadebas test, as long as the clothing or skin has not been washed. Cellular material within the saliva, if present in sufficient quantities, can be used to obtain a DNA profile.

JUSTICES ACT 1886

I acknowledge by virtue of Section 110A (6C) (c) of the Justices Act 1886 that:

(i) This written statement by me dated 17 June 2021 and contained in the pages numbered 1 to 9 is true to the best of my knowledge and belief; and

(ii) I make it knowing that, I would be liable to prosecution for stating anything that I know is false.

..[redacted].....

Angelina KELLER

Signed at BRISBANE on 17 June 2021

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AK-12

appliedbiosystems

Project: [redacted]

GeneMapper™ ID-X 1.6

Sample File	Sample Name	Panel
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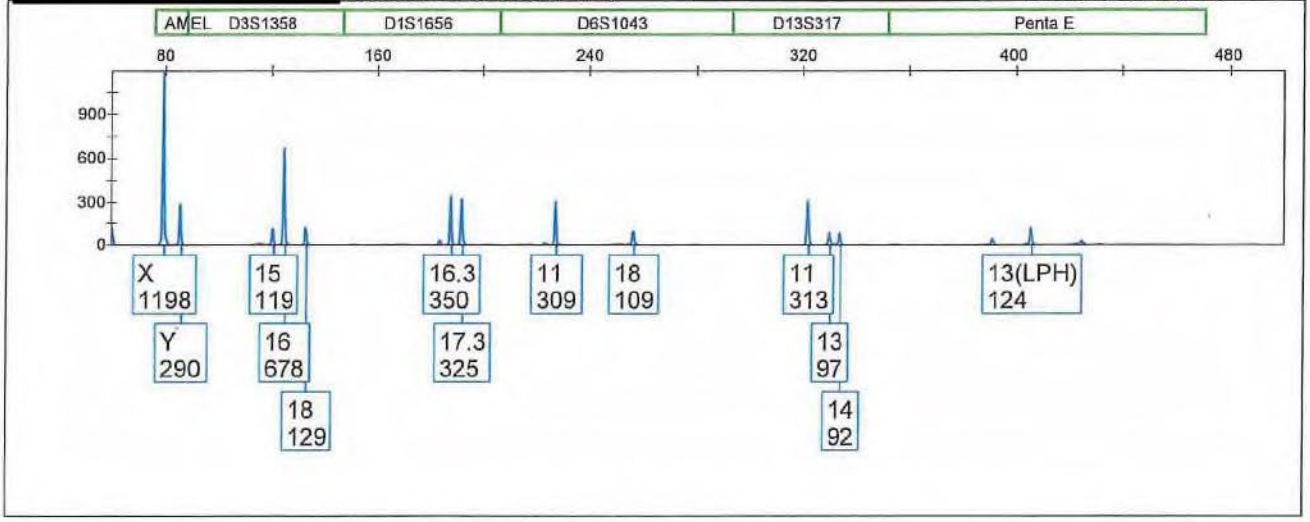

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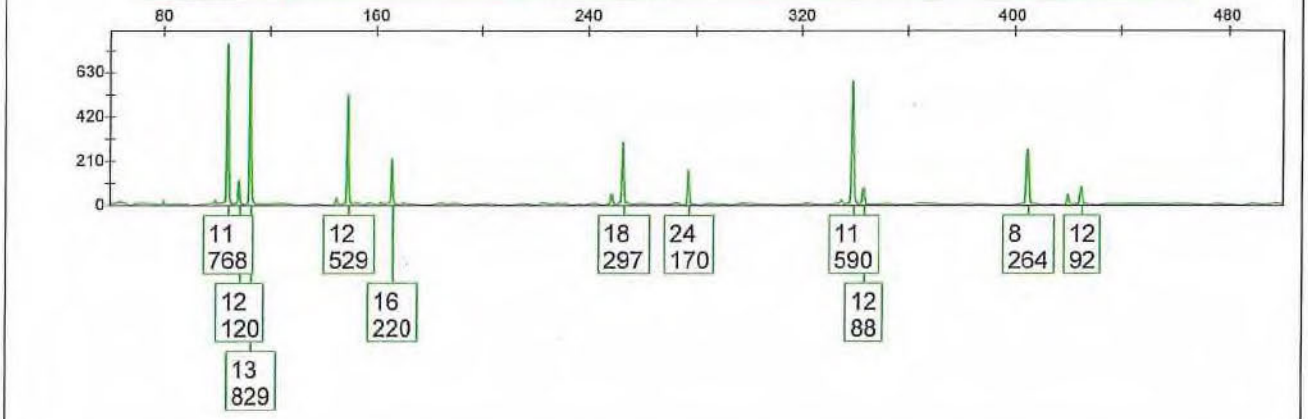

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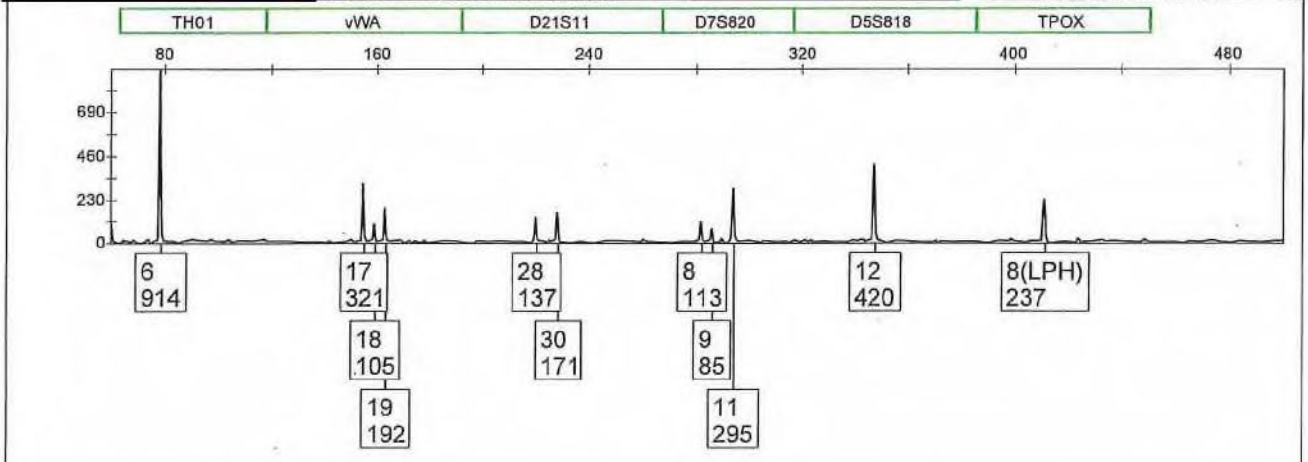

applied biosystems

Project: [redacted]

GeneMapper™ ID-X 1.6

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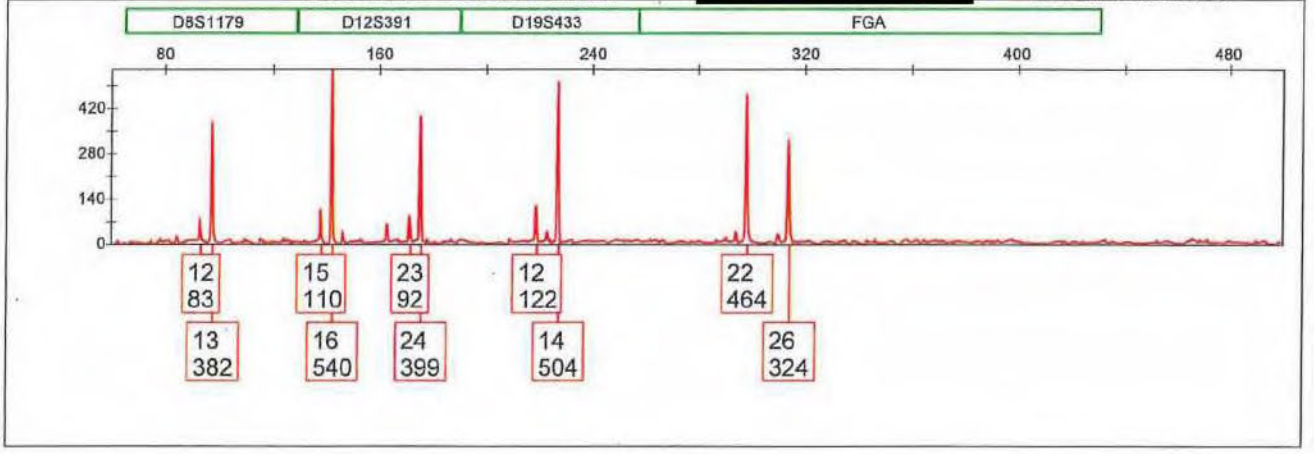

appliedbiosystems

Project: [redacted]

GeneMapper™ ID-X 1.6

Sample File	Sample Name	Panel
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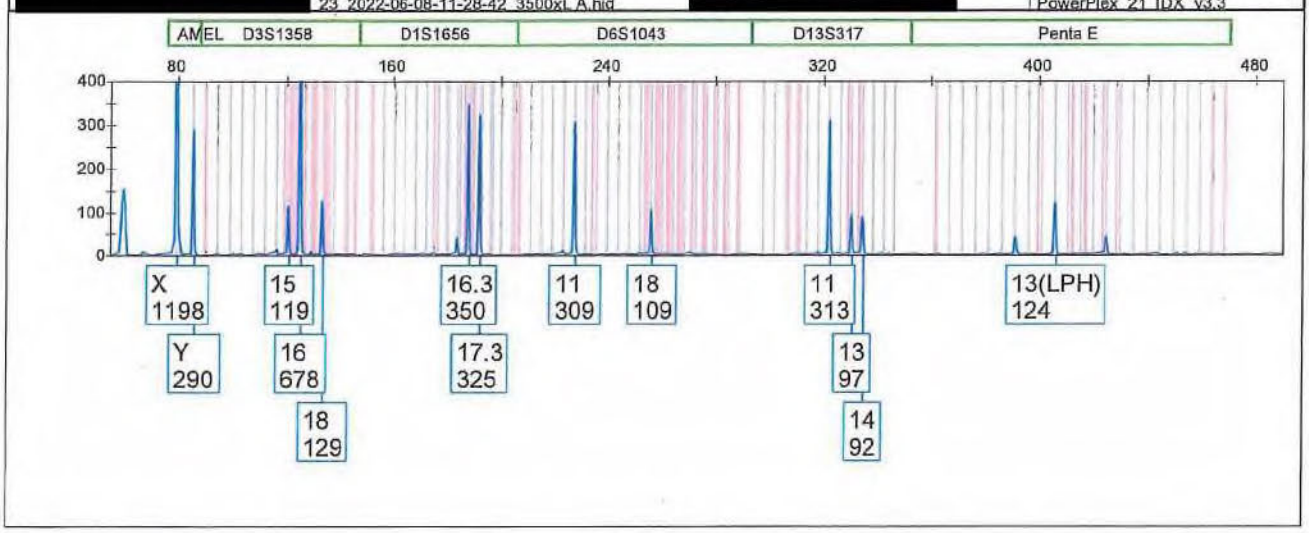

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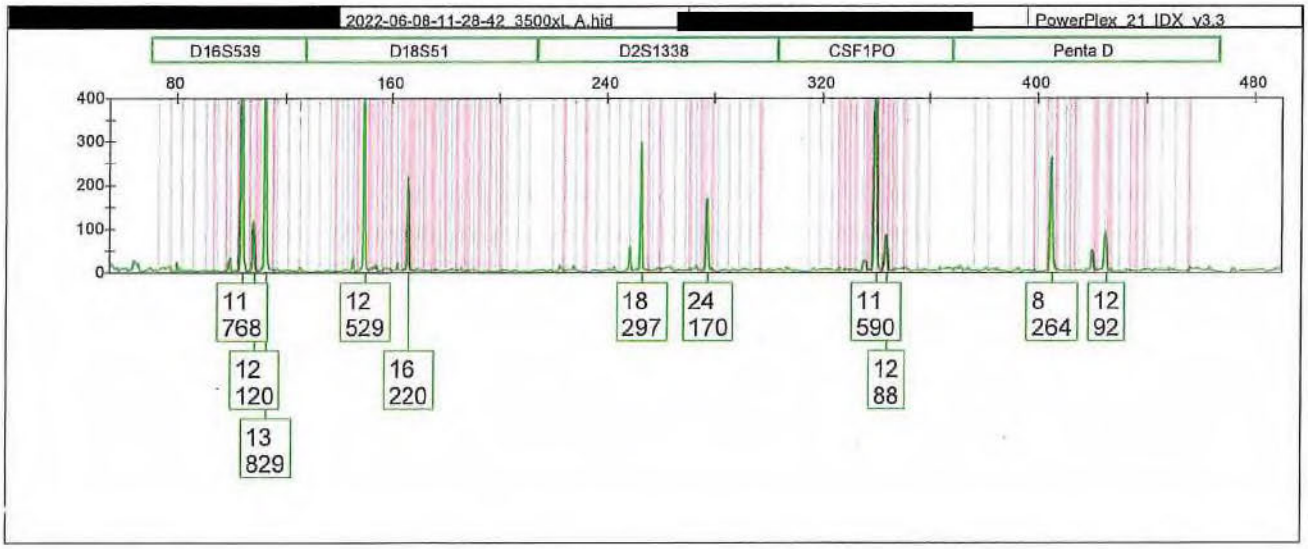

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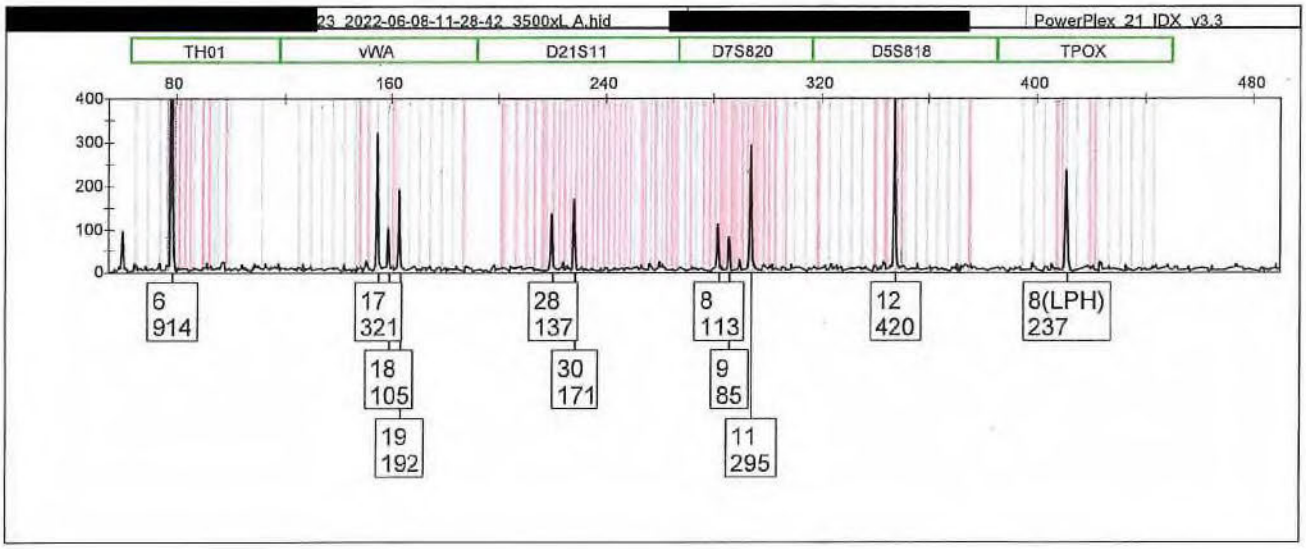

appliedbiosystems
by ThermoFisher Scientific

Project: [redacted]

GeneMapper™ ID-X 1.6

Sample File	Sample Name	Panel
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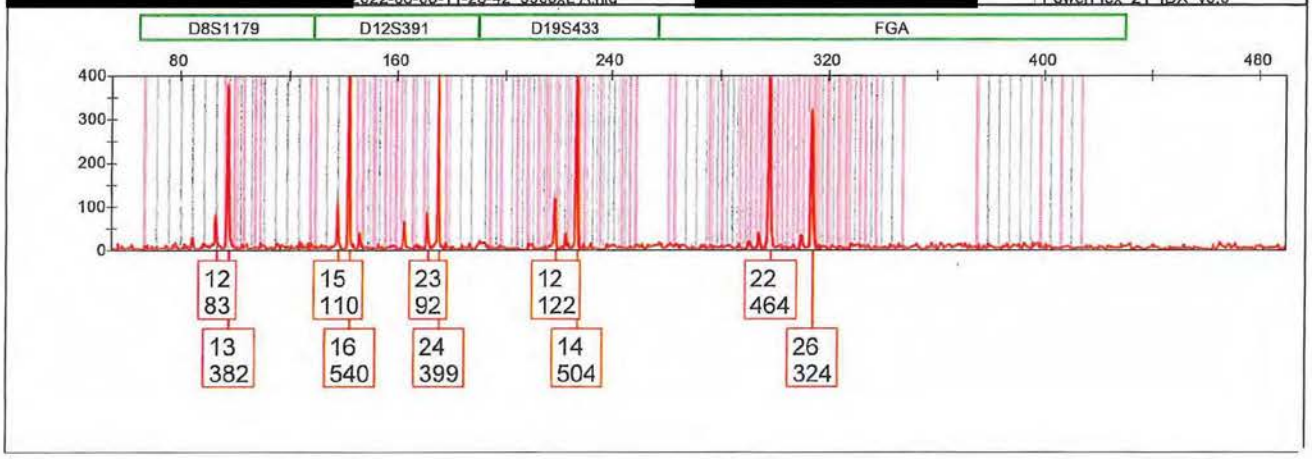

AK-13

7/21/22, 9:19 AM [redacted]

Exhibit Analytical Detail

Search...

Exhibit Detail

Barcode No: [redacted] Forensic No: [redacted] QPRIME No: [redacted]
Operation:
Offence: Child Offences Complainant: [redacted]
Batch No: Subject: [redacted]
Category Swab perianal SAIK-[redacted]

Case Scientist: Review Scientist: Status: P2 15/06/2022 14:27 Profile Review

Exhibit Testing / Examinations

Date / Time	Technique	Testing Summary	Linked No	Employee	Reviewer
03/03/2021 10:50	DNA - Item Exam	Swab labelled hand written [redacted] 9/10/12 [redacted]		440126	440132
03/03/2021 11:28	DNA - Result	Submitted-results pending		440126	440132
03/03/2021 11:57	DNA - DNAEXT [WL]	Retain Supernatant DNA IQ		440132	
04/03/2021 07:04	DNA - Subsample	[redacted] SPIN]		600154	440114
04/03/2021 07:04	DNA - Subsample	[redacted] SUPNAT]		600154	440114
04/03/2021 07:31	DNA - DNAEXT	[redacted] - Retain Supernatant DNA IQ		440191	
04/03/2021 13:02	DNA - SNTTST [WL]	[redacted] Phadebas; [redacted]		440114	
04/03/2021 13:02	DNA - DNAQUA [WL]	Quantifiler Trio; [redacted]		440114	
09/03/2021 08:15	DNA - DNAQUA	[redacted] - Quantifiler Trio		440220	
09/03/2021 11:41	DNA - Result	DNA insufficient for further processing (QTY: 0.00200)		440215	440214
10/03/2021 10:58	DNA - SNTTST	[redacted] - Phadebas		440123	
10/03/2021 11:50	DNA - Result	Presump saliva test negative		600154	440123
02/06/2022 15:47	DNA - Result	This sample has undergone further processing		440112	440190
02/06/2022 15:49	DNA - PSTEXT [WL]	Microcon PowerPlex 21		440112	
02/06/2022 15:53	DNA - Analytical Note	M'con to full		600154	440112
[redacted]6/2022 12:57	DNA - PSTEXT	[redacted] - Microcon PowerPlex 21		440236	
[redacted]/2022 13:58	DNA - STRAMP [WL]	PowerPlex21 3500xL Manual - [SV1: 11.0] [TV1: 4.0] [SV2: 0.0] [TV2: 0.0]		440185	
[redacted]06/2022 16:35	DNA - STRAMP	[redacted] PowerPlex21 3500xL Manual - [SV1: 11.0] [TV1: 4.0] [SV2: 0.0] [TV2: 0.0]		440252	
[redacted]/06/2022 09:10	DNA - CE	[redacted] - PowerPlex21 3500xL		440214	
10/06/2022 07:31	DNA - Result	MIX [redacted]		600154	440185
10/06/2022 09:34	DNA - PDA [WL]	GeneMapper IDX; [redacted]		440266	
15/06/2022 12:55	DNA - STRMix [WL]	Deconvolution		440112	
15/06/2022 13:10	DNA - STRMix			440066	440066
15/06/2022 13:53	DNA - PDA [WL]	GeneMapper IDX; [redacted]		440066	
15/06/2022 14:25	DNA - Result	[redacted] 09/10/2012		440112	440190
15/06/2022 14:26	DNA - Result	Three person mixed DNA profile		440112	440190
15/06/2022 14:26	DNA - Result	3 person mixed profile - conditioned on	[redacted]	440112	440190
15/06/2022 14:26	DNA - Result	3 person mix remaining - supports non contribution		440112	440190
15/06/2022 14:27	DNA - Profile Review			440112	440190

Exhibit Movement

Date / Time	Status	Location Details	Continuity Officer	Forensic Officer
08/06/2022 08:26	IN	FSS Forensic DNA Analysis [redacted] - Perm 6029	440236	440236
06/06/2022 16:35	IN	FSS Forensic DNA Analysis [redacted] PowerPlex21 3500xL Manual	440252	440252
06/06/2022 13:59	IN	FSS Forensic DNA Analysis [redacted] DNA Ext Temp 14	440185	440185
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09/03/2021 13:19	IN	FSS Forensic DNA Analysis [redacted] 08 - DNA Ext Temp 2	440263	440263
09/03/2021 08:15	IN	FSS Forensic DNA Analysis [redacted] Quantifiler Trio	440267	440267
04/03/2021 14:09	IN	FSS Forensic DNA Analysis [redacted] - DNA Ext Temp 24	440250	440250
04/03/2021 13:06	IN	FSS Forensic DNA Analysis [redacted] - DNA Ext Temp 1	440114	440114
[redacted]/2021 13:46	IN	FSS Forensic DNA Analysis [redacted] For Processing Storage	440191	440191
[redacted]/2021 13:35	IN	FSS Forensic DNA Analysis [redacted] - ERT-AS Trans Box 34	440191	440191
03/03/2021 10:51	IN	FSS Forensic DNA Analysis [redacted] - ERT-AS Trans Box 16	440126	440126
03/03/2021 10:41	IN	FSS Forensic DNA Analysis	440126	440126

AK-14

Kylie Rika

From: Justin Howes
Sent: Monday, 22 August 2022 10:53 AM
To: Kylie Rika
Cc: Angelina Keller
Subject: RE: [redacted] - DG MEMO - from Dr David Rosengren, Acting Director-General, Queensland Health - Subject of memorandum

Hi
The memo mentions the range, so if bone/teeth are in the range, then they would be microconned in the way the memo describes. If at examination, an analytical note of a different approach is made, then that could be made. This would be similar to cold case Q&H processes where the note is made to hold and consult after quant.

Justin

Justin Howes
Team Leader - Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream, Forensic & Scientific Services
Prevention Division, Queensland Health

p [redacted] m [redacted]
a 39 Kessels Road, Coopers Plains, QLD 4108
e [redacted] w www.health.qld.gov.au/fss

Please note that I may be working from a different location during the COVID-19 Pandemic. The best contact method is via email.

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.

From: Kylie Rika <[redacted]>
Sent: Monday, 22 August 2022 9:59 AM
To: Justin Howes <[redacted]>
Cc: Angelina Keller <[redacted]>
Subject: FW: [redacted] - DG MEMO - from Dr David Rosengren, Acting Director-General, Queensland Health - Subject of memorandum

Hi Justin

Please see query below from Angelina. Are we able to get some clarification on this please?

Thanks
Kylie

From: Angelina Keller <[redacted]>
Sent: Monday, 22 August 2022 9:51 AM
To: Kylie Rika <[redacted]>
Subject: FW: [redacted] - from Dr David Rosengren, Acting Director-General, Queensland Health - Subject of memorandum

Hi Kylie,

Is it possible to clarify the sample categories affected by this latest direction. For example I would assume bone / teeth aliquots are exempt as well as No DNA samples.

Kind regards,
Angelina

Angelina Keller
Reporting Scientist

Forensic DNA Analysis, Forensic & Scientific Services
Prevention Division, Queensland Health

p [redacted]
a 39 Kessels Road, Coopers Plains, Qld, 4108
e [redacted] w www.health.qld.gov.au/fss

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.

From: Helen Gregg <[redacted]>
Sent: Friday, 19 August 2022 3:33 PM
To: Abigail Ryan <[redacted]>; Adam Kaity <[redacted]>; Adrian Pippia <[redacted]>; Alanna Darmanin <[redacted]>; Alicia Quartermain <[redacted]>; Allan McNevin <[redacted]>; Allison Lloyd <[redacted]>; Amy Cheng <[redacted]>; Amy Morgan <[redacted]>; Angela Adamson <[redacted]>; Angelina Keller <[redacted]>; Anne Finch <[redacted]>; Belinda Andersen <[redacted]>; Biljana Micic <[redacted]>; Cassandra James <[redacted]>; Cathie Allen <[redacted]>; Cecilia Flanagan <[redacted]>; Chantal Angus <[redacted]>; Chelsea Savage <[redacted]>; Cindy Chang <[redacted]>; Claire Gallagher <[redacted]>; Dasuni Harmer <[redacted]>; Deborah Nicoletti <[redacted]>; Emma Caunt <[redacted]>; FSS.FDNA.Admin <[redacted]>; Generosa Lundie <[redacted]>; Helen Williams <[redacted]>; Ingrid Moeller <[redacted]>; Jacqui Wilson <[redacted]>; Janine Seymour-Murray <[redacted]>; Josie Entwistle <[redacted]>; Julie Brooks <[redacted]>; Justin Howes <[redacted]>; Kerry-Anne Lancaster <[redacted]>; Kevin Avdic <[redacted]>; Kim Estreich <[redacted]>; Kirsten Scott <[redacted]>; Kristina Morton <[redacted]>; Kylie Rika <[redacted]>; Lai-Wan Le [redacted]>; Lisa Farrelly <[redacted]>; Luke Ryan <[redacted]>; Madison GULLIVER <[redacted]>; Maria Aguilera <[redacted]>; Matthew Hunt <[redacted]>; Melissa Cipollone <[redacted]>; Michael Goodrich

<[redacted]>; Michael Hart <[redacted]>; Michelle Margetts <[redacted]>; Naomi French <[redacted]>; Nicole Roselt <[redacted]>; Paula Brisotto <[redacted]>; Penelope Taylor <[redacted]>; Phillip McIndoe <[redacted]>; Pierre Acedo <[redacted]>; Rhys Parry <[redacted]>; Ryu Eba <[redacted]>; Sandra McKean <[redacted]>; Sharelle Nydam <[redacted]>; Sharon Johnstone <[redacted]>; Stephanie Waiariki <[redacted]>; Suzanne Sanderson <[redacted]>; Tara Prowse <[redacted]>; Tegan Dwyer <[redacted]>; Thomas Nurthen <[redacted]>; Valerie Caldwell <[redacted]>; Vicki Pendlebury-Jones <[redacted]>; Wendy Harmer <[redacted]>; Yvonne Connolly <[redacted]>
Cc: Alison Slade <[redacted]>; FSS Corro <[redacted]>; Lara Keller <[redacted]>; Keith McNeil <[redacted]>; Petra Derrington <[redacted]>
Subject: FW: [redacted] DG MEMO - from Dr David Rosengren, Acting Director-General, Queensland Health - Subject of memorandum

Good afternoon everyone,

Please see attached memo. I have asked for an enhancement to FR to assist with this change.

Please hold all quants effective immediately, until the FR enhancement is complete. Paula has specific details for the analytical team.
For batches that have already progressed beyond quant, proceed as per this morning's processes.

Could you please update SOPs asap.

Contact me if you have any queries.

Regards
Helen

Helen Gregg
A/Executive Director

Forensic and Scientific Services
Prevention Division, Queensland Health

p [redacted] m [redacted]
e [redacted] w www.health.qld.gov.au/fss

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.

Good Afternoon

Please see attached the Memorandum from Dr David Rosengren, Acting Director-General, Queensland Health, for your attention.

Should you have any questions in relation to this advice, please contact Professor Keith McNeil, Acting Deputy Director-General on telephone [redacted]

Kind Regards

Queensland Government

Ministerial & Executive Services Unit, Office of the Director-General | Queensland Health

[redacted]@[redacted]

W health.qld.gov.au

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

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AK-15

Project: [redacted]

GeneMapper™ ID-X 1.6

Sample File	Sample Name	Panel
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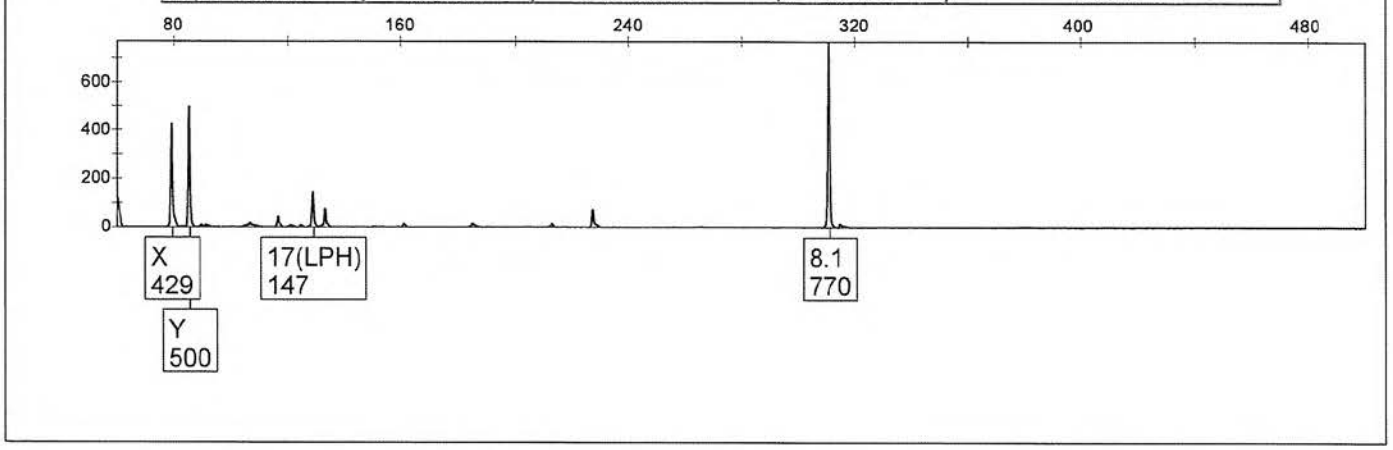

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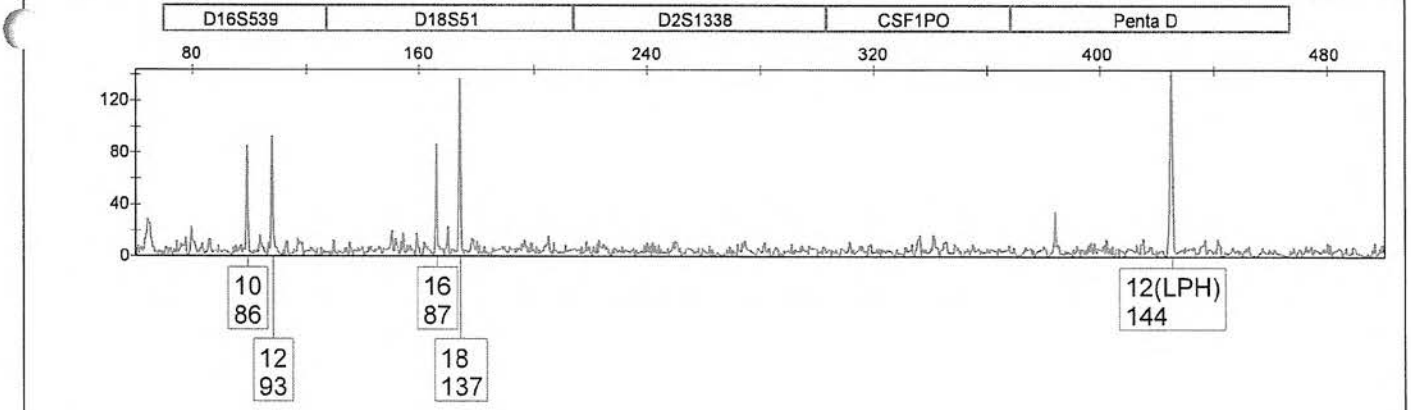

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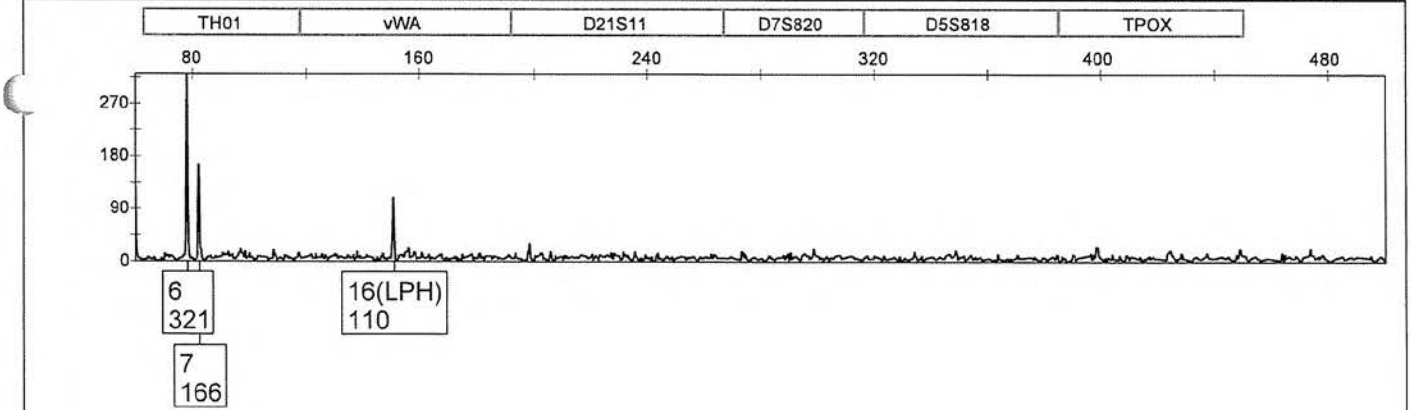

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by Thermo Fisher Scientific

Project: [redacted]

GeneMapper™ ID-X 1.6

Sample File	Sample Name	Panel
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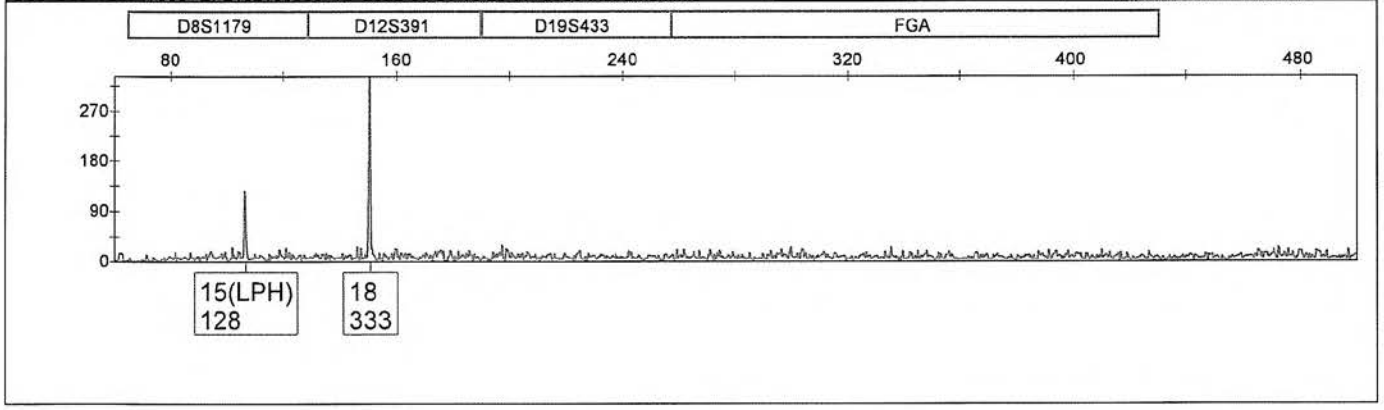

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by Thermo Fisher Scientific

Project: [redacted]

GeneMapper™ ID-X 1.6

Sample File	Sample Name	Panel
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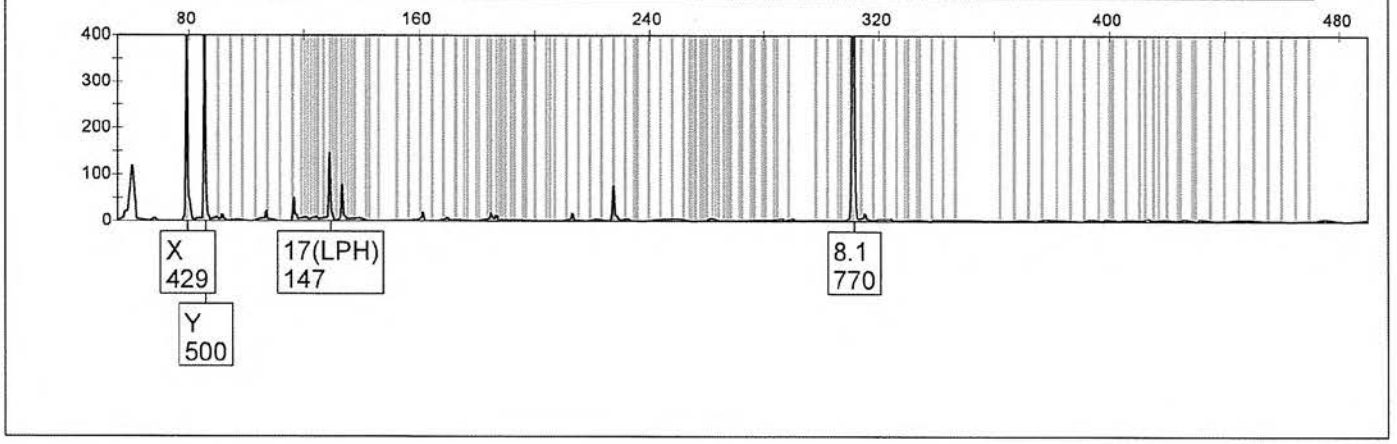

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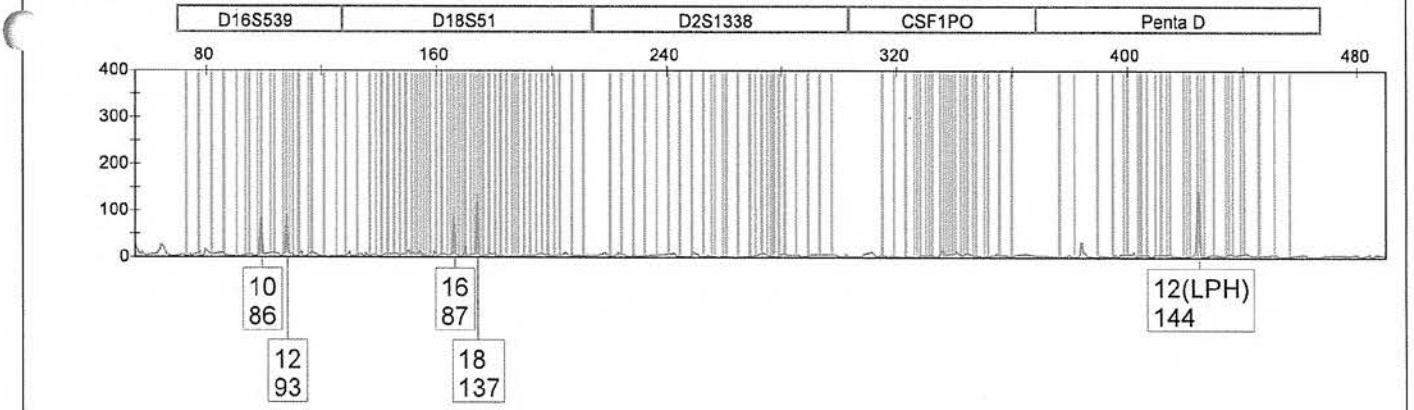

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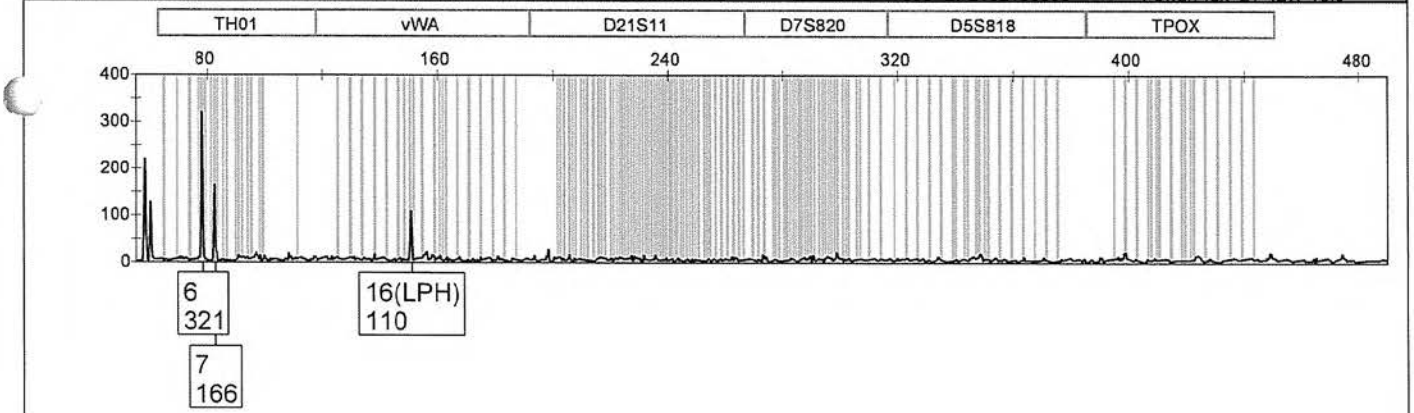

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Project: [redacted]

GeneMapper™ ID-X 1.6

Sample File	Sample Name	Panel
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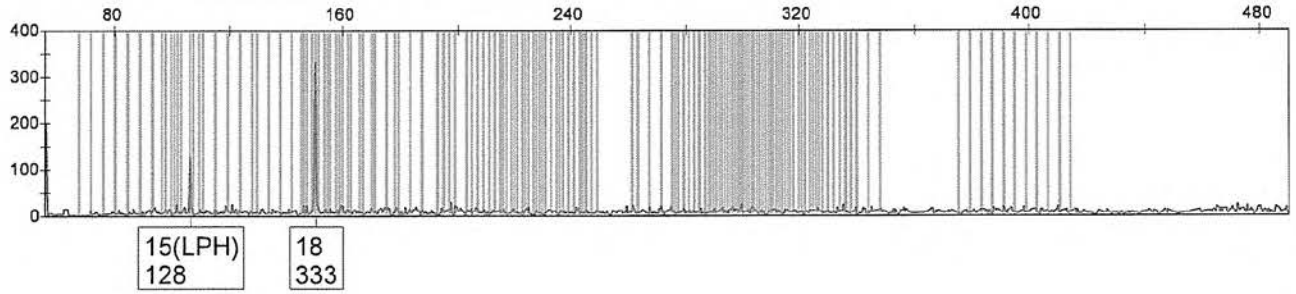

AK-16

Project: [redacted]

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GeneMapper™ ID-X 1.6

Sample Name	Panel
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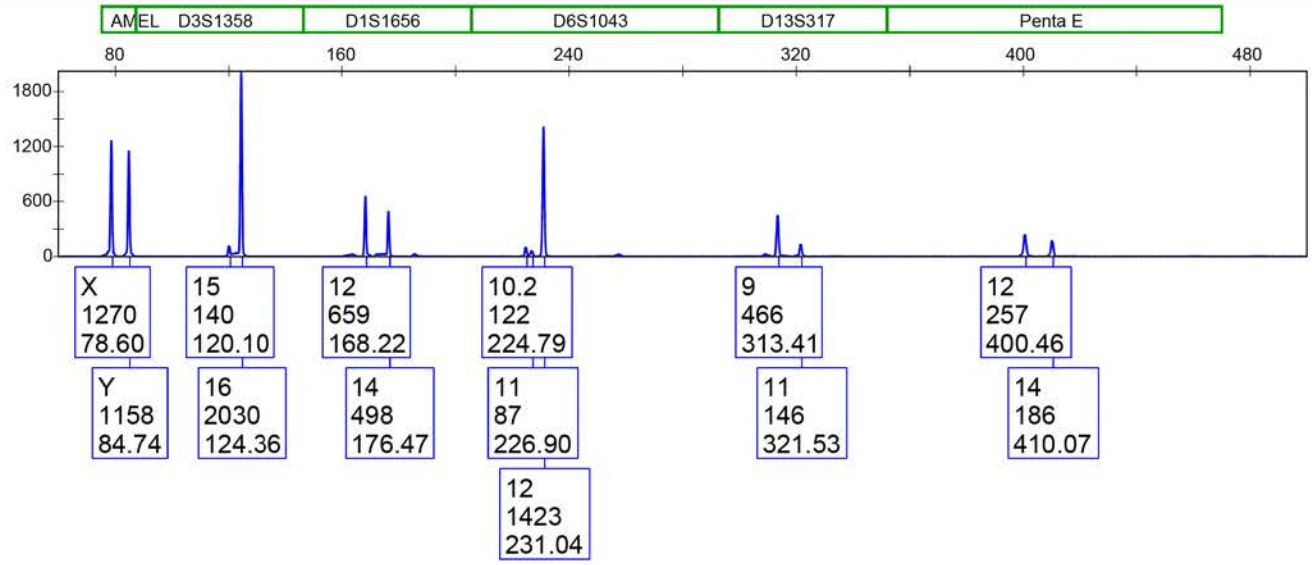

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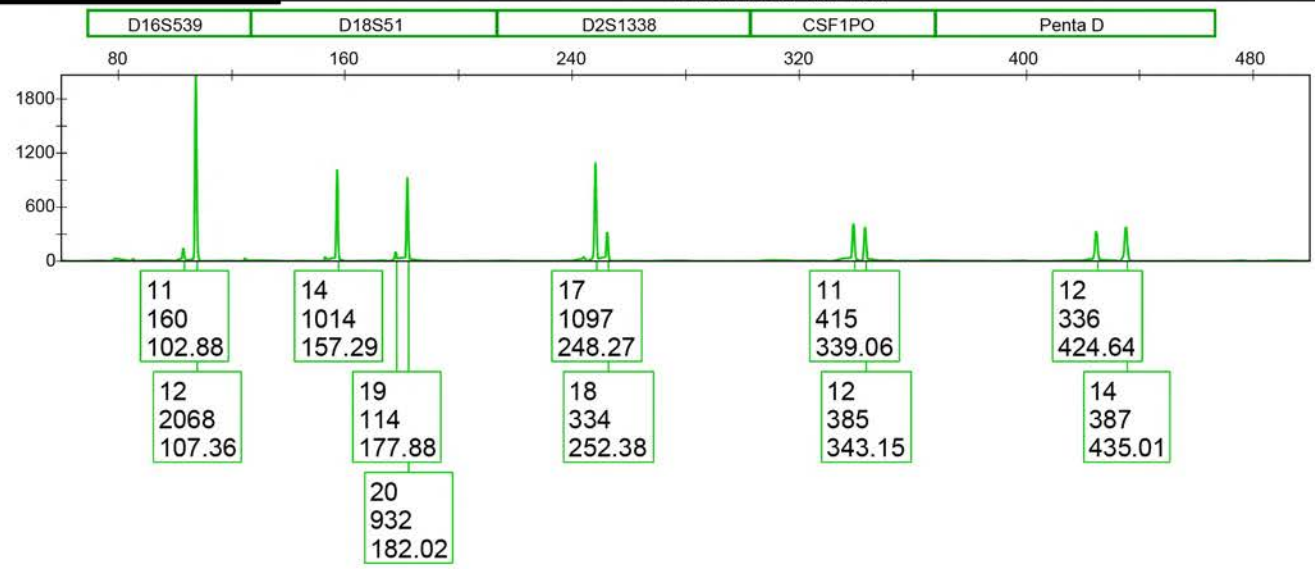

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Project: [redacted]

GeneMapper™ ID-X 1.6

Sample Name	Panel
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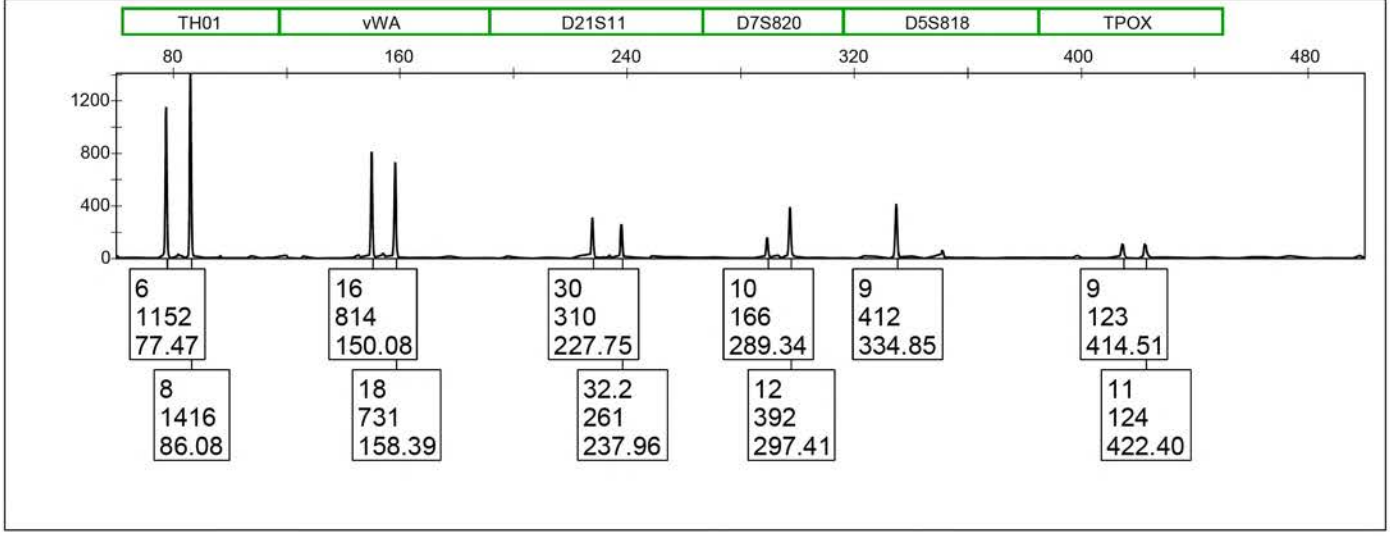

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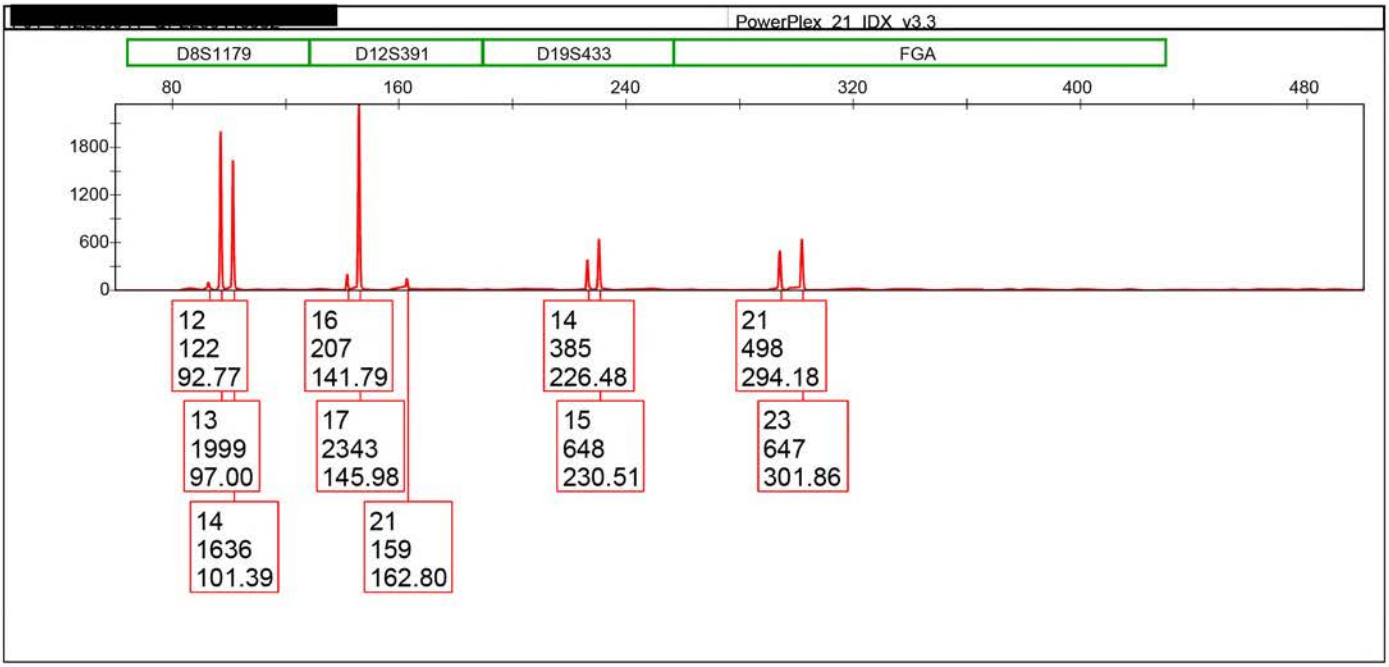

AK-17

appliedbiosystems

Project: [redacted]

GeneMapper™ ID-X 1.6

[redacted]

Sample File	Sample Name	Panel
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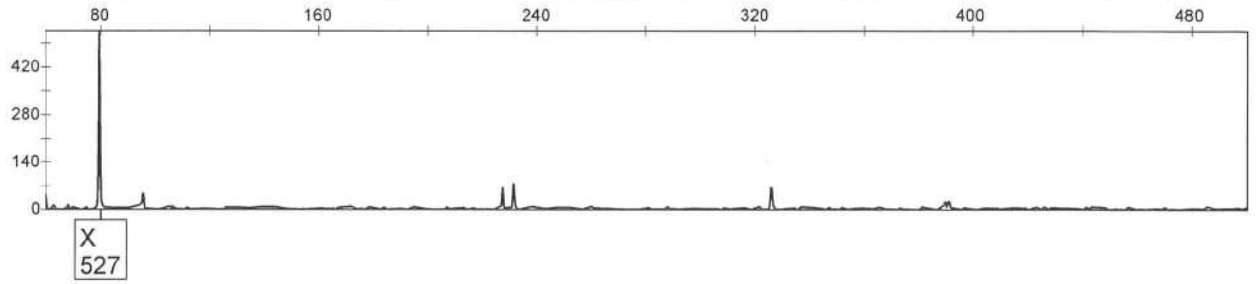

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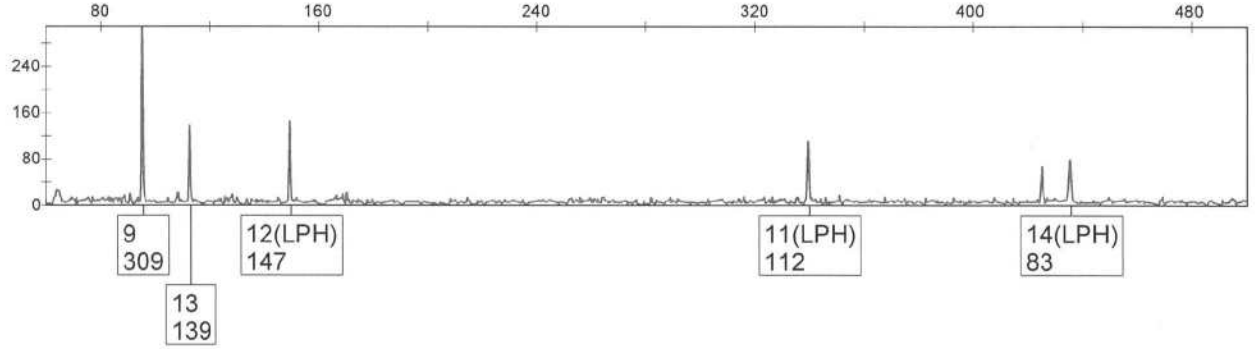
D16S539 | D18S51 | D2S1338 | CSF1PO | Penta D



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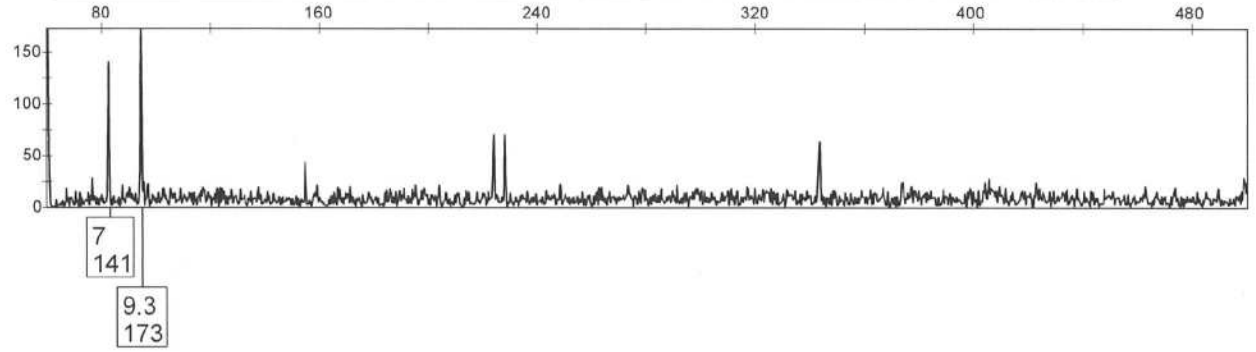
TH01 | vWA | D21S11 | D7S820 | D5S818 | TPOX



Uncleared as at 18.08.2022

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Project: [redacted]

GeneMapper™ ID-X 1.6

Sample File	Sample Name	Panel
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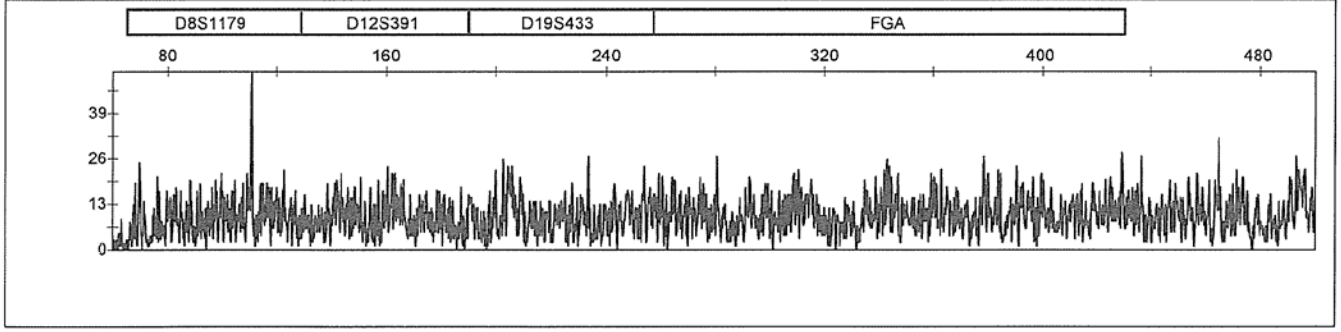

applied biosystems
by Thermo Fisher Scientific

Project: [redacted]

GeneMapper™ ID-X 1.6

Sample File	Sample Name	Panel
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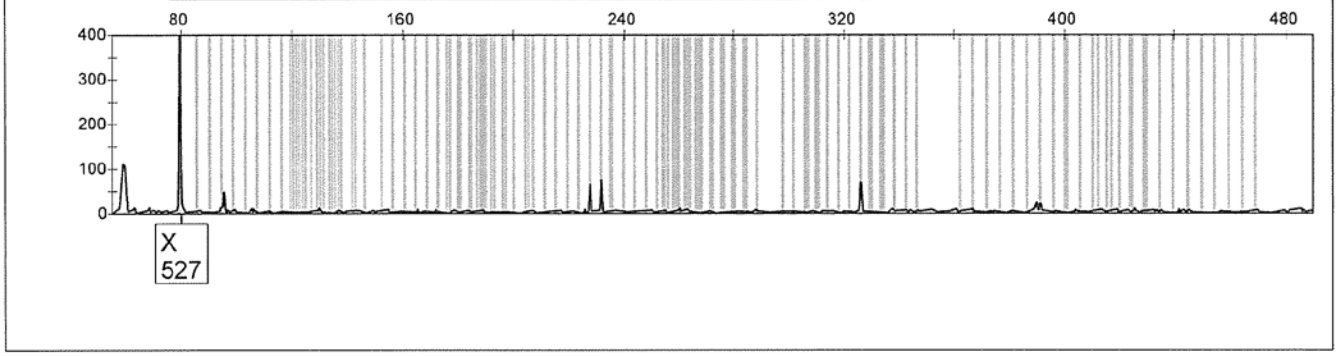

Sample File	Sample Name	Panel
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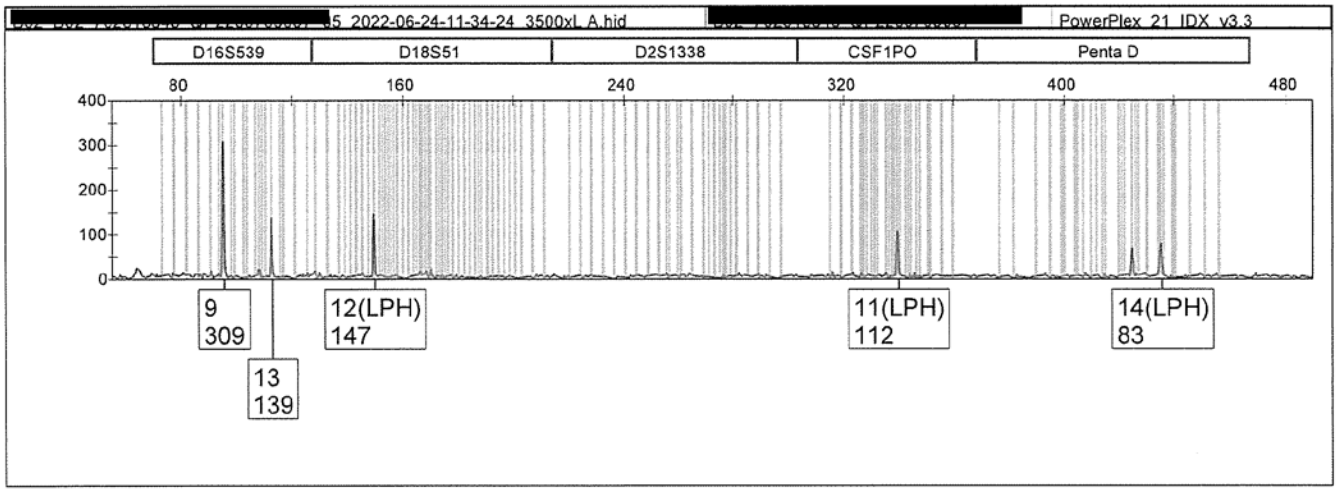

Sample File	Sample Name	Panel
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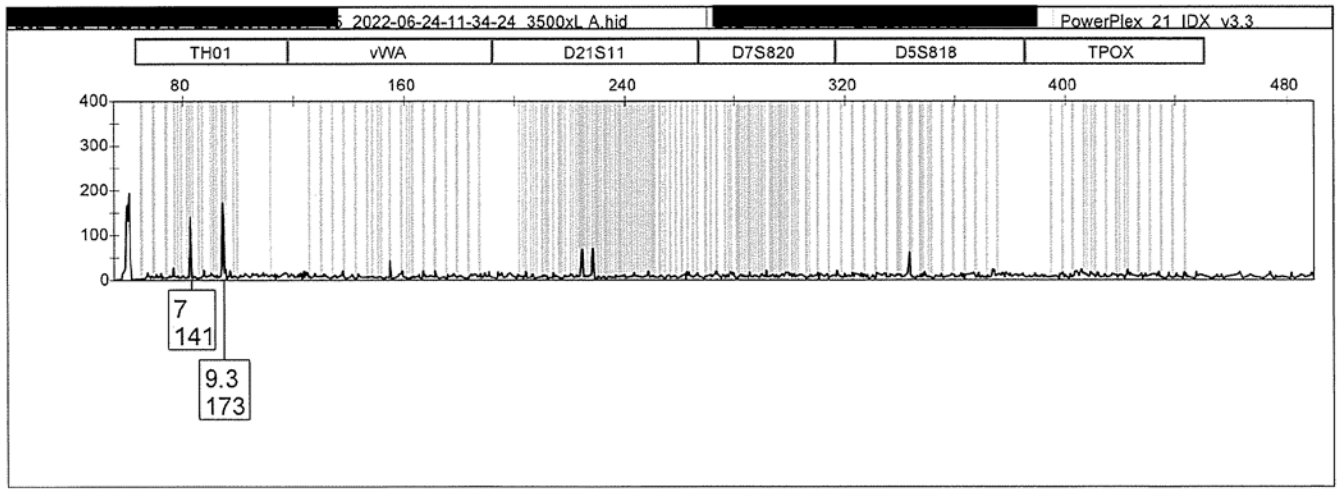

Unreviewed as at
13.08.2022

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Project: [redacted]

GeneMapper™ ID-X 1.6

Sample File	Sample Name	Panel
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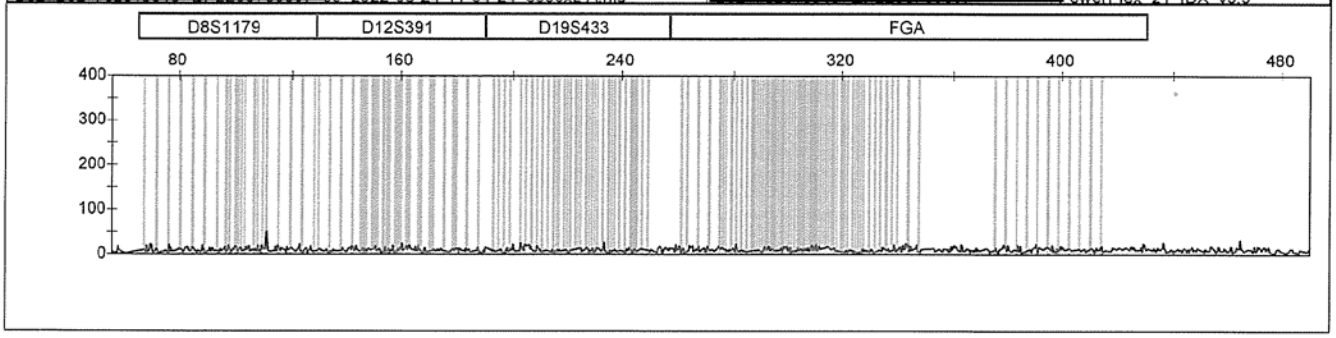

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by Thermo Fisher Scientific

Project: [redacted]

GeneMapper™ ID-X 1.6

[redacted]

Sample File	Sample Name	Panel
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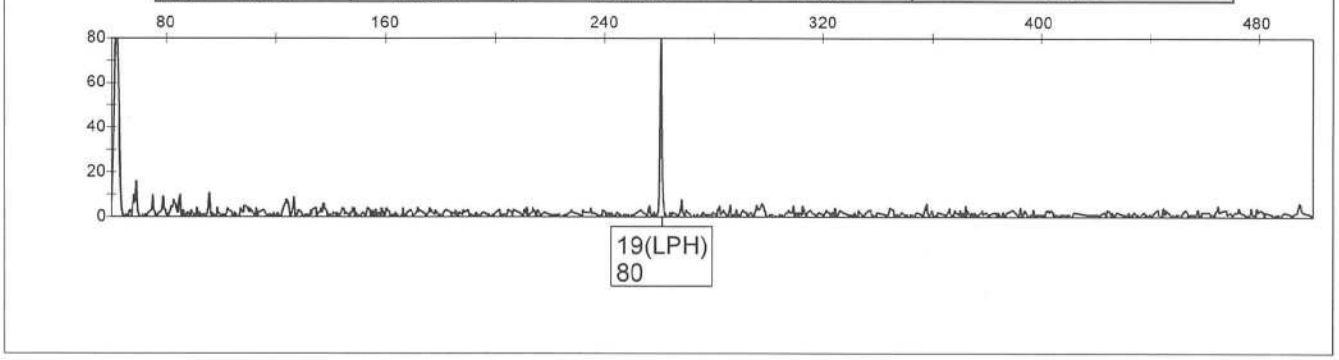

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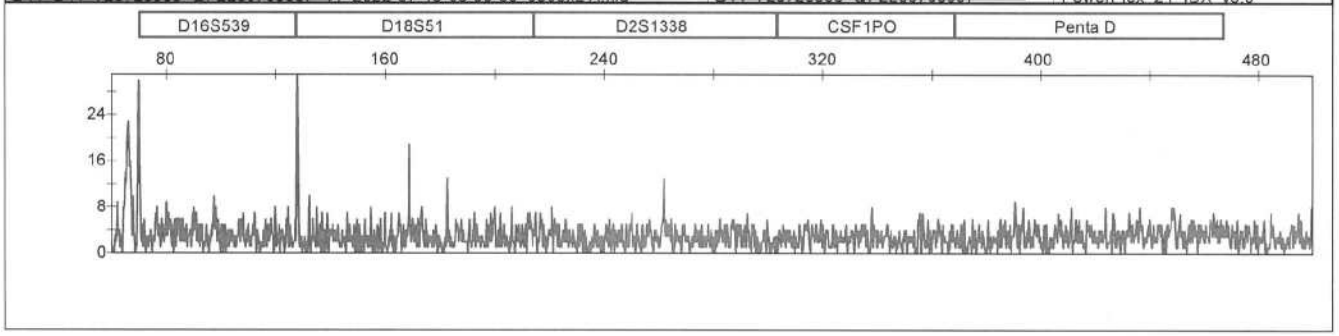

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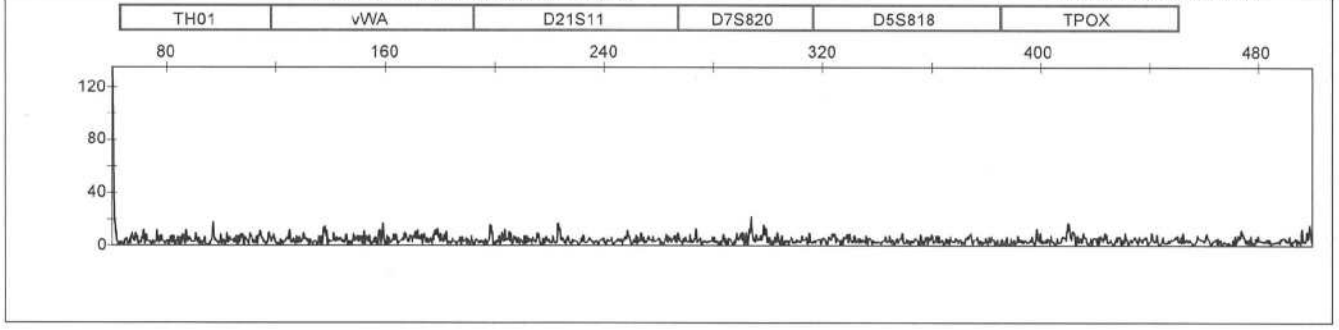

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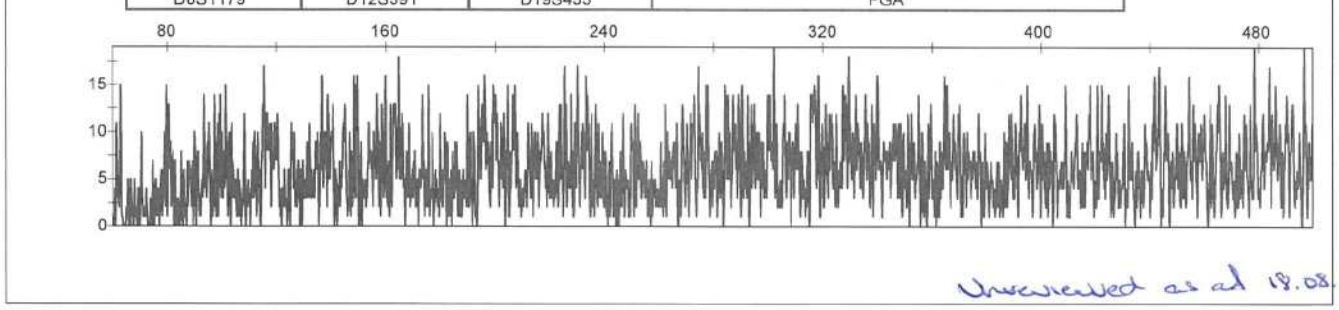

Unreviewed as at 18.08.2022

appliedbiosystems

Project: [redacted]

GeneMapper™ ID-X 1.6

Sample File	Sample Name	Panel
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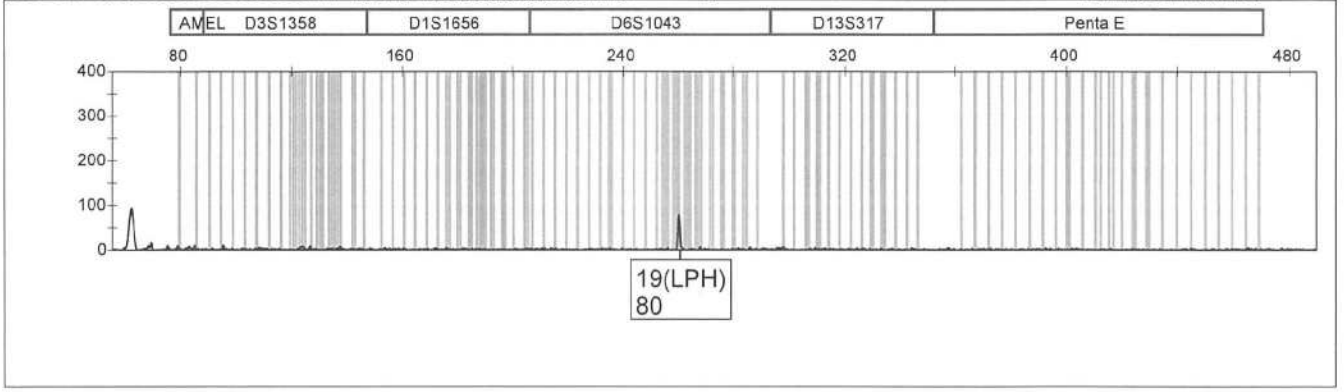

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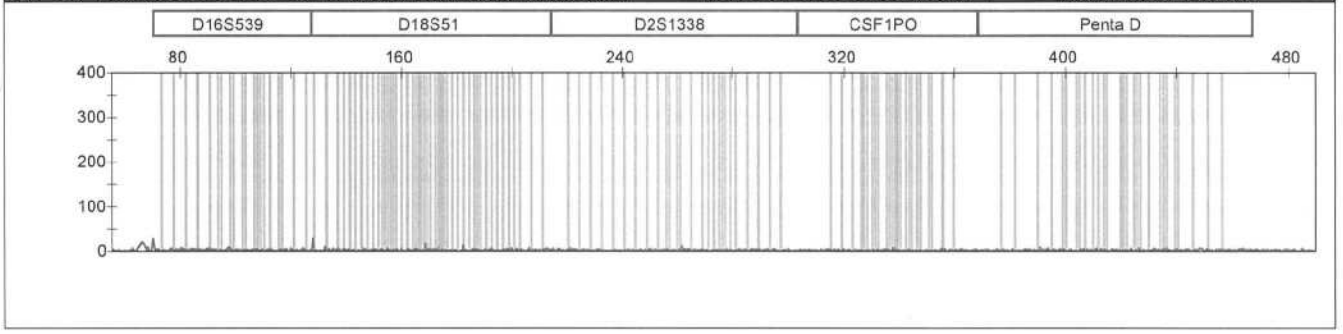

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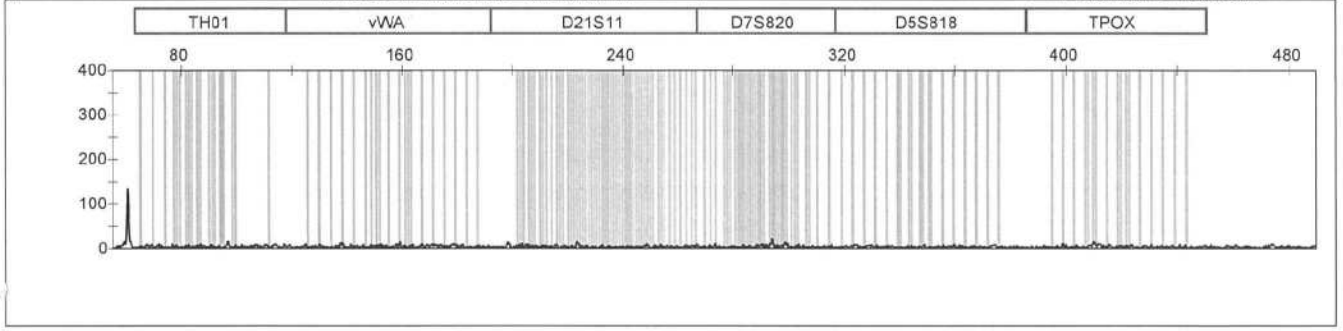

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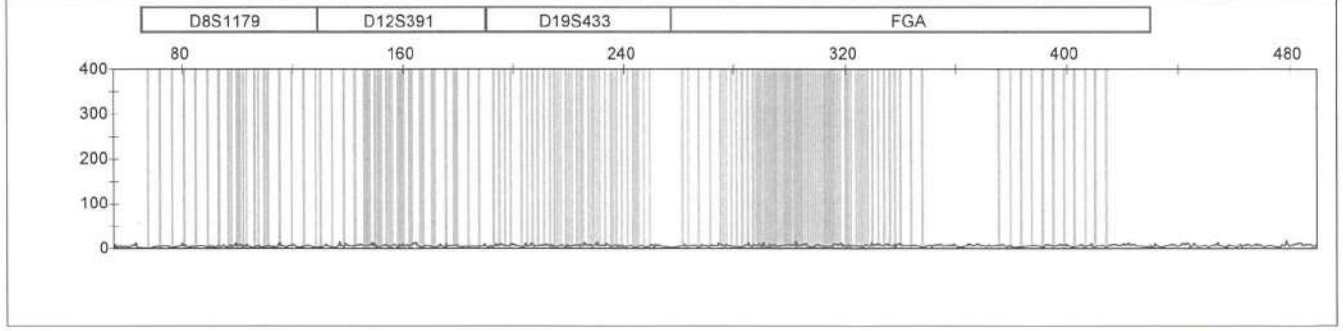

Unreviewed as at 18.08.2022

AK-18

Project [redacted]

applied biosystems

GeneMapper™ ID-X 1.6

Sample Name	Panel
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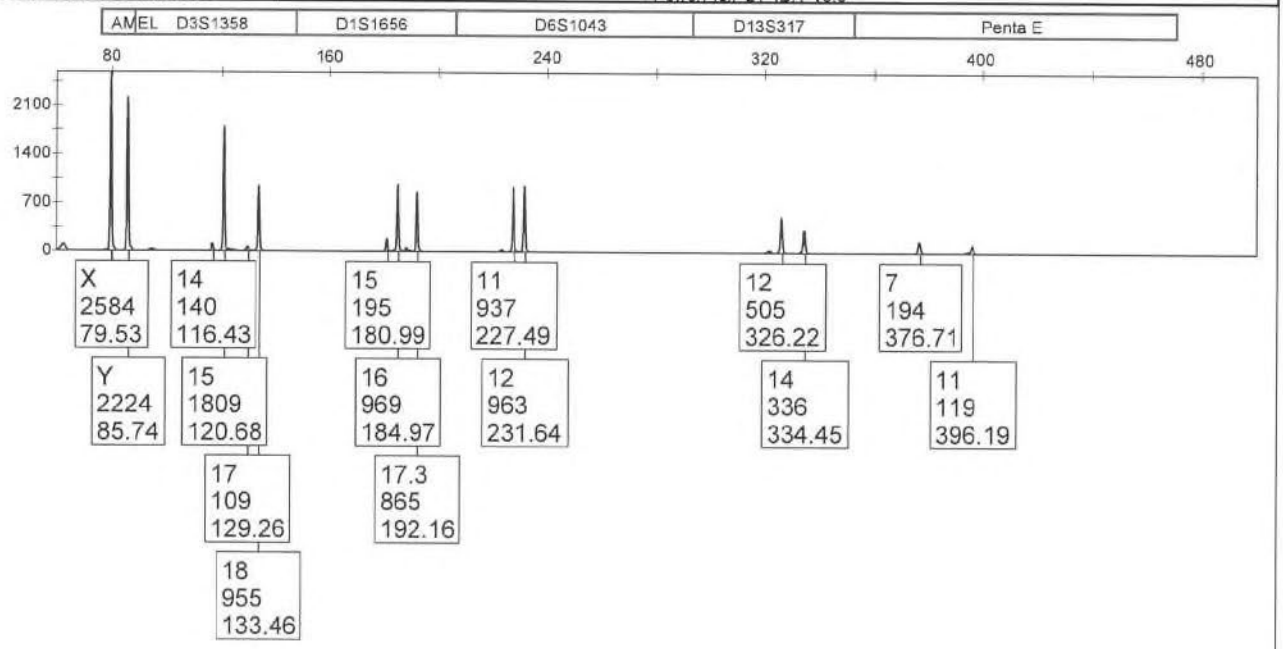

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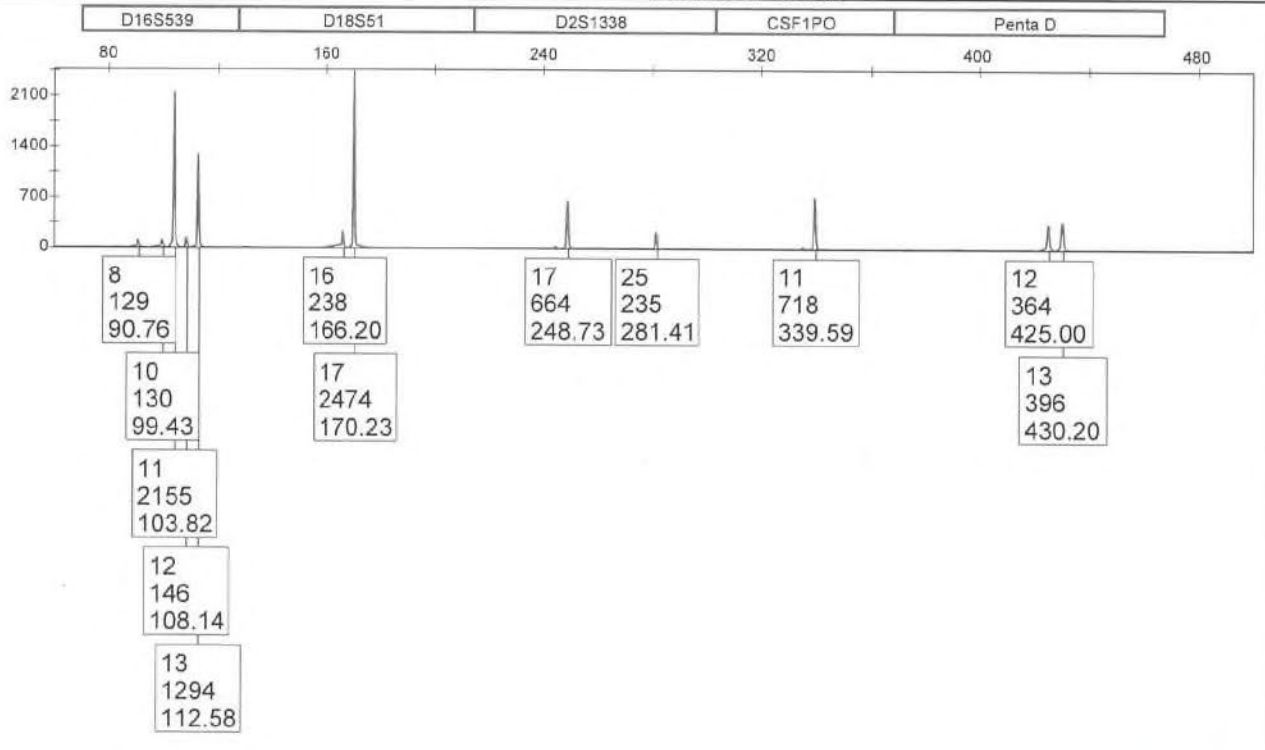

Unreviewed as at 18.09.2022

Mixture @ D16
RSP 15.08.2022

applied biosystems
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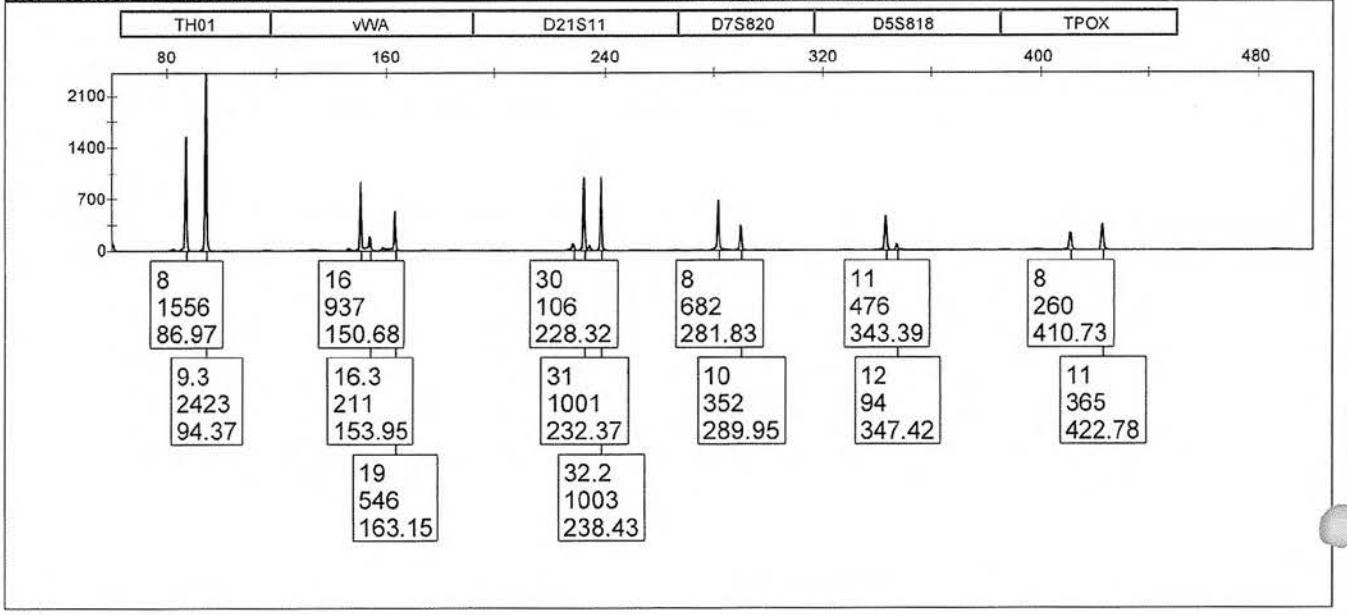
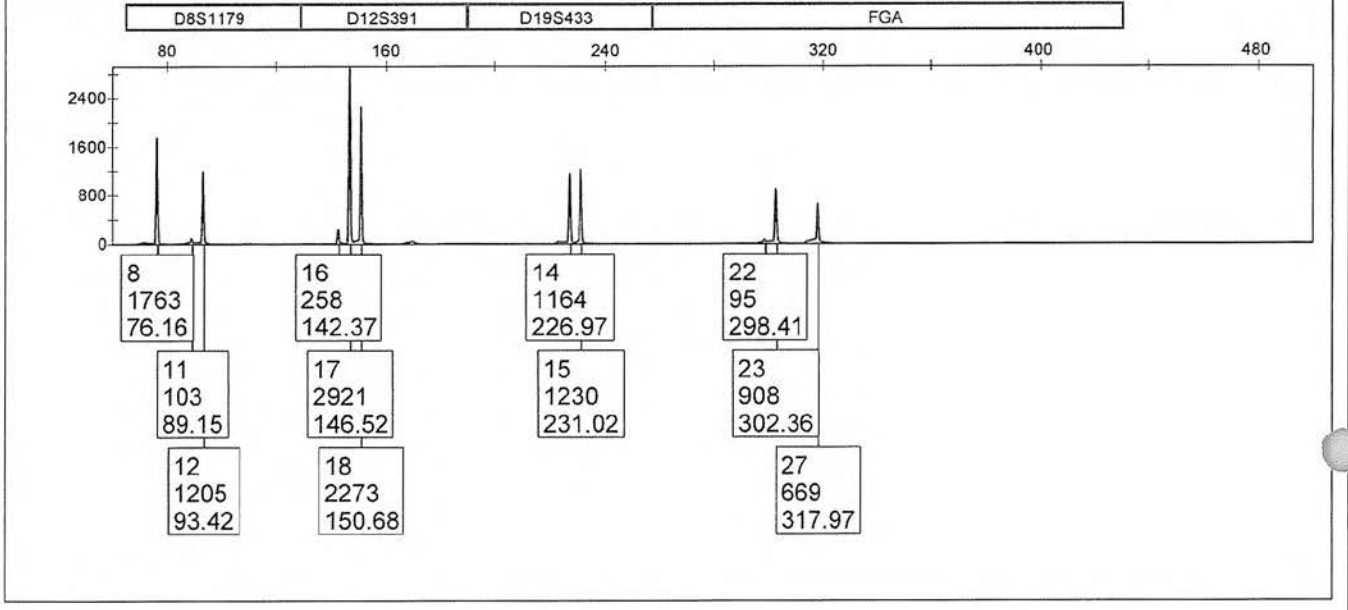
Project: [redacted]

GeneMapper™ ID-X 1.6

Sample Name	Panel
[redacted]	PowerPlex 21 IDX v3.3



PowerPlex 21 IDX v3.3



applied biosystems

Project: [redacted]

GeneMapper™ ID-X 1.6

Sample File	Sample Name	Panel
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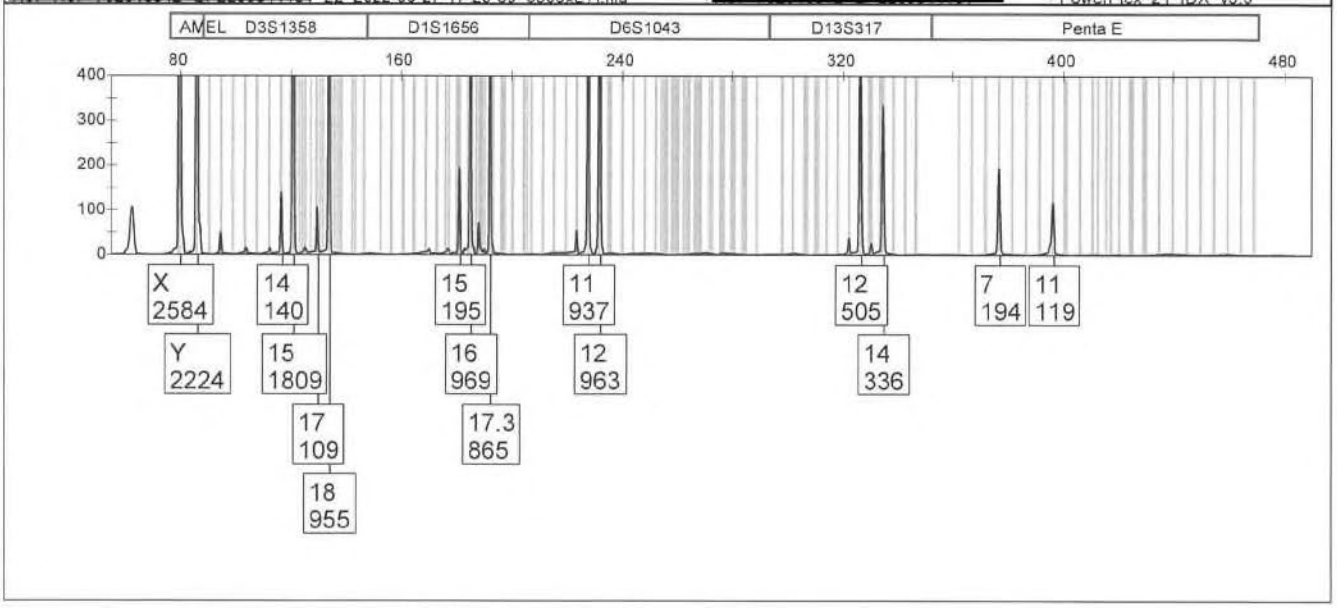
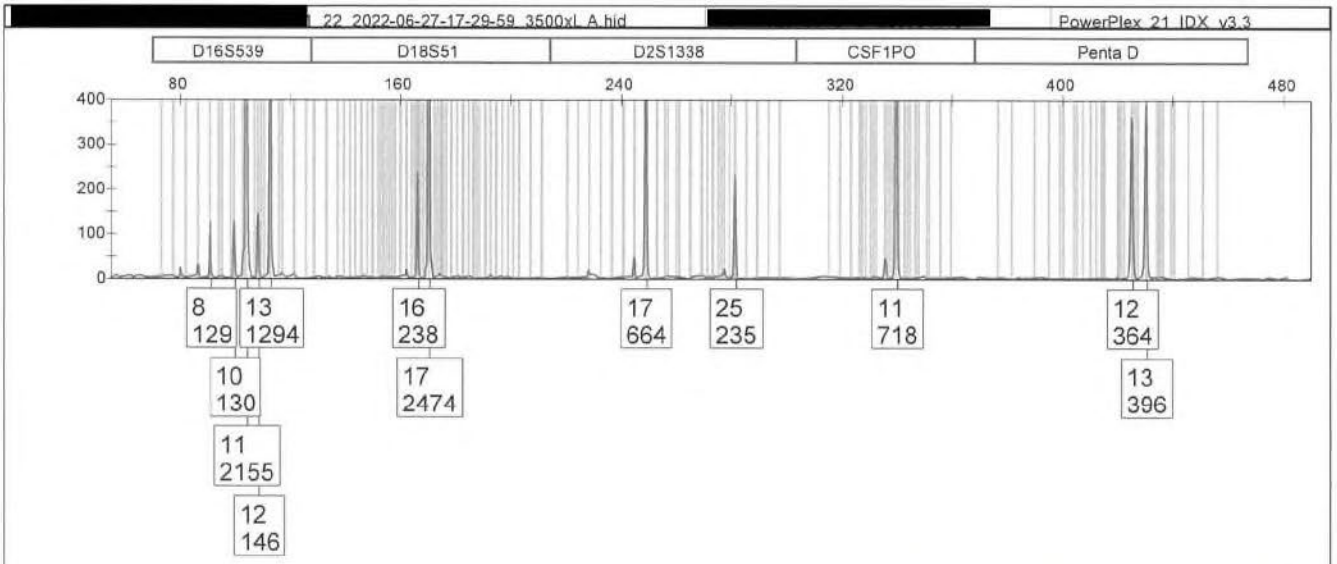


[redacted] 22 2022-06-27-17-29-59 3500xL A.hid	[redacted]	PowerPlex 21 IDX v3.3



Unreviewed as at 15.08.2022

applied biosystems
by Thermo Fisher Scientific

Project: [redacted]

GeneMapper™ ID-X 1.6

Sample File	Sample Name	Panel
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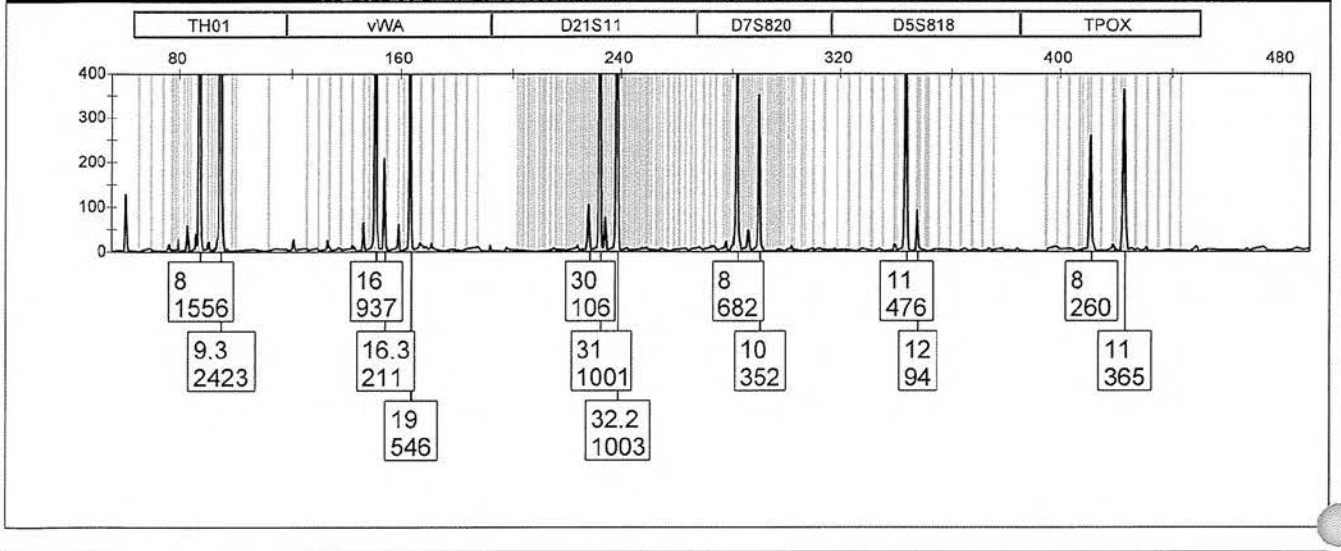

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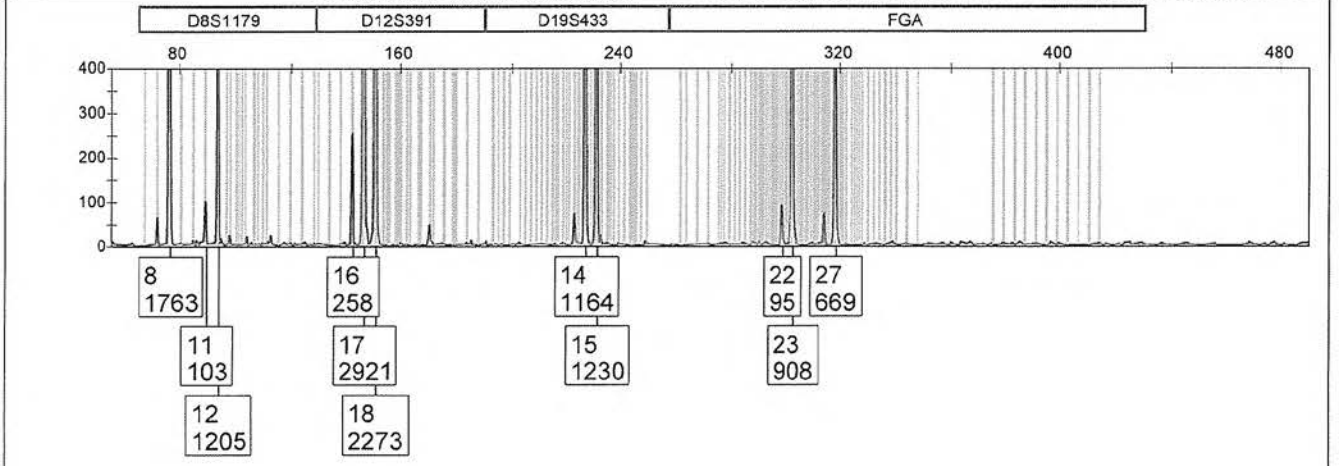


AK-19

	Date sampled	QP number	FR number	CA number	Tissue	Result	
	10/04/2019	[redacted]	[redacted]	[redacted]	Bone	SS	
	30/04/2019	[redacted]			Bone	SS	
	12/06/2019	[redacted]			Bone	SS	
	22/05/2019	[redacted]			Tooth	SS	
05/07/2019 - C							
	26/11/2019	[redacted]			Bone	SS	
	07/02/2020	[redacted]			Tooth	No DNA	
	12/03/2020	[redacted]			Bone	Partial SS	
	12/03/2020	[redacted]			Tooth	No DNA	
25/03/2020- E							
	03/08/2020	[redacted]			Bone	No DNA	Linked
	02/09/2020	[redacted]			Bone	SS	Fresh
	13/10/2020	[redacted]			Bone	No DNA	Linked
	19/10/2020	[redacted]			Bone	No DNA	
	02/11/2020	[redacted]			Teeth	SS	
	02/11/2020	[redacted]			Teeth	Complex unsuitable	
	26/11/2020	[redacted]			Bone	Complex unsuitable	
15/02/2021 - CE							
	15/03/2021	[redacted]			Bone	1 x mix, 3 x SS	Fresh
	15/09/2021	[redacted]			Bone	SS	
	04/02/2022	[redacted]			Teeth	Complex unsuitable	Linked
	07/03/2022	[redacted]			Bone	SS	
	24/03/2022	[redacted]			Bone	2 x mix, 2 x SS	Linked
	24/03/2022	[redacted]			Bone	3 x SS, 1 x mix	Linked
	24/03/2022	[redacted]			Bone	4 x mix	Linked
	25/03/2022	[redacted]			Teeth	Complex unsuitable	Linked
	25/03/2022	[redacted]			Teeth	Complex unsuitable	Linked
	08/04/2022	[redacted]			Bone	Complex unsuitable	[redacted]
	20/05/2022	[redacted]			Bone	SS, Mix	Linked
	31/05/2022	[redacted]			Bone	Complex unsuitable	Linked
	01/06/2022	[redacted]			Bone	8 x mix	Link
	30/06/2022	[redacted]			Bone	Complex unsuitable	Linked
	30/06/2022	[redacted]			Bone	Complex unsuitable	Linked
	08/08/2022	[redacted]			Bone	No DNA	

AK-20

Report for QIS OQI as of 21/09/2022 2:45:52 PM

Report for QIS OQI - [redacted] Mixtures in Bones

OQI Details

Status	Investigation
Subject	Multiple cases involving bones have generated mixed DNA profiles.
Source of OQI	Internal Problem
Date Identified	17/06/2022

OQI Creator Contact Details

Creator	Angelina KELLER
Organisational Unit/s	Reporting 2
Service/s	Forensic and Scientific Service
Site Location/s	Coopers Plains

Investigator/Actioner Contact Details

Actioner	Allison LLOYD
Organisational Unit/s	Evidence Recovery
Service/s	Forensic and Scientific Service
Site Location/s	Coopers Plains

Investigation Details

No Investigations found

Action Details

No Actions found

Task Details

No Tasks found

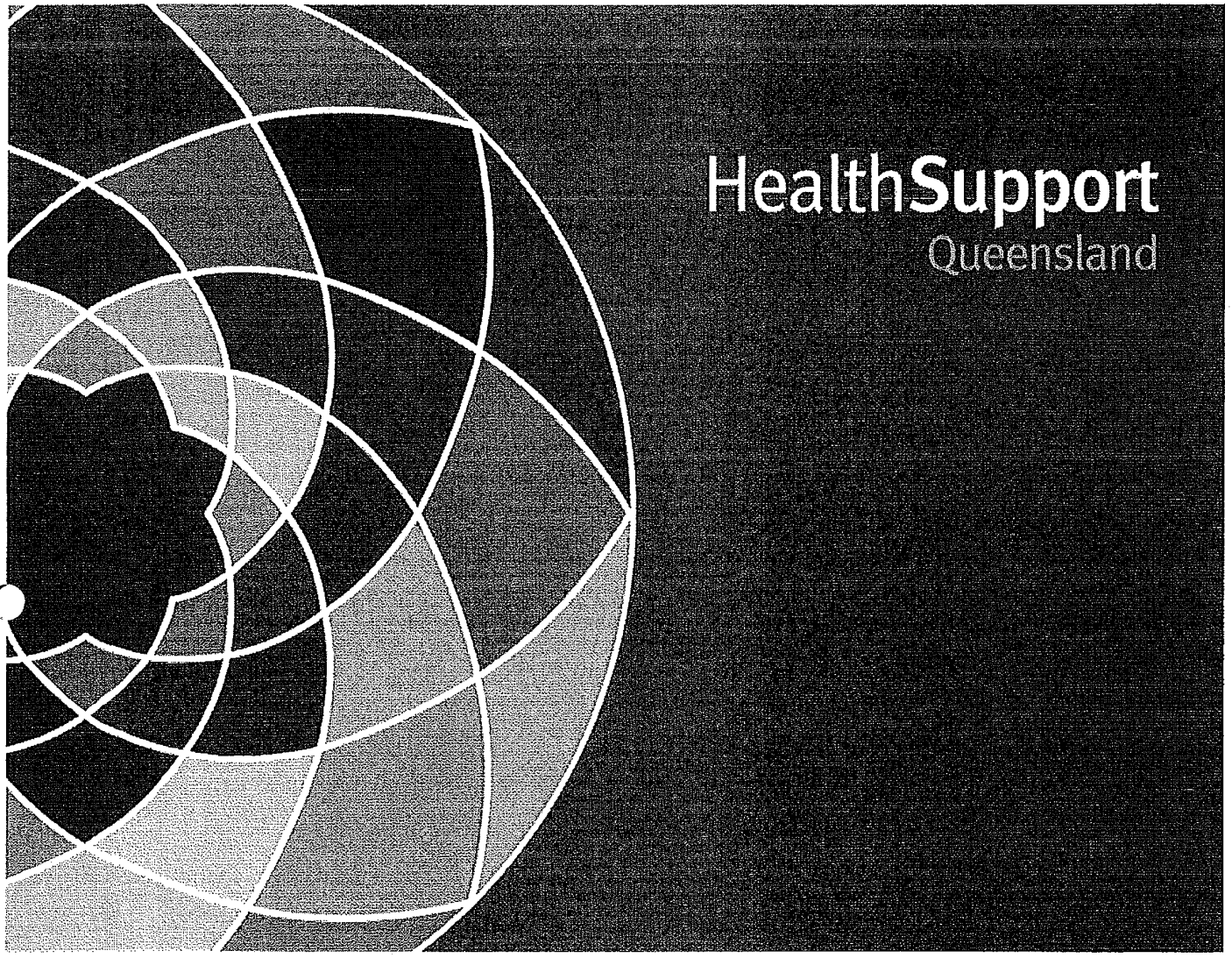
Follow-up And Approval

No Follow Up and Approval Information Available for this OQI

Associations

No Associations found

AK-21



Project #148 – to optimise the cleaning protocol for bone crusher vials

Timothy GARDAM, Sharon JOHNSTONE, Cathie ALLEN

May 2015

Project #148 – to optimise the cleaning protocol for bone crusher vials

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Version	Date	Changed by	Description
1	08-May-2015	Timothy GARDAM	1st Version
1.1	23-Jun-2015	Timothy GARDAM	Incorporated Management Team feedback

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Contents

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1. Abstract

The Forensic DNA Analysis laboratory prepares bone samples by crushing in a SPEX 6750 Freezer Mill. The vials where the crushing takes place are a re-usable component. The manufacturer lists basic cleaning protocols for the crushing vials but no specific protocols suitable for Forensic DNA identification purposes are listed, nor are they present in the literature.

Before crushing a bone sample in the Forensic DNA Analysis laboratory the crushing vial components are swabbed and this swab is submitted for DNA profiling along with the bone sample. The purpose of this 'Equipment Control' is to show that the crushing vial is free from contaminating DNA. The laboratory has recently changed from the Profiler Plus multiplex to the PowerPlex 21 (PP21) multiplex. PP21 appears to have much greater sensitivity for low levels of DNA than Profiler Plus and we are now frequently seeing low-level DNA profiles in Equipment Controls – approximately 70% of Equipment Controls amplified with PP21 have one or more peaks above our limit of detection as compared to less than 10% for those amplified with Profiler Plus.

To have confidence in our results for crushed bones we investigated alternative cleaning protocols to try to ensure that the amount of contaminating DNA in the crushing vials was sufficiently reduced. This experiment compared several alternative cleaning protocols to the current cleaning protocol to see whether they reduced the amount of contaminating DNA.

The use of the autoclave as part of bone vial cleaning was dropped from the project plan early on as initial testing showed that it had only minimal impact on the cleaning process.

Any suitable cleaning protocol must not damage the stainless steel components of the crushing vials by causing rusting or pitting. Such damage weakens the vials and increases the risk that they will break during crushing. It also makes them far more difficult to clean properly, increasing retention of contaminating DNA.

This project found that Tergazyme (the current detergent) was the most effective cleaning agent but the Miele dishwasher 'SPECIAL' cycle offered equivalent performance with the added benefit of being automated.

2. Materials

The following resources are required for this project and are currently in use within the Forensic DNA Analysis Laboratory:

2.1 Reagents

- Terg-a-zyme® enzyme detergent (Alconox Inc.)
- Decon 90 Cleaning solution (Decon Laboratories Ltd.)
- Trigene Advance (CEVA DEIVET Pty. Ltd., Seven Hills, NSW, AU)
- Miele Dishwasher Detergent: Asepti Advantage and Asepti Neutraliser (Miele Australia Pty. Ltd., AU)
- Promega 2800M Positive Control DNA (Promega Corporation, Sydney, AU)
- 5% v/v Hypo 10 bleach (elite Chemicals Pty. Ltd.,Lytton, QLD, AU)
- Proteinase K (20 mg/mL) (Sigma-Aldrich® Corporation, St Louis, MO, US)
- Dithiothreitol (Sigma-Aldrich® Corporation, St Louis, MO, US)
- Ethanol (Recochem Incorporated, Wynnum, QLD,AU)
- Amphyl (Reckitt Benckiser Inc. Parsippany, NJ, US)
- Sarcosyl (Sigma-Aldrich® Corporation, St Louis, MO, US)
- Nanopure water: from Millipore Milli-Q Advantage A10
- Positive controls (Forensic DNA Analysis Unit, Brisbane, QLD, AU)
- TNE (Forensic DNA Analysis Unit, Brisbane, QLD, AU)
- 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)
- 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)
- Water amplification grade (Promega Corp., Madison, WI, US)
- DNA IQ™ Casework Pro Kit for Maxwell® 16 (Promega Corp., Madison, WI, US)

2.2 Materials

- Tubed Sterile DrySwab™ MW1048, wood shaft, cotton bud (Medical Wire & Equiment, Wiltshire, UK)
- Small Stainless Steel End Plugs 6751E (SPEX SamplePrep, Metuchen, NJ, US)
- 96-well PCR micro-plates (Axygen Scientific Inc., Union City, CA, US)
- Tape pads (Qiagen Pty. Ltd., Doncaster, VIC, AU)
- 96-well plate Septa mats (Life Technologies Applied Biosystems, Foster City, CA, USA)
- Sterile 2 mL screw-cap tubes (Axygen Scientific Inc., Union City, CA, US)
- ART Filtered 1000, 300 & 20p pipette tips (Molecular BioProducts Inc., San Diego, CA, US)
- One Touch filtered 10 µL and 200 µL pipette tips (Quantum Scientific Lab Advantage, Murrarie, QLD, AU)
- Rediwipes (Cello Paper Pty. Ltd., Fairfield, NSW, AU)
- Adhesive film (QIAGEN, Hilden, DE)
- Sterile conductive filtered Roborack 25 µL disposable tips (PerkinElmer, Downers Grove, IL, USA)

2.3 Equipment

- Sonicator: Elma Transsonic T310 (Elma Hans Schmidbauer GmbH & Co. KG, Singen, Germany)
- Dishwasher: Miele Professional G7883 CD (Miele Professional USA, Princeton, USA)
- LaboGene Scanspeed 1248 Centrifuge (Labgear, Lynge, Denmark)
- Hot-block (Ratek Instruments Pty. Ltd., Boronia, VIC, AU)
- Biological safety cabinets class II (Westinghouse Pty. Ltd., Newport, AU)
- Refrigerators and freezers (Westinghouse Pty. Ltd., AU)
- GeneMapper-IDX ver.1.4 (Life Technologies Applied Biosystems, Foster City, CA, USA)
- AB 7500 Real Time PCR System (Life Technologies Applied Biosystems, Foster City, CA, USA)
- GeneAmp PCR system 9700 (Life Technologies Applied Biosystems, Foster City, CA, USA)
- ABI 3130*xl* Genetic Analyzer (Life Technologies Applied Biosystems, Foster City, CA, USA)
- STORstar instrument (Process Analysis & Automation, Hampshire, GB)

- MultiPROBE II PLUS HT EX with Gripper Integration Platform (PerkinElmer, Downers Grove, IL, US)
- Thermomixer (Eppendorf AG, Hamburg, DE)
- MixMate (Eppendorf AG, Hamburg, DE)
- Vortex (Ratek Instruments Pty Ltd, Melbourne, VIC, AU)
- Micro centrifuge (Tomy, Tokyo, JP)
- Pipettes (Eppendorf, Hamburg, DE and Thermo Fisher Scientific(Finnpipette), Waltham, MA, US)

3. Methods

3.1 Sample Selection

For Experiment 1 buccal swabs and saliva from donor [redacted] were used. Each end plug was coated with approximately 250 µL of saliva and smeared with buccal cells from a fresh buccal swab (one swab per end plug). The end plugs were air-dried for 24 hours to ensure the saliva-buccal cell mix was adhered to them.

For experiment 2 a dilution of the Promega 2800M Positive Control was used as template DNA. This ensured improved consistency for the inhibition test. A Positive control DNA concentration of 0.3 ng/µL using a total of 0.3 ng per reaction was used to maximise the amount of sample from the end plug that could be added and therefore maximise the sensitivity of inhibition detection.

For each of the experiments crushing vial end plugs were tested by swabbing with a swab moistened with Nanopure water as per SOP 22904 "Procedure for Crushing Bone and Teeth using the SPEX 6750 Freezer Mill". These swabs were submitted for DNA profiling as outlined below (Methods 3.4 to 3.8).

3.2 Reagent Preparation

- Tergazyme was prepared as a saturated solution.
- 5% v/v Decon 90 was prepared as per SOP 17165 "Receipt, Storage & Preparation of Chemicals, Reagents & Kits"
- 5% v/v Trigene Advance was prepared as per the procedure for Trigene II in SOP 17165 "Receipt, Storage & Preparation of Chemicals, Reagents & Kits" but with Trigene Advance rather than Trigene II.
- 0.3 ng/µL Promega 2800M Positive Control was prepared as per the procedure in SOP 19994 "Procedure for testing DNA Quantification Standards, DNA Quantification and Amplification kits & Reagents, and Quality Control Samples" except that 3 µL of 10 ng/µL control was added to 97 µL water.

3.3 Miele Dishwasher "SPECIAL" cycle

Main Wash:	Cold Water, Detergent, 93 °C 10 min
Rinse:	Hot Water, Detergent Neutraliser
Rinse:	Hot Water
Final Rinse:	Distilled Water, 75 °C 3 min
Drying:	99 °C 35 min

3.4 DNA Extraction

Swabs were submitted for bone extraction as per "Extracting DNA from Bone and Teeth" (QIS 17182). This is the same method used for Equipment Controls.

3.5 DNA Quantification

All reactions were prepared by manual methods or using the MultiPROBE II plus HT EX platform according to QIS 19977 "Quantification of Extracted DNA using the Quantifiler™ Human DNA Quantitation Kit".

3.6 DNA Amplification

All amplification set ups were performed using the MultiPROBE II plus HT EX platform and amplification using the GeneAmp PCR system 9700 according to QIS 31511 "Amplification of Extracted DNA using the PowerPlex®21 System".

Table 1 lists the PCR cycling conditions used for this project.

Table 1 **PCR cycling conditions for PowerPlex®21 System.**

PowerPlex® 21 Kit	Standard
GeneAmp 9700 mode	Max
	30 cycles
Activation	96 °C for 1 minute
Cycling	94 °C for 10 seconds
	59 °C for 1 minute
	72 °C for 30 seconds
Extension	60 °C for 10 minutes
	4 °C Soak

3.7 DNA Fragment Analysis

The plates for DNA fragment analysis were prepared and the PCR fragments separated by capillary electrophoresis (CE) using a 3130*xl* Genetic Analyser according to QIS 15998 "Procedure for the Use and Maintenance of the AB 3130*xl* Genetic Analysers". Table 2 outlines the 3130*xl* Genetic Analyser running conditions used.

Table 2 **CE protocol conditions**

Injection time	Injection voltage	Run time
5 s	3 kV	1500 s

3.8 Profile Interpretation

All samples were analysed with GeneMapper ID-X v1.4 under current casework conditions by an independent scientist with no knowledge of the profiles being analysed. The "16RFU Negative Controls" analysis method was used as this is the analysis method used for Equipment Controls.

3.9 Experimental Design

For each experiment 25x bone crusher end vials were cleaned using the current cleaning procedure then labelled '1' to '25' with black marker pen. All of the end vials were cleaned at the same time in individual 70 ml plastic screw-cap vials, except for the dishwasher samples which were instead washed together in a wire basket in the dishwasher.

3.9.1 Experiment 1 – Detergent

Bone crusher vial end plugs were subjected to a 15 minute soak in the following cleaning reagents:

a) Nanopure Water
b) Tergazyme (current procedure)
c) 5% v/v Decon 90
d) 5% v/v Trigene Advance
e) Miele Dishwasher "SPECIAL" program*

* These end plugs were not soaked and scrubbed but were instead washed in the dishwasher using the "SPECIAL" program

With the exception of e) – washed in the dishwasher – the end plugs were subjected to the following physical cleaning measures after the 15 minute detergent soak:

- Sonication (15 min) in Nanopure water
- Scrub with clean nail brush under hot tap water

25x bone crusher vial end plugs were coated with a mixture of buccal cells/saliva and air-dried for a period of 24 hours. One of the above cleaning reagents (a. to e.) was applied to each end plug (5x end plugs for each cleaning reagent). End plugs were swabbed and submitted for DNA Profiling.

NOTE: A 15 minute soak was chosen as that is the period that is used currently for crushing vial components. Longer periods (e.g. overnight) significantly increase the risk of corrosion and rusting.

3.9.2 Experiment 2 – Inhibition Test

Bone crusher vial end plugs were subjected to a 15 minute soak in the following cleaning reagents:

a) Nanopure Water
b) Tergazyme (current procedure)
c) 5% v/v Decon 90
d) 5% v/v Trigene Advance
e) Miele Dishwasher "SPECIAL" program*

* These end plugs were not soaked and scrubbed but were instead washed in the dishwasher using the "SPECIAL" program

With the exception of e) – washed in the dishwasher – the end plugs were subjected to the following physical cleaning measures after the 15 minute detergent soak:

- Sonication (15 min) in Nanopure water
- Scrub with clean nail brush under hot tap water

25x bone crusher vial end plugs were cleaned by one of the cleaning reagents listed above. One of the above cleaning reagents (a. to e.) was applied to each end plug (5x end plugs for each cleaning reagent). End plugs were swabbed and submitted for DNA Profiling. After extraction and quantitation and prior to the amplification step each sample was spiked with 1 µL of 0.3 ng/µL Promega positive control DNA to test for inhibition from detergent residues.

3.9.3 Acceptance Criteria

The optimum cleaning reagent was selected based on the combination of:

- No rusting or damage to the end plugs during the 15 minute soak
- No indication of inhibition (Experiment 2), and
- The lowest amount of DNA detected by quantitation and the fewest amplified peaks (Experiment 1).

4. Results

4.1 Experiment 1 – Detergent

The quantitation values are listed in Table 3 and plotted with standard deviation in Figure 1. For clarity the quant values are shown in pg/µL rather than the usual ng/µL.

Table 3 **Quantitation Values (pg/µL)**

	a. Nanopure Water	b. Tergazyme	c. Decon 90 (5% v/v)	d. Trigene Advance (5% v/v)	e. Dishwasher "SPECIAL"
	28.100	0.000	1.890	41.600	0.000
	4.780	0.000	1.680	65.300	0.490
	0.110	0.000	1.100	138.000	0.000
	14.900	0.000	0.000	68.300	0.000
	18.700	0.000	0.000	17.500	0.000
Average:	**13.318**	**0.000**	**0.934**	**66.140**	**0.098**

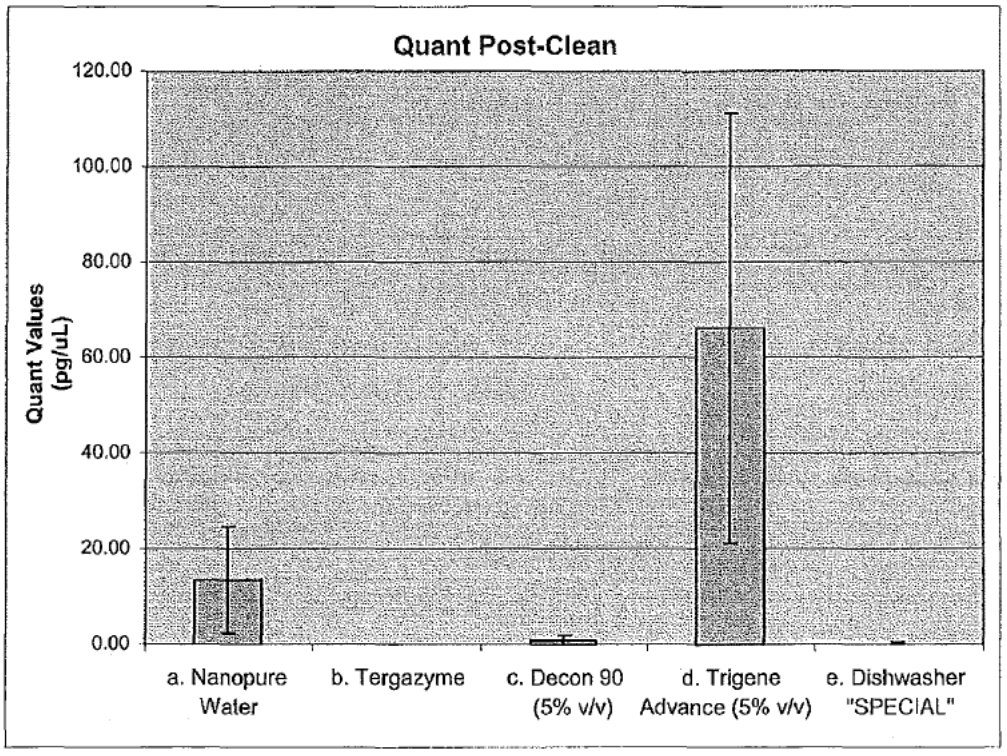


Figure 1 **Quantitation Values (pg/µL) with standard deviation**

The extracts above were DNA profiled. The DNA profiles were read at a threshold of 16 rfu – the more sensitive level used for checking negative controls such as bone vial equipment controls. The number of peaks seen (maximum of 40) is listed in Table 4 and plotted in Figure 2 with standard deviation.

Table 4 **Alleles (≥16 RFU)**

	a. Nanopure Water	b. Tergazyme	c. Decon 90 (5% v/v)	d. Trigene Advance (5% v/v)	e. Dishwasher "SPECIAL"
	40	0	7	40	0
	40	0	13	40	2
	18	1	6	40	0
	40	2	1	40	0
	38	0	1	39	0
Average:	**35.2**	**0.6**	**5.6**	**39.8**	**0.4**

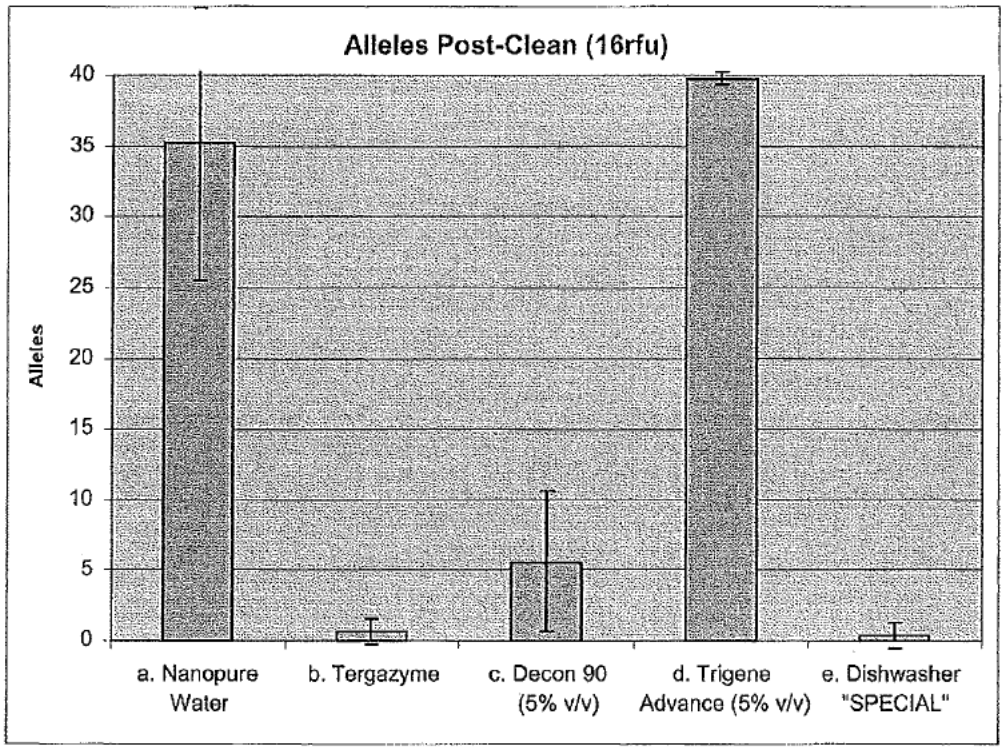


Figure 2 **Alleles (≥16 RFU) with standard deviation**

5% Trigene Advance (average 66.1 pg/µL, 39.8 peaks) gave a greater DNA yield and a greater number of peaks than water (average 13.3 pg/µL, 35.2 peaks). Because of this result Trigene Advance was considered not suitable for cleaning bone vials and so was not tested in Experiment 2.

5% Decon 90 (average 0. 9 pg/µL, 5.6 peaks) gave a much lower DNA yield and fewer peaks than water (average 13.3 pg/µL, 35.2 peaks). Tergazyme (average 0 pg/µL, 0.6 peaks) and the Miele dishwasher "SPECIAL" cycle (average 0.1 pg/µL, 0.4 peaks) gave a much lower yield and fewer peaks again than 5% Decon 90.

4.2 Experiment 2 – Inhibition Test

After soaking, sonicating and scrubbing then DNA profiling, no inhibition was noted – all samples gave full 40-allele DNA profiles from the positive control (all peaks ≥40 RFU).

To assess more subtle effects of inhibition the average Peak Height, Heterozygote Balance and Stutter Percentage for each reagent were calculated from the peak height data (Table 5). Stutter percentage could only be calculated for stutter peaks ≥6 RFU but these peaks were considered representative. The Peak Height data is shown in Figure 3 with standard deviation.

Table 5 Summary of Peak Data from Experiment 2

	a. Nanopure Water	b. Tergazyme	c. Decon 90 (5% v/v)	e. Dishwasher "SPECIAL"
Average Peak Heights (RFU)	658	544	594	510
Average Heterozygote Balance (%)	82.8	82.7	81.4	80.6
Average Stutter (%)	7.83	7.81	7.59	7.67

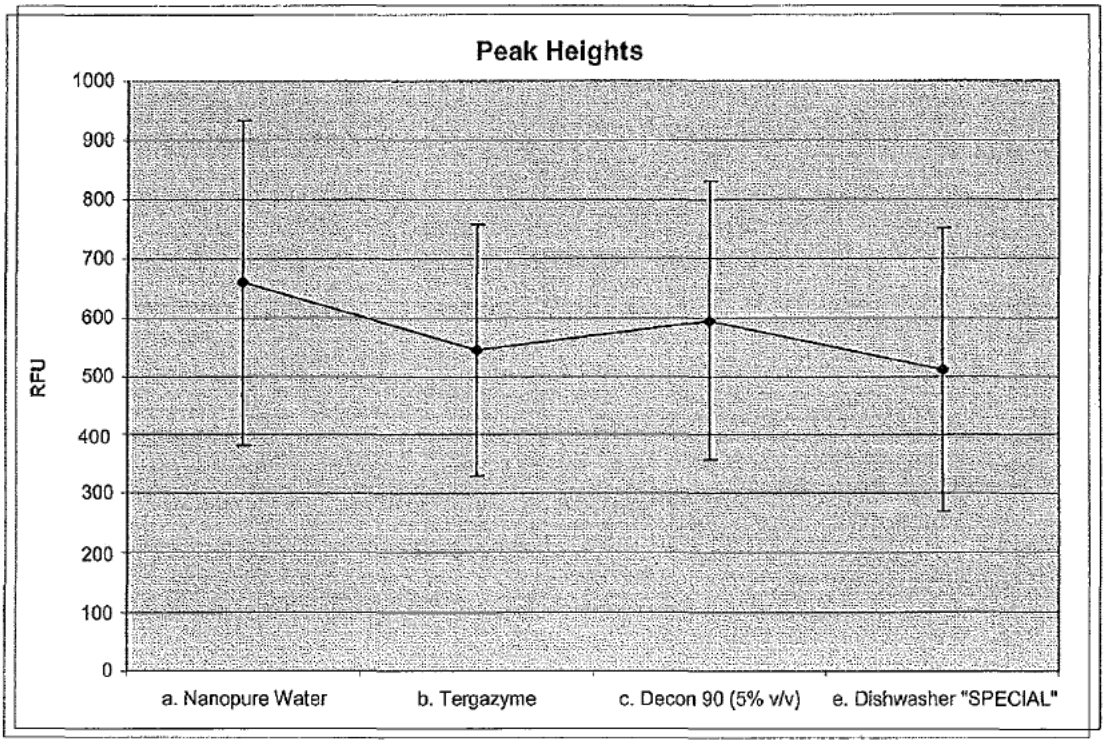


Figure 3 Peak Heights with Standard Deviation

Although there appeared to be some difference in average peak heights between the different reagents the variance within each group was very high (CV~40%) making it difficult to assess whether this difference was significant or not.

A two-tailed student's t-test was used to compare the results for the Miele "SPECIAL" cycle and Tergazyme to assess whether there was likely to be a significant difference between the data sets. A p-value of < 0.05 was considered to be significantly different. For Heterozygote Balance ($p=0.27$), Stutter percentage ($p=0.65$) and Peak Heights ($p=0.13$) there was no significant difference between the Miele "SPECIAL" cycle and Tergazyme.

Another possible indicator of inhibition is the pattern of 'degradation' seen in the DNA profiles. All of the DNA profiles from Experiment 2 were run through the STRmix software[3] to assess the degree of degradation. For all samples STRmix modelled degradation of 0.0 RFU/bp – meaning no degradation.

No significant difference was noted between the Miele "SPECIAL" cycle and Tergazyme (the current cleaning method) in Experiment 2 with regard to peak heights, heterozygote balance, stutter or degradation.

5. Summary & discussion

The results of Experiment 1 showed a large variation between the different cleaning reagents in their ability to remove DNA from dried-on saliva. Although there was significant variation between the quantitation values for some of the replicates – especially those with higher quant values – there was certainly a clear trend for each of the reagents. The number of alleles seen after amplification (Alleles (≥6 RFU)Table 4, Figure 2) was consistent with the quant value results (Table 3, Figure 1) and was a more sensitive measure for differentiating between the reagents with lower quant values.

The most striking results are those of 5% Trigene Advance which gave significantly higher DNA yields and peak counts than water. This surprising result is similar to what was found by Ballantyne *et al.* (2015) when testing Virkon with wet and dry saliva samples[1]. They speculated that cellular and extracellular components of the saliva may inhibit the active ingredients in Virkon. In this case it appears that the Trigene Advance is actually increasing the yield from dried saliva stains, relative to water. This may be because Trigene Advance is not damaging the DNA but is damaging some of the 'stickier' proteins so more nuclei and nuclear fragments are released to the swab after treatment with Trigene Advance versus washing with water. This result also conflicts with other testing at this laboratory using whole blood dried onto petri dishes, where it was found that Trigene Advance and Virkon are the most effective cleaning agents[2]. It is possible that depending on the surface to be cleaned (metal, plastic) and the contaminant (dried saliva, dried whole blood, extracted DNA, amplified DNA) all of the different cleaning agents will perform quite differently. It may be that no one cleaning agent will be suitable for all cleaning tasks in a forensic DNA laboratory. Because of the results in Experiment 1, Trigene Advance was not considered suitable for cleaning the bone vials.

Although the Decon 90 was considerably more effective than Nanopure water for removing dried saliva stains the Tergazyme and the Miele "SPECIAL" cycle were much more effective again.

From Experiment 2 none of the reagents tested appears to show any significant inhibition at the concentrations tested. Additionally after analysing peak heights, heterozygote balance, stutter percentage and degradation there was no significant difference seen between the Miele "SPECIAL" cycle and Tergazyme (the current cleaning method).

All of these detergents are classified as 'Corrosive' and 'Irritant' and there is no significant safety benefit to using any one over any other, with the exception of the dishwasher where operator exposure to the detergent is minimal. None of these cleaning agents caused rusting or damage to the bone crusher end plugs during the 15-minute soak steps.

The suitability of these reagents for cleaning bone vials then comes down to their performance in Experiment 1. Because of their lower quantitation results and lower peak counts, Tergazyme and the Miele "SPECIAL" cycle are the preferred options. The Miele "SPECIAL" cycle is an automated process whereas cleaning with Tergazyme is a manual process. The automated process requires less operator hands-on time, less risk of operator exposure to detergents, and is likely to be subject to less operator-to-operator variability than the manual cleaning process. The Miele "SPECIAL" cycle is therefore the preferred option.

6. Recommendations

From the outcome of this report, the authors suggest the following recommendations for implementation within the Forensic DNA Analysis laboratory:

1. The Miele "SPECIAL" cycle is recommended as the primary cleaning method as it requires less operator hands-on time, less operator exposure to detergents, and is likely to be less susceptible to operator variation.
2. Cleaning with Tergazyme should remain a viable backup method if the dishwasher is unavailable for any reason.

7. Abbreviations / Glossary

DNA	Deoxyribonucleic Acid.
PCR	Polymerase chain reaction.
Quant	DNA Quantification/Quantitation – determining the amount of amplifiable DNA present in the sample.
RFU	Relative fluorescence units – a unit-less measure of peak intensity for DNA profiles.
CV	Coefficient of Variation – the ratio of the standard deviation to the mean.
bp	Base pairs (of a DNA strand).
Degradation	When referring to a DNA profile, a pattern of sharply decreasing peak heights as peak molecular weight increases. Seen when amplifying poor quality degraded DNA where shorter fragments predominate, but also if the PCR is impaired for other reasons (e.g. PCR inhibitors are present in the sample).

8. References

1. Ballantyne K, Salemi R, Guarino F, Pearson J, Garlepp D, Fowler S, van Oorschot R. "DNA contamination minimisation – finding an effective cleaning method". *Australian Journal of Forensic Sciences*, DOI: 10.1080/00450618.2015.1004195
2. Thompson S, Kaity A, Mathieson M, Ryan L, McNevin A, Allen C. *Project#153 - Verification of Cleaning Reagents (Trigene Advance, Viraclean, Virkon, Pyroneg, Decon, Cavicide, F10SC) for use in Forensic DNA Analysis*. 2015
3. STRmix™ software v2.06, http://strmix.esr.cri.nz

Implementation Date	Details	Project Leader
5/07/2019	Change in bone processing equipment cleaning protocol: Cleaning of the bone crushing equipment using the dishwasher as per Proposal #148; Use bleach and / or Trigene followed by 70% ethanol (as appropriate) to clean the remaining equipment in line with other Evidence Recovery and Analytical laboratory equipment protocols	ARM

AK-22

[redacted] AK-23

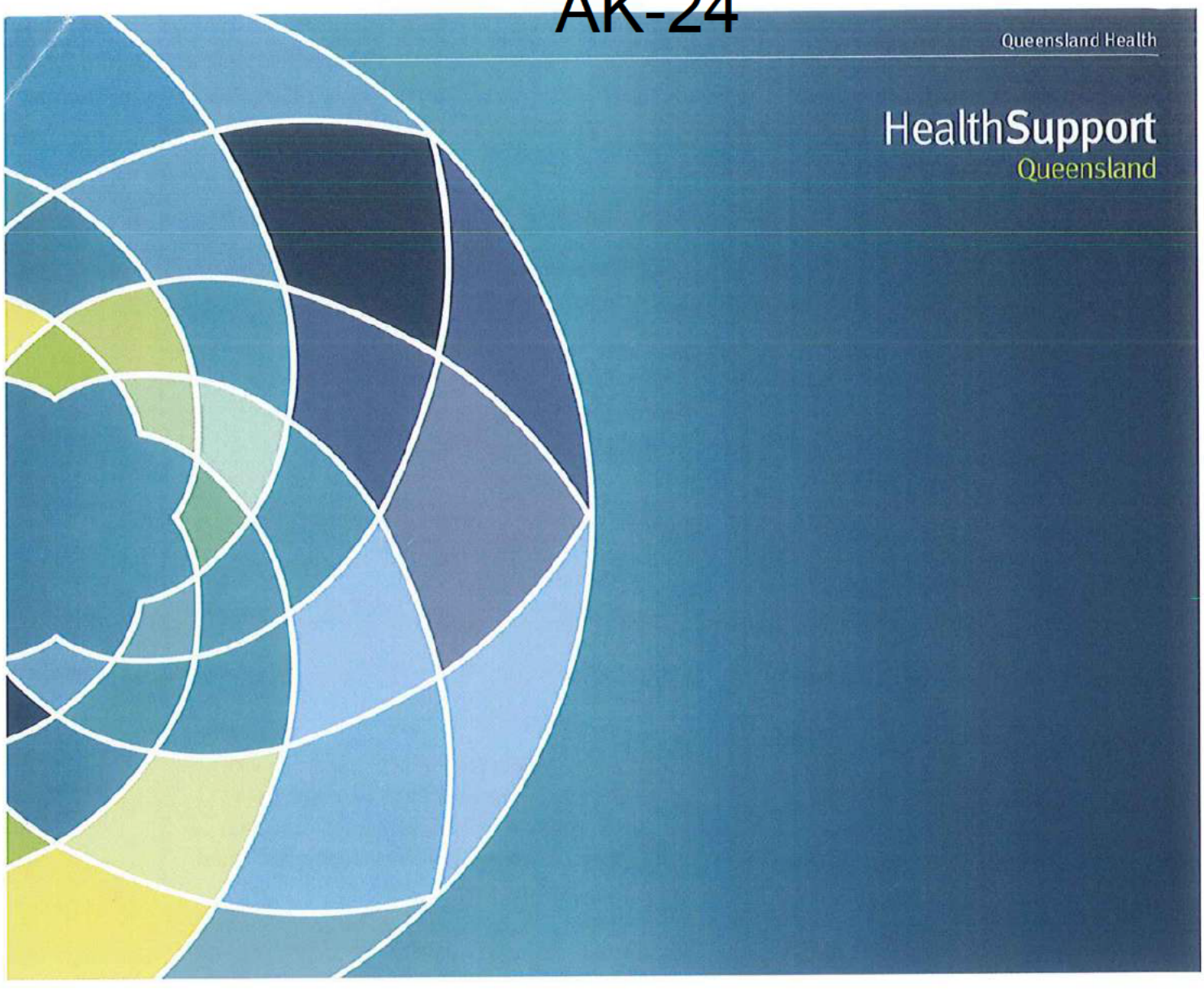
Coronial ID 4

Tues 6 March 2018	Bone sampled by AR (refresher from maternity leave), JSM,VC (trainer)	4 x ~0.4 g of bone powder from left femur (1st crush)
		Organic extraction (96% success rate)
Fri 9 March 2018		Quantification values 0.02549, 0.04019, 0.03256, 0.02619
Tues 13 March 2018		4 x DNA profiles with extra peaks / mixtures
Thurs 29 March 2018	Saw JAH about re-sampling bone to confirm mixtures	
Fri 6 April 2018	Saw JW about re-sampling bone to confirm mixtures JW (reporter for case) requested resampling with AM	
Mon 9 April 2018	Bone re-sampled by AR, JSM, NG (new); no consultation about re-sampling or new sampler (AK, AL bone sampling trainers; VC too; All were absent from this re-sampling)	4 x ~0.4 g of bone powder from left femur (2nd crush)
Fri 13 April 2018		QIAsymphony extraction (newly validated, organic replaced without notification)
Tues 17 April 2018		Quantification values 0.01387, 0.00056, 0.00127, 0.00015 (3 x no DNA or insufficient DNA results)
Wed 18 April 2018	Saw JH with JW about new bone extraction not working; Told due to sample to sample variation, JH did not think there was a problem, JW conceeded, AK disagreed ~ do think there is a problem and am concerned about future bone profiling also not working	
	Saw LR in hallway; Asked if LR had any ideas why new bone extraction not working, shrugged shoulders, did not think there was a problem	
	Saw KR; Looked for new bone extraction validation, found she was the only manager who had been left off the approvals list; KR sent JH and e-mail raising my concerns and hers; No response	
Thurs 19 April 2018	Asked RP to look at new bone extraction validation; Within 5 mins RP had found experimental design was invalid with N=1; RP went to see JH but not in office so RP saw PB who supported RP concerns	
	As far as I am aware via RP, a group of people are reassessing new bone validation, meanwhile back to organic extraction, no further involvement or notification	
Tues 24 April 2018	Saw JH, LR, PB, AR, with JW to get more bone aliquots submitted from 2nd resampling for organic bone extraction	2 x ~0.4 g of bone powder from left femur (2nd crush revisited)
Mon 30 April 2018		Organic extraction
Thurs 3 May 2018	Whole laboratory suppression issue but organic extraction not affected (all processing temporarily ceased)	Quantification values 0.05370, 0.04142
Fri 8 June 2018	Single source DNA profiles obtained	Contamination confirmed from 1st crush
Tues 12 June 2018	Quality search conducted, source of contamination in 1st crush unknown	
Thurs 14 June 2018	Identification statement released	AK reporter (JW on leave), JH reviewer

Additional discussions

Wed 11 April 2018	AK, AL saw MH as acting line manager about AM not includng AK, AL in re-sampling as per entry 9 April 2018; MH supportive, wants to discuss with AM and JAH on our behalf; Rotating roster
Thurs 12 April 2018	MH discussed with AM, JH; Limited support; AM words repeated by JH

AK-24



Project Report #192

Validation of QIAsymphony SP for Bone Extraction

April 2018

Melissa Cipollone, Luke Ryan, Megan Mathieson and Cathie Allen

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:

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Title: Senior Scientist – Analytical
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Email:

Version history

Version	Date	Changed by	Description
1.0	March 2018	Melissa Cipollone	Creation of Document
1.1	April 2018	Luke Ryan	Feedback incorporated

Document sign off

A quorum was reached for the approval of this Project Report in accordance with Section 4.1 of QIS 22871 'Procedure for Change Management in Forensic DNA Analysis'. The quorum was reached based on those officers who have provided feedback on this document and includes those members of the Management Team who have approved and/or endorsed this document below.

This document has been **approved** by:

Name	Position	Signature	Date
Cathie Allen	Managing Scientist	[redacted]	10/04/2018

The following officers have endorsed **this document**

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Matthew Hunt	A/Senior Scientist Reporting	[redacted]	06/04/2018

Contents

Abstract

The purpose of this project was to validate bone DNA extractions on the QIAsymphony SP instrument, as this would dramatically increase the efficiency and processing capacity of extracting DNA from bone samples when compared to the current organic extraction protocol. Further, organic extraction involves the use of phenol chloroform isoamyl alcohol which is a chemical hazard, therefore implementing an alternative protocol would remove this hazard.

This project had quantitative acceptance criteria, and these criteria were assessed on completion of the data analysis for each experiment performed. The following experiments were performed to test and compare a number of extraction protocols:

- Experiment 1: Current Organic Extraction
- Experiment 2: QIAGEN Pre-Lysis with Overnight Incubation and QIAsymphony SP Extraction
- Experiment 3: QIAGEN Pre-Lysis with 5 hour Incubation and QIAsymphony SP Extraction
- Experiment 4: Organic Pre-Lysis with Overnight Incubation and QIAsymphony SP Extraction
- Experiment 5: Organic Pre-Lysis with 5 hour Incubation and QIAsymphony SP Extraction

The results of this project have shown the protocol tested in Experiments 2 and 3 (QIAGEN Pre-Lysis with 5 hour and overnight Incubation and QIAsymphony® SP Extraction) should be implemented as they gave DNA quantification and final profile results which were comparable to or better than the other tested protocols. Further, use of the Experiment 3 protocol (5 hour pre-lysis incubation) would dramatically increase the efficiency and processing capacity of bone DNA extractions from 24 hours (using current organic extraction) to under 8 hours which may be critical for disaster victim identification (DVI) cases.

Introduction

Forensic DNA Analysis currently performs automated DNA extractions on a range of sample types and substrates using a QIAGEN® QIAsymphony® SP/AS instrument. The original validation of the QIAsymphony® SP/AS did not include bone or teeth extraction. Forensic DNA Analysis currently have two QIAsymphony® SP/AS instruments the use of these instruments for bone/teeth extraction would be particularly beneficial in the event of a large scale DVI as it will dramatically increase the efficiency and processing capacity of bone/teeth DNA extractions.

The QIAsymphony® SP/AS instrument is a modular automated system which enables the processing of up to 96 samples on a single run. The QIAsymphony® SP module is used for the extraction and purification of DNA from forensic casework and reference samples. It uses pre-programmed optimized protocols and the QIAGEN® cartridge-based magnetic-particle chemistry kit, the QIAsymphony® DNA Investigator Kit. The SP module was the only module tested in this validation.

The original scope of this validation included both teeth and bone extractions. However due to time and resource limitations, only bone extractions were tested. Teeth extractions will be tested in the future and reported in a separate report.

Resources and Methods

All reagents, materials and equipment used in this project were as specified in the approved in-house document Project Proposal #192: Validation of QIAsymphony SP for bone extraction (November 2017). This document will be referred to as the Experimental Design. The following QIS documents are referenced throughout this report:

- QIS 34039 Extracting DNA from Bone and Teeth
- QIS 34045 Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit
- QIS 34052 Amplification of Extracted DNA using the PowerPlex®21 System
- QIS 34112 STR fragment analysis of PowerPlex® 21 profiles using GeneMapper® ID-X software
- QIS 34131 Capillary Electrophoresis Quality (CEQ) Check

- QIS 34132 DNA Extraction and Quantification of samples using the QIAsymphony® SP and AS – FR

Sample Selection

Ten powdered bone samples were retrieved from storage (from previously examined case files) and used as per the experimental design. The quantification results from original testing of these bone sample aliquots was used to ensure that sample selection included a range of sample qualities. Table 1 contains the bones selected for this project (for simplicity in the remainder of this document, these samples will be referred to using their Sample Number rather than Lab Number).

Please note that for each of these samples a number of bone aliquots were originally tested, and differing quantification results were obtained from each aliquot. For this reason, quantification results are given as a range.

Table 1: Bone samples used in this validation

Lab Number	Sample Number	Original Quant Range (ng/µL)
[redacted]	Sample 1	>50
	Sample 2	10 – 20
	Sample 3	20 – 30
	Sample 4	0.10 – 0.15
	Sample 5	7 – 12
	Sample 6	0.08 – 0.40
	Sample 7	4 – 5
	Sample 8	0.03 – 0.30
	Sample 9	0.00
	Sample 10	0.001 – 0.003

Experiments and Results

Experiment 1: Current Organic Extraction

Purpose

The purpose of this experiment was to extract human genomic DNA from powdered bone using the current validated method of extracting DNA from bone and teeth using phenol chloroform isoamyl alcohol (PCIA), this is also referred to as an organic extraction.

Results

The results from Experiment 1 provide a benchmark to compare the results from Experiments 2, 3, 4 and 5.

Samples 1 and 3 gave average quantification values >10 ng/µL. These samples were diluted and quantified. The quantification values were multiplied by the dilution factor to give a more accurate final quantification result. The average quantification results and allele counts obtained for all the samples that underwent organic extraction are outlined in Table 2.

Table 2: Average quantification results and allele count for Experiment 1

Sample	Initial Average Quant Result (ng/µL)	Dilution factor	Dilution Quant Result (ng/µL)	Final Quant Result (ng/µL)	Allele Count
Positive Control	1.648			1.648	40
Negative Control	0.001			0.001	9
Sample 1	53.879	1:150	0.319	47.793	40
Sample 2	1.883			1.883	32
Sample 3	58.155	1:150	0.321	48.174	40
Sample 4	0.072			0.072	35
Sample 5	4.448			4.448	40
Sample 6	0.004			0.004	12
Sample 7	1.618			1.618	39
Sample 8	0.189			0.189	40
Sample 9	0.000			0.000	0
Sample 10	0.002			0.002	0

The positive extraction control gave a full expected profile, however given this is a blood sample, this was not used to compare/assess the bone extraction protocols. The negative extraction control gave a 0.001 ng/µL quantification result and a DNA profile with 9 peaks present (analysed at 16 RFU as per QIS #34131). The negative extraction control was reamplified and the contamination was reproduced, confirming

the contamination is present in the extract. A quality search was conducted and the peaks were found to be consistent with an Analytical Team staff member. Sample 4 on this batch gave a low level mixed DNA profile, which was consistent with a mixture of the expected profile and the staff sample which had contaminated the negative extraction control.

No other samples on this batch show the presence of contamination from this staff member or from any other source. The staff member whose DNA has contaminated these samples did not process any of these batches, and was not rostered in the main laboratory during the processing of these samples. Given two samples on this batch have been contaminated from the same staff member, it is likely the mechanism of contamination is the same for both samples. However, as only two samples were contaminated, and the remaining samples showed no evidence of contamination (even those samples which gave No DNA Profile final results), it is unlikely one of the reagents used for the entire batch was contaminated. The contamination may be due to a pipette, tip, tube or other consumable used in the process. The exact mechanism of contamination was not able to be determined.

Given the Experimental Design called for each protocol to be tested with at least four different bone samples, and that ten samples were actually tested in this validation, Sample 4 has been excluded from the remainder of this validation. Given that the remainder of samples on this batch gave expected profiles and did not show signs of contamination, they were accepted as valid.

Experiment 2: QIAGEN Pre-Lysis with Overnight Incubation and QIAsymphony® SP Extraction

Purpose

The purpose of this experiment was to test the extraction of human genomic DNA from powdered bone using the QIAGEN pre-lysis method with the samples being incubated overnight and then extracted on the QIAsymphony® SP instrument.

Results

Samples 1 and 3 gave quantification values >10 ng/µL. These samples were diluted and quantified. The quantification value was then multiplied by the dilution factor to give a more accurate overall quantification value.

The average quantification results and allele counts for all the samples in Experiment 2 are outlined in Table 3.

Table 3: Average quantification results and allele counts for Experiment 2

Sample	Initial Average Quant Result (ng/µL)	Dilution factor	Dilution Quant Result (ng/µL)	Final Quant Result (ng/µL)	Allele Count
Positive Control	0.844			0.844	40
Negative Control	0.000			0.000	0
Sample 1	49.623	1:150	0.329	49.341	40
Sample 2	1.831			1.831	40
Sample 3	13.800	1:20	0.773	15.465	40
Sample 5	8.088			8.088	40
Sample 6	0.236			0.236	40
Sample 7	1.447			1.447	40
Sample 8	0.120			0.120	40
Sample 9	0.000			0.000	0
Sample 10	0.007			0.007	18

The positive extraction control gave a full expected profile, however given that this is a blood sample, this was not used to compare/assess the bone extraction protocols. The negative extraction control gave a 0.000 ng/µL quantification result and No DNA Profile final result as expected.

Experiment 3: QIAGEN Pre-Lysis with 5 hour Incubation and QIAsymphony® SP Extraction

Purpose

The purpose of this experiment was to test the extraction of human genomic DNA from powdered bone using the QIAGEN Pre-lysis method with the samples being incubated for 5 hours and then extracted on the QIAsymphony® SP instrument.

Results

Samples 1 and 3 gave quantification values >10 ng/µL. These samples were diluted and quantified. The quantification value was then multiplied by the dilution factor to give a more accurate overall quantification value.

The average quantification results and allele counts for all the samples in Experiment 3 are outlined in Table 4.

Table 4: Average quantification results and allele counts for Experiment 3

Sample	Initial Quant Result (ng/µL)	Dilution factor	Dilution Quant Result (ng/µL)	Final Quant Result (ng/µL)	Allele Count
Positive Control	0.920			0.920	40
Negative Control	0.000			0.000	0
Sample 1	57.257	1:150	0.382	57.257	40
Sample 2	2.344			2.344	40
Sample 3	25.345	1:100	0.253	25.345	40
Sample 5	8.101			8.101	40
Sample 6	0.261			0.261	39
Sample 7	2.299			2.299	40
Sample 8	0.073			0.106	40
Sample 9	0.000			0.000	0
Sample 10	0.261			0.009	12

The positive extraction control gave a full expected profile, however given that this is a blood sample, this was not used to compare/assess the bone extraction protocols. The negative extraction control gave a 0.000 ng/µL quantification result and No DNA Profile final result as expected.

Experiment 4: Organic Pre-Lysis with Overnight Incubation and QIAsymphony® SP Extraction

Purpose

The purpose of this experiment was to test the extraction of human genomic DNA from powdered bone using the current organic extraction pre-lysis method with the samples being incubated overnight and then extracted on the QIAsymphony® SP instrument.

Results

Sample 1 gave a quantification value >10 ng/µL. This sample was diluted and quantified. The quantification value was then multiplied by the dilution factor to give a more accurate overall quantification value.

The average quantification results and allele counts for all the samples in Experiment 4 are outlined in Table 5.

Table 5: Average quantification results and allele counts for Experiment 4

Sample	Initial Average Quant Result (ng/µL)	Dilution factor	Dilution Quant Result (ng/µL)	Final Quant Result (ng/µL)	Allele Count
Positive Control	2.218			2.218	40
Negative Control	0.000			0.000	1
Sample 1	14.430	1:20	0.721	14.430	39
Sample 2	1.184			1.184	39
Sample 3	8.468			8.468	40
Sample 5	3.259			3.259	40
Sample 6	0.047			0.047	34
Sample 7	0.512			0.512	35
Sample 8	0.015			0.015	35
Sample 9	0.000			0.000	0
Sample 10	0.000			0.000	0

The positive extraction control gave a full expected profile, however given that this is a blood sample, this was not used to compare/assess the bone extraction protocols. The negative extraction control gave a 0.000 ng/µL quantification result and a DNA profile with one peak present. The negative extraction control was accepted as per routine negative extraction control acceptance criteria (see QIS #34131).

Experiment 5: Organic Pre-Lysis with 5 hour Incubation and QIAsymphony® SP Extraction

Purpose

The purpose of this experiment was to test the extraction of human genomic DNA from powdered bone using the current organic extraction pre-lysis method with the samples being incubated for 5 hours and then extracted on the QIAsymphony® SP instrument.

Results

Samples 1 and 3 gave quantification values >10 ng/µL. These samples were diluted and quantified. The quantification value was then multiplied by the dilution factor to give a more accurate overall quantification value.

The average quantification results and allele counts for all the samples in Experiment 5 are outlined in Table 6.

Table 6: Average quantification results and allele counts for Experiment 5

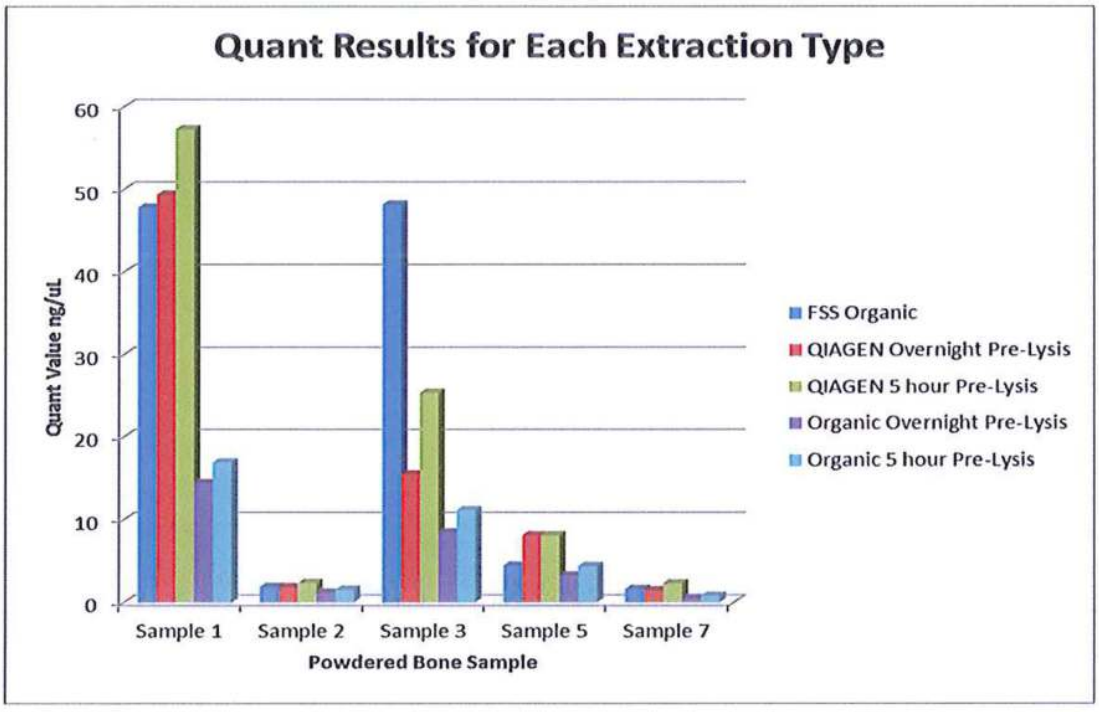
Sample	Initial Average Quant Result (ng/µL)	Dilution factor	Dilution Quant Result (ng/µL)	Final Quant Result (ng/µL)	Allele Count
Positive Control	0.481			0.481	40
Negative Control	0.000			0.000	0
Sample 1	16.935	1:20	0.847	16.935	40
Sample 2	1.508			1.508	40
Sample 3	11.148	1:20	0.557	11.148	40
Sample 5	4.405			4.405	40
Sample 6	0.047			0.047	35
Sample 7	0.779			0.779	39
Sample 8	0.014			0.014	40
Sample 9	0.000			0.000	0
Sample 10	0.000			0.000	0

The positive extraction control gave a full expected profile, however given that this is a blood sample, this was not used to compare/assess the bone extraction protocols. The negative extraction control gave a 0.000 ng/µL quantification result and No DNA Profile final result as expected.

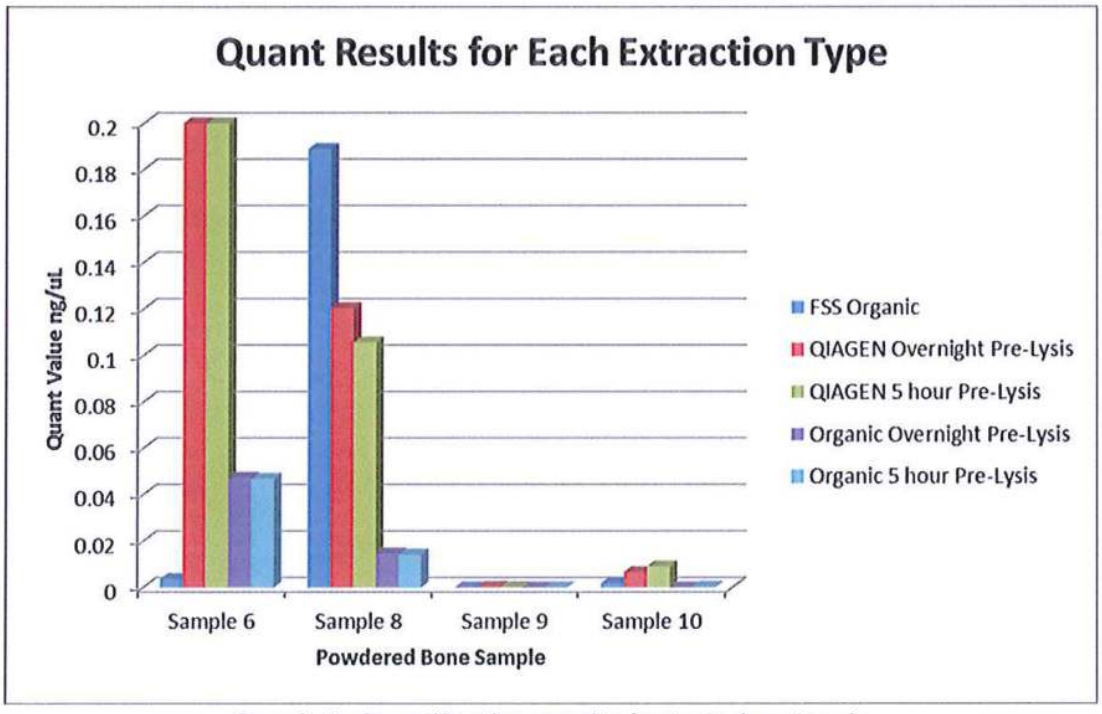
Discussion

The protocols tested in Experiments 1, 2, 3, 4 and 5 were compared using DNA quantification results and allele counts obtained in the final DNA profile. Generally, an extraction protocol was assessed as performing better if it gave higher DNA quantification results and more alleles in the final DNA profile. Graphs have been prepared to display and compare the quantification and allele count results for each of the protocols.

Graphs 1 and 2 below provide a summary of the average quantification results for the samples tested in this validation.

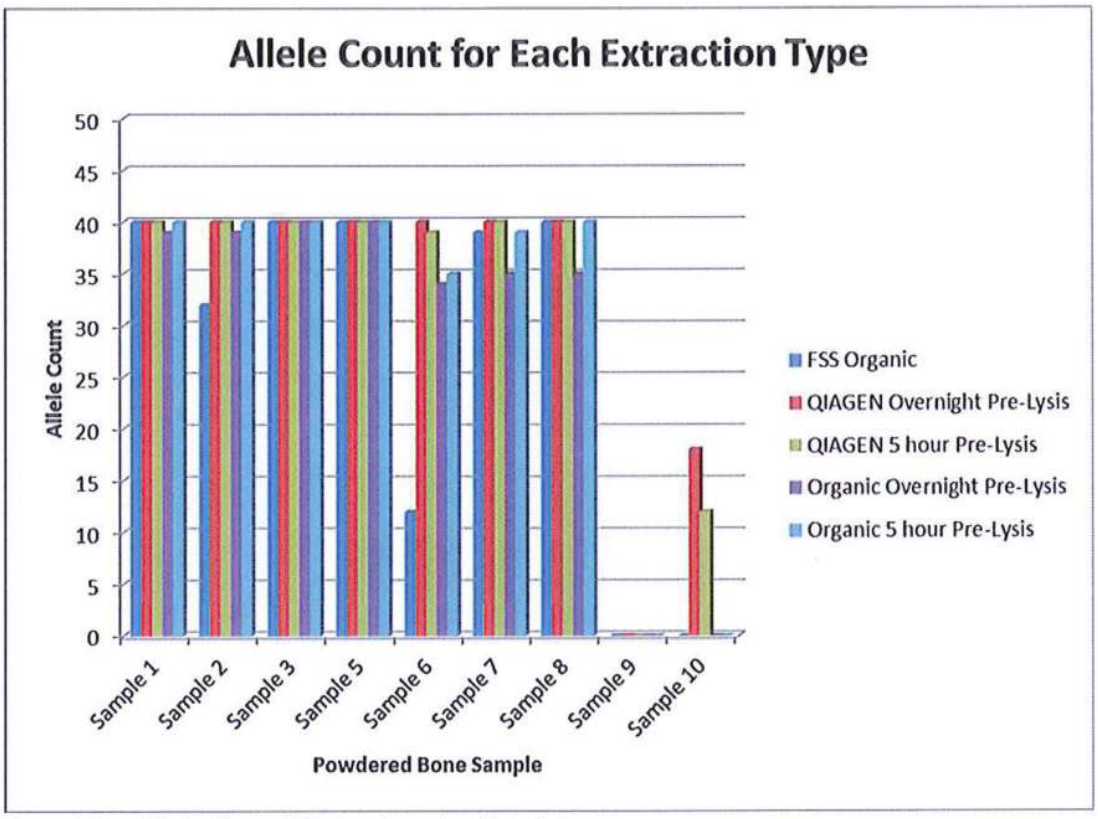


Graph 1: Quantification results for tested protocols



Graph 2: Quantification results for tested protocols

Graph 3 below provides a summary of the alleles obtained in final DNA profiles for each of the samples included in this validation.



Graph 3: Allele Count for each extraction type

Results showed that Organic Pre-Lysis with 5 hour and overnight incubations with QIAsymphony® SP extraction (Experiments 4 and 5) gave the lowest DNA quantification results when compared to other protocols. This may be due to incompatibility between the Organic Pre-Lysis reagents and QIAsymphony® reagents. These two protocols have therefore been excluded as possible options for implementation.

The QIAGEN 5 hour and/or overnight Pre-Lysis with QIAsymphony® SP extraction (Experiments 2 and 3) gave DNA quantification results which were comparable to or higher than the Organic extraction for Samples 1, 2, 5, 6, 7, 9 and 10. The QIAGEN protocols also gave the same or higher number of alleles in the final DNA profile:

- Sample 1 – all protocols gave full profiles (40 alleles)
- Sample 2 – both QIAGEN protocols (Experiments 2 and 3) gave full profiles (40 alleles), whereas the Organic extraction gave 32 alleles.
- Sample 5 - all protocols gave full profiles (40 alleles)
- Sample 6 – the QIAGEN overnight incubation (Experiment 2) gave a full profile (40 alleles) and the QIAGEN 5 hour (Experiment 3) gave 39 alleles, whereas the Organic extraction gave 12 alleles.
- Sample 7 – both QIAGEN protocols (Experiments 2 and 3) gave full profiles (40 alleles) and the Organic extraction gave 39 alleles.

- Sample 9 – both QIAGEN protocols (Experiments 2 and 3) and the Organic extraction gave undetermined quantification result and No DNA Profile final result.
- Sample 10 – the QIAGEN overnight incubation (Experiment 2) gave 18 alleles and the QIAGEN 5 hour (Experiment 3) gave 12 alleles, whereas the Organic extraction gave No DNA Profile.

The Organic extraction gave higher DNA quantification results than both the QIAGEN 5 hour and overnight Pre-Lysis with QIAsymphony® SP extraction (Experiments 2 and 3) for Samples 3 and 8. Of these samples, there was no difference in the number of alleles obtained in the final DNA profiles:

- Sample 3 - all protocols gave full profiles (40 alleles)
- Sample 8 - all protocols gave full profiles (40 alleles)

In addition to allele count data, profile quality (i.e. preferential amplification, degradation and peak morphology) were not noticeably different for the samples in Experiments 1, 2 and 3.

When comparing the quantification results for the two QIAGEN protocols (Experiments 2 and 3):

- The 5 hour pre-lysis incubation protocol (Experiment 3) gave higher DNA quantification results for Samples 1, 2, 3 and 7
- The 5 hour and overnight pre-lysis incubations gave similar DNA quantification results for Samples 5, 6, 8, 9 and 10.

When comparing the number of alleles in final DNA profiles for QIAGEN pre-lysis 5 hour and overnight incubations:

- Both the 5 hour pre-lysis and overnight incubations gave full DNA profiles (40 alleles) for Samples 1, 2, 3, 5, 7 and 8.
- The overnight pre-lysis incubation (Experiment 2) gave more alleles than the 5 hour incubation for Sample 6 (40 and 39 alleles respectively) and Sample 10 (18 and 12 alleles respectively).
- Both the 5 hour pre-lysis and overnight incubations gave No DNA Profile for Sample 9.

T-tests were conducted to compare the quantification results for the two QIAGEN and Organic extraction protocols (see Table 7 below). The T-test results show although there were differences between the Organic and QIAGEN protocols (overnight and 5 hour incubations), these differences were not statistically significant. Further, the T-test results also showed any differences between the two QIAGEN protocols (overnight and 5 hour incubations) were not significantly different.

Table 7: T-tests comparing QIAGEN and Organic extraction protocols

Comparison of Quantification Results	T-test
Organic Extraction – QIAGEN 5 hour Incubation	0.435044
Organic Extraction – QIAGEN Overnight Incubation	0.922513
QIAGEN Overnight – 5 hour Incubation	0.802843

The quantification results, final DNA profile allele counts and T-test results support the conclusion that the QIAGEN protocols (Experiments 2 and 3) performed as well as or better than the Organic extraction protocol (Experiment 1).

Conclusion

Overall, the QIAGEN protocols tested in Experiments 2 and 3 were shown to be comparable to or better than the current Organic extraction protocol (Experiment 1) in terms of DNA quantification and final profile allele count.

The protocols tested in Experiments 4 and 5 (Organic pre-lysis with QIAsymphony® SP extraction gave poorer quantifications results overall when compared to the results of Experiments 1, 2 and 3.

Implementation of either QIAGEN protocol (Experiments 2 and 3) would increase efficiency and throughput of bone extractions and also eliminate the use of the hazardous phenol chloroform isoamyl alcohol (used in Organic extractions).

Recommendations

It is recommended that:

- The protocols tested in Experiments 2 and 3 are both implemented and can be selected based on staffing and operational requirements.
- Organic extractions are ceased, QIS# 34039 archived and phenol chloroform isoamyl alcohol is disposed.
- Further validation is conducted to assess the ability to store lysates for up to 8 days.

References

Aguilera, M., Micic, B., Acedo, P., Ryan, L. and Allen, C. (2016) Validation of the QIAsymhpony SP/AS Modules [Final Report].

AK-25

Kylie Rika

From: Kylie Rika
Sent: Wednesday, 18 April 2018 3:29 PM
To: Justin Howes
Subject: Project 192

Hi Justin

I've been made aware of a bone sample that had four aliquots that gave quants of 0.025, 0.04, 0.03, 0.02 – all done via organic extn.

Another sample of bone from the same femur was taken and aliquots of four gave: 0.013, 0.0005, 0.00127, 0.00015 – via QIAsymphony.

This seemed like a big difference to me and made me think about looking at the project for bone symphony.

I noticed that for all 3 reports – plan, proposal and final report, I was the only mgmt. team member not included.

But looking at the final report today I notice that for sample 8 (which had similar quant to the first set of four above), the original organic extraction gave the best quant values cf. the 4 symphony protocols.

I guess I am worried that perhaps for samples in the quant range ~0.03, symphony may not be the best option

Symphony might be good for DVIs that are fresh but maybe not coronials?

Angelina has also shown me data that suggests a success rate of about 96% for obtaining profiles from bone/teeth when using phenol/organic.

I understand that getting rid of phenol is great for safety purposes but if it is the better option for bones/teeth perhaps we should keep it or do more testing of samples in the quant range around 0.03.

Thanks

Kylie Rika DipMgt PGradDipForensic BSc
Senior Scientist Reporting – Forensic DNA Analysis

Forensic & Scientific Services,
Health Support Queensland, **Department of Health**

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w | www.health.qld.gov.au e | [redacted]

AK-26

Queensland Health

HealthSupport Queensland

Project Report #192

Validation of QIAsymphony® SP for Bone Extraction

Supplementary Repeatability and Reproducibility

Melissa Cipollone, Luke Ryan, Megan Mathieson and Cathie Allen

March 2020
Version 2.0

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: Luke Ryan
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Version history

Version	Date	Changed by	Description
1.0	December 2019	Melissa Cipollone	Creation of Document
2.0	March 2020	Melissa Cipollone, Luke Ryan, Megan Mathieson	Document Feedback

Document sign off

This document has been **approved** by:

Name	Position	Signature	Date
Cathie Allen	Managing Scientist	[redacted]	24/03/2020

The following officers have **endorsed** this document

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Sharon Johnstone	Senior Scientist Reporting 1		23/03/2020
Kylie Rika	Senior Scientist Reporting 2		24/03/2020

Contents

Abstract

Forensic DNA Analysis currently uses an organic extraction for the extraction of DNA from bone and teeth. Phenol chloroform isoamyl alcohol is used in the organic extraction process and is a chemical hazard to the operator. The organic extraction process is time consuming and labour intensive. One organic extraction batch contains a maximum of 12 bone/teeth samples and takes an operator a full day to complete which is relatively inefficient and is the rate limiting step in the processing of bone/teeth samples in the Analytical Team.

Forensic DNA Analysis currently uses the QIAsymphony® SP instrument for automated DNA extraction of a range of substrate and sample types (QIS# 33758), but not for bones/teeth. QIAsymphony® SP DNA extractions can process up to 96 samples per batch, and it is possible for one operator to run up to two full runs of 96 samples in a day. QIAGEN have developed protocols for pre-lysis and on-deck protocols for bones/teeth and other casework samples which have been used as the basis for the protocols to be tested in this validation.

The purpose of this project was to conduct further repeatability and reproducibility experiments for the QIAsymphony® SP bone extraction using both the 5 hour and overnight pre-lysis protocols, and to compare these results to the current organic extraction protocol.

The results obtained from this experiment show the 5 hour and overnight pre-lysis QIAsymphony® extractions are comparable to the current organic extraction with the overnight pre-lysis QIAsymphony® protocol the preferred method for routine processing.

Introduction

Forensic DNA Analysis currently performs automated DNA extractions on a range of sample types and substrates using a QIAGEN® QIAsymphony® SP/AS instrument. The QIAsymphony® SP/AS instrument is a modular automated system which enables the processing of up to 96 samples on a single run. The QIAsymphony® SP module is used for the extraction and purification of DNA from forensic casework and reference samples. It uses pre-programmed optimized protocols and the QIAGEN® cartridge-based magnetic-particle chemistry kit, the QIAsymphony® DNA Investigator Kit.

The original validation of the QIAsymphony® SP/AS did not include bone or teeth extraction. Forensic DNA Analysis currently have two QIAsymphony® SP/AS instruments and the use of these instruments for bone/teeth extraction would be particularly beneficial in the event of a large scale disaster victim identification (DVI), as it will dramatically increase the efficiency and processing capacity of bone/teeth DNA extractions. Furthermore, organic extraction involves the use of phenol chloroform isoamyl alcohol which is a chemical hazard, therefore implementing an alternative protocol would remove this hazard.

Processing bone extractions on the QIAsymphony® SP would also provide benefits and efficiencies to training and maintenance of competency. The low numbers of routinely submitted bones/teeth make initial training, and subsequent maintenance of competency, lengthy and difficult to coordinate. Extraction of bones/teeth on the QIAsymphony® would be included in the standard QIAsymphony® casework training module, and not a separate organic extraction competency as it currently is.

Following the completion of the first validation experiments it was decided additional repeatability and reproducibility experiments were required. The following experiments were performed to test and compare repeatability and reproducibility of three extraction protocols:

- Repeatability Experiment:
  - Current organic extraction
  - QIAGEN pre-lysis with overnight incubation and QIAsymphony® SP extraction
  - QIAGEN pre-lysis with 5 hour incubation and QIAsymphony® SP extraction
- Reproducibility Experiment over 5 days:
  - Current organic Extraction
  - QIAGEN pre-lysis with overnight incubation and QIAsymphony® SP extraction
  - QIAGEN pre-lysis with 5 hour incubation and QIAsymphony® SP extraction

Resources and Methods

All reagents, materials and equipment used in this project were as specified in the approved in-house document Project #192 Validation of QIAsymphony® Bone Extraction - Supplementary R&R. This document will be referred to as the experimental design.

All samples used in this verification were selected, analysed and interpreted as outlined in the experimental design.

Sample Selection

Five powdered bone samples were retained from the Freezer Mill Project #209.

Bone Sample	Laboratory Number
Bone 1	[redacted]
Bone 2	
Bone 3	
Bone 4	
Bone 5	

(*Exhibit registered in Auslab)

Table 1: Bone samples used in this Validation

Experiments and Results

Experiment 1 – Repeatability

Purpose

The purpose of the repeatability experiment was to extract human genomic DNA from powdered bone using three different extraction methods and compare the results. The compared methods were:

- The current validated method of extracting DNA from bone and teeth using organic extraction.
- The QIAGEN pre-lysis method with the samples being incubated for 5 hours only and then extracted on the QIAsymphony® SP instrument.
- The QIAGEN pre-lysis method with the samples being incubated overnight and then extracted on the QIAsymphony® SP instrument.

Results

Tabulated results are provided in Appendices 1-5. The repeatability quantification results for bones 1-5 are shown in Figures 1-5. The number of alleles obtained for bones 1 to 5 are shown in Figure 6. It should be noted the allele count for some samples were obtained after a microcon concentration procedure (refer to tabulated results in Appendices 1-5).

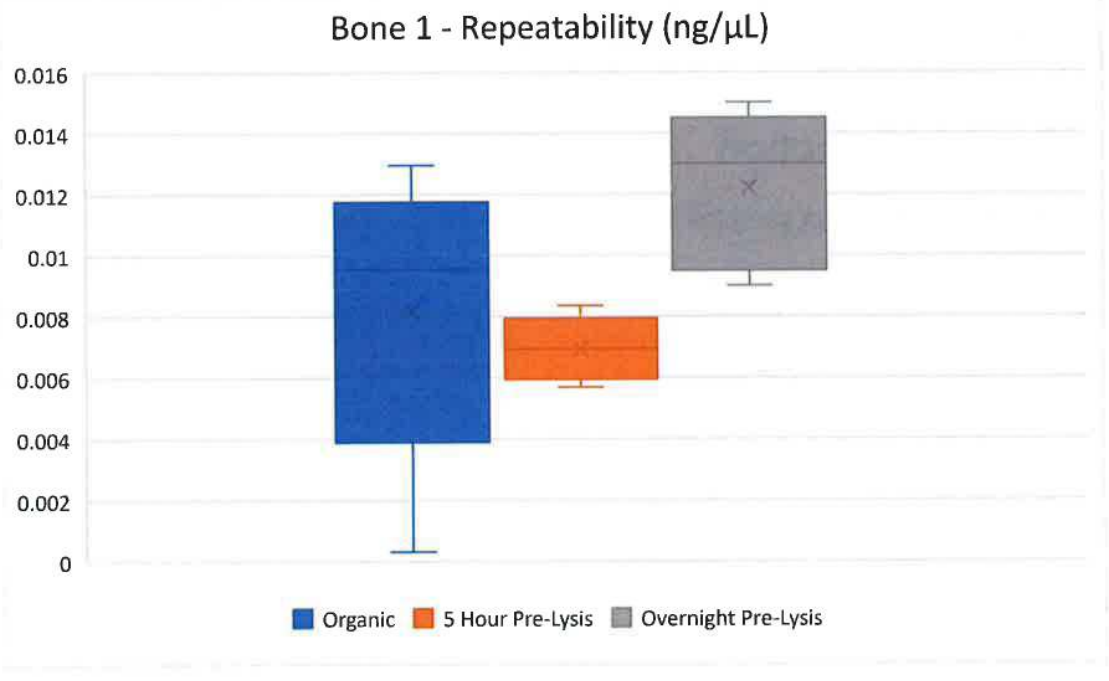

Figure 1: Representation of repeatability data for Bone 1 using Quant Values

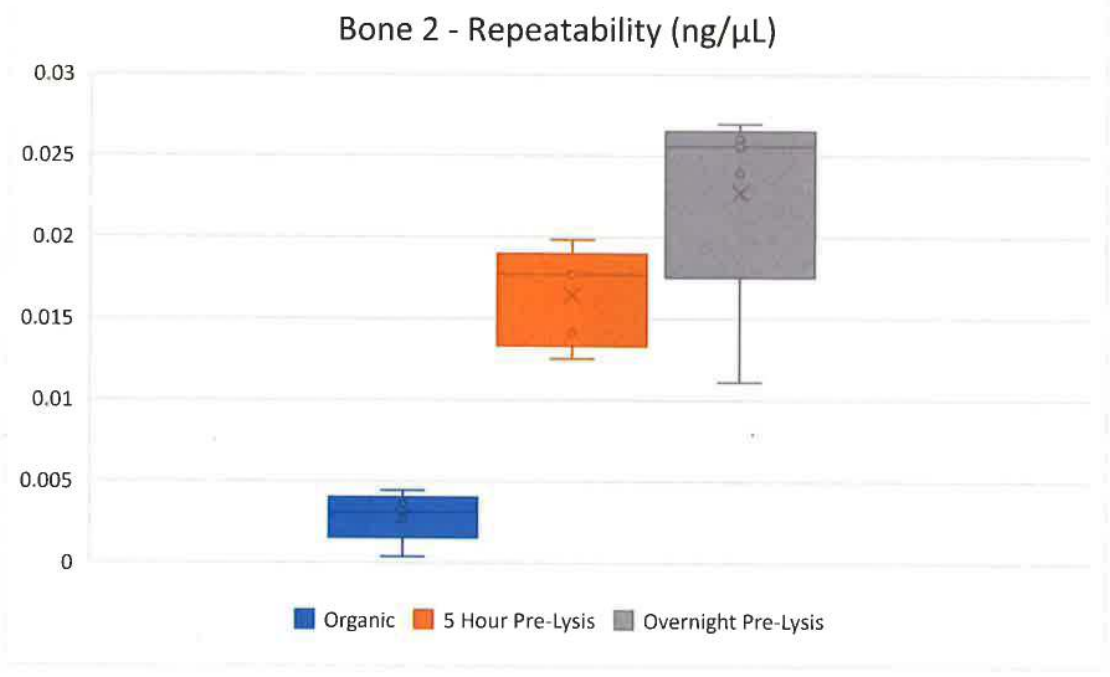

Figure 2: Representation of repeatability data for Bone 2 using Quant Values

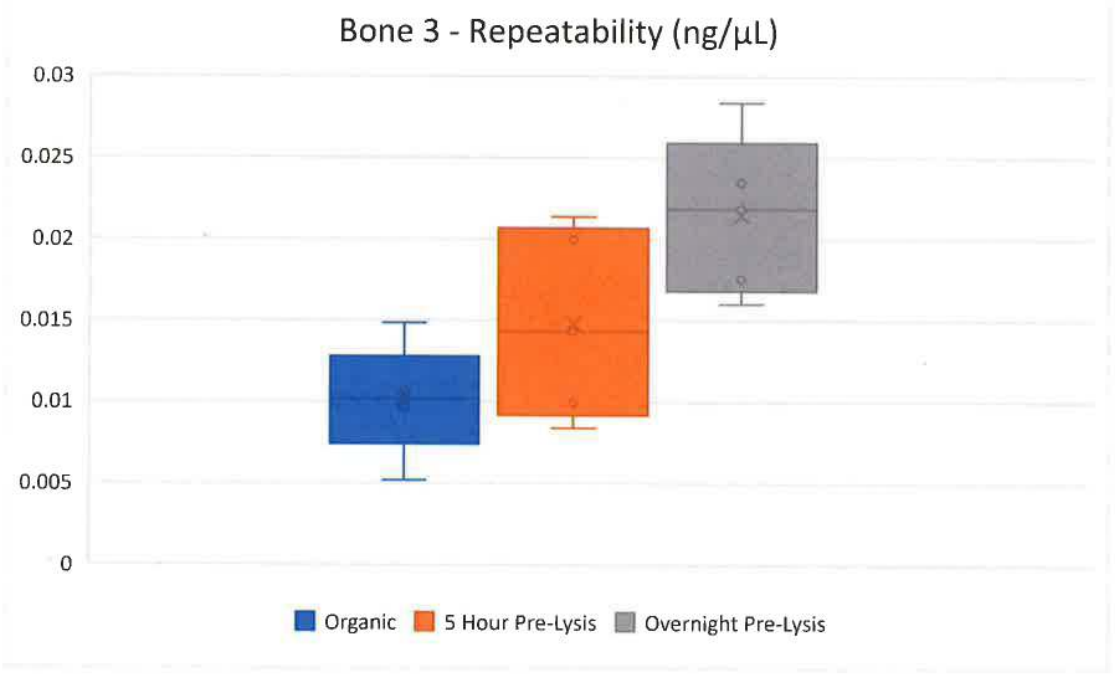

Figure 3: Representation of repeatability data for Bone 3 using Quant Values

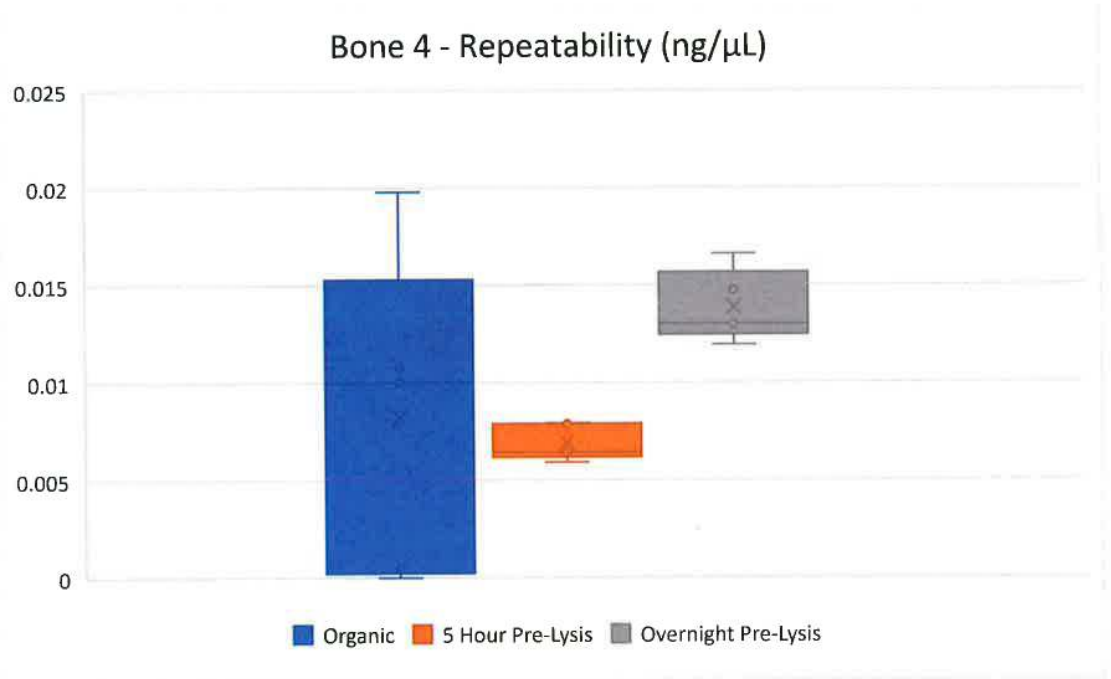

Figure 4: Representation of repeatability data for Bone 4 using Quant Values

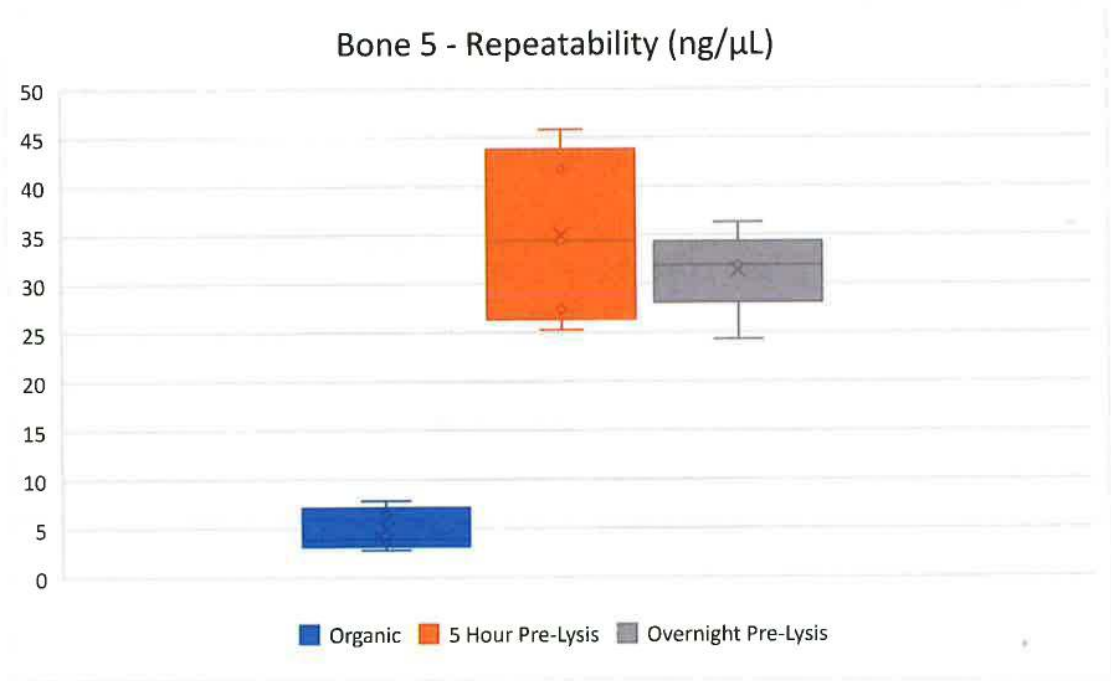

Figure 5: Representation of repeatability data for Bone 5 using Quant Values

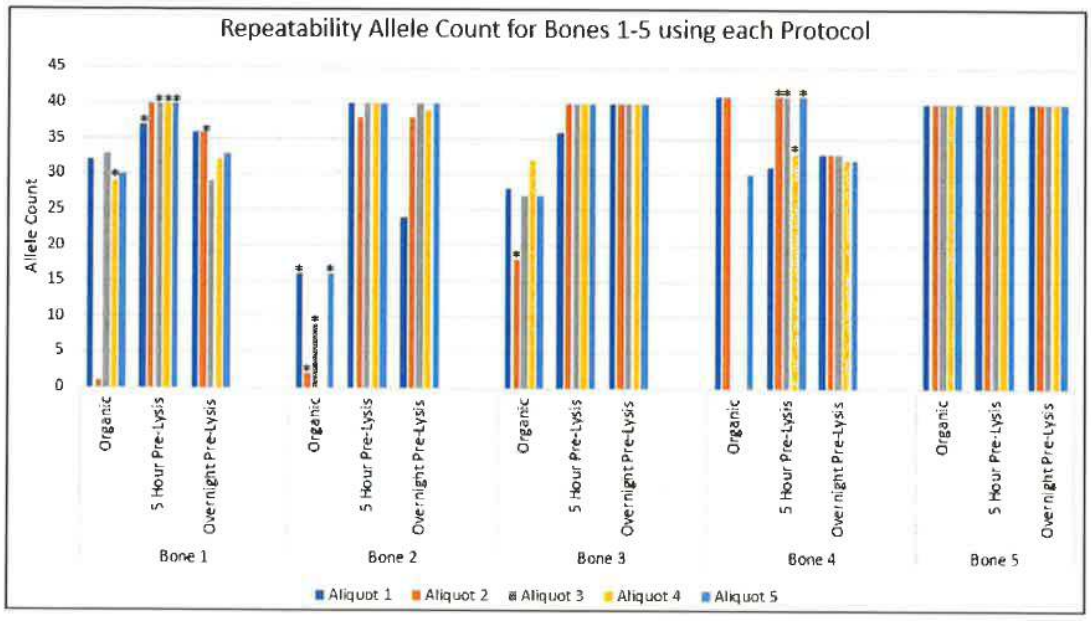

Figure 6: Representation of repeatability data for Bones 1-5 using Allele counts. * indicates samples which have undergone microcon concentration.

Discussion

The results from the current validated method (organic extraction) were used as a benchmark to compare the results from the QIAsymphony® protocols.

Repeatability for each extraction protocol varied between samples with no apparent consistency or trend. The organic and both QIAsymphony® protocols appeared to have a comparable level of repeatability with no one protocol being more or less repeatable consistently across the 5 samples (refer to Figures 1 - 5).

The quantification results for bones 1-4 were lower than bone 5 for all extraction protocols. This is likely due to the quality of the bone samples given the consistency across each of the three extraction protocols.

The overnight pre-lysis QIAsymphony® extraction gave higher quantification results for each of the 5 replicates of bones 2, 3 and 5 (as per Figures 2, 3 and 5) than the organic extraction. For bones 1 and 4, the mean quantification results (across the 5 replicates) were higher for the overnight pre-lysis QIAsymphony® than the organic extraction (as per Figures 1 and 4).

The QIAsymphony® extraction with 5 hour pre-lysis gave higher mean quantification results (across the 5 replicates) than the organic extraction for bones 2, 3 and 5. For bones 1 and 4, although the mean quantification result was lower for the QIAsymphony® extraction, quantification results overall overlapped and were comparable. It should be noted for bones 1 and 4, the range of results for the organic extraction were much wider than the QIAsymphony® extraction with 5 hour pre-lysis, which meant that although some organic replicates gave higher quantification results, some also gave lower quantification results.

Sample extracts quantified in the range 0.001-0.0088 ng/µL underwent microcon concentration prior to amplification to mimic real processing conditions. As stated

previously, bones 1-4 gave low quantification results which resulted in a number of samples undergoing microcon concentration. Across all samples tested, 6 organic extraction samples and 8 QIAsymphony® 5 hour pre-lysis extraction samples underwent microcon concentration. No QIAsymphony® overnight pre-lysis samples underwent microcon concentration (see Appendices 1 – 5). Given the final DNA profile results include samples which have and have not undergone microcon concentration, the final profile and allele count results (refer to Figure 6) have only been used to assess any negative impact the extraction protocols may have had on profile quality. No negative impact on profile quality was noted for any of the extraction protocols.

Overall this repeatability experiment has shown that the organic and both QIAsymphony® protocols are comparable, with the overnight lysis generally giving higher quantification results than the 5 hour lysis. This fits with intuitive expectations as increased reaction time could be expected to give higher yields.

Experiment 2 - Reproducibility

Purpose

The purpose of the reproducibility experiment is to test the reproducibility of results from each extraction protocol when performed by five independent scientists. One aliquot from each sample was tested per protocol for the reproducibility experiments (75 aliquots in total not including controls).

The compared methods were done over a 5 day period by 5 different operators:

- Current organic extraction
- The QIAGEN pre-lysis method with the samples being incubated for 5 hours only and then extracted on the QIAsymphony® SP instrument.
- The QIAGEN pre-lysis method with the samples being incubated overnight and then extracted on the QIAsymphony® SP instrument.

The five independent analytical scientists who conducted each of the reproducibility experiments are:

1	Scientist 1
2	Scientist 2
3	Scientist 3
4	Scientist 4
5	Scientist 5

Table 2: The five independent scientists used for the reproducibility validation

Results

Tabulated results are provided in Appendices 6-8. The reproducibility quantification results for bones 1-5 are shown in figures 7-11. The number of alleles obtained for bones 1-5 are shown in Figure 12. It should be noted the allele count for some samples were obtained after a microcon concentration procedure (refer to tabulated results in Appendices 6-8).

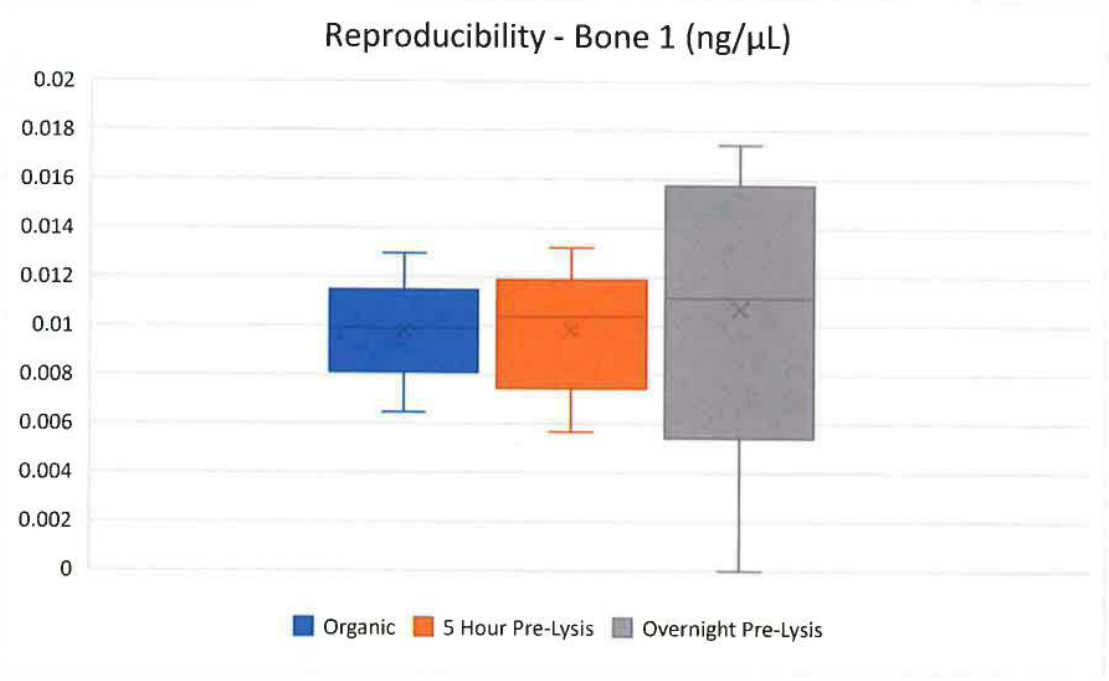

Figure 7: Reproducibility results for Bone 1 using each protocol and quant values

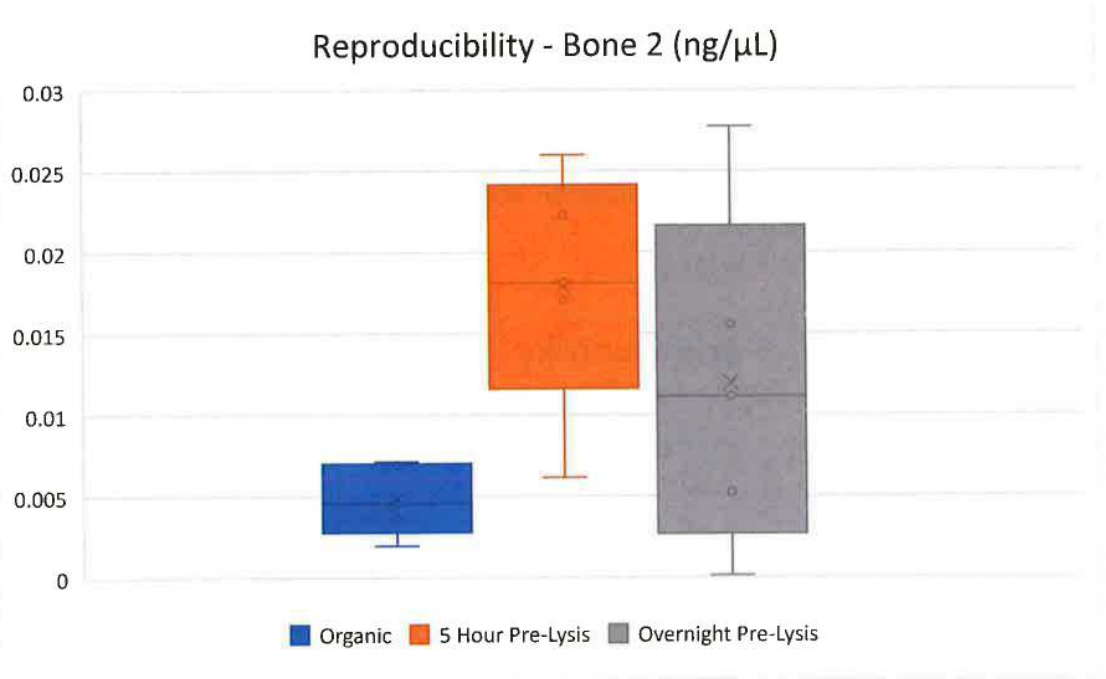

Figure 8: Reproducibility results for Bone 2 using each protocol and quant values

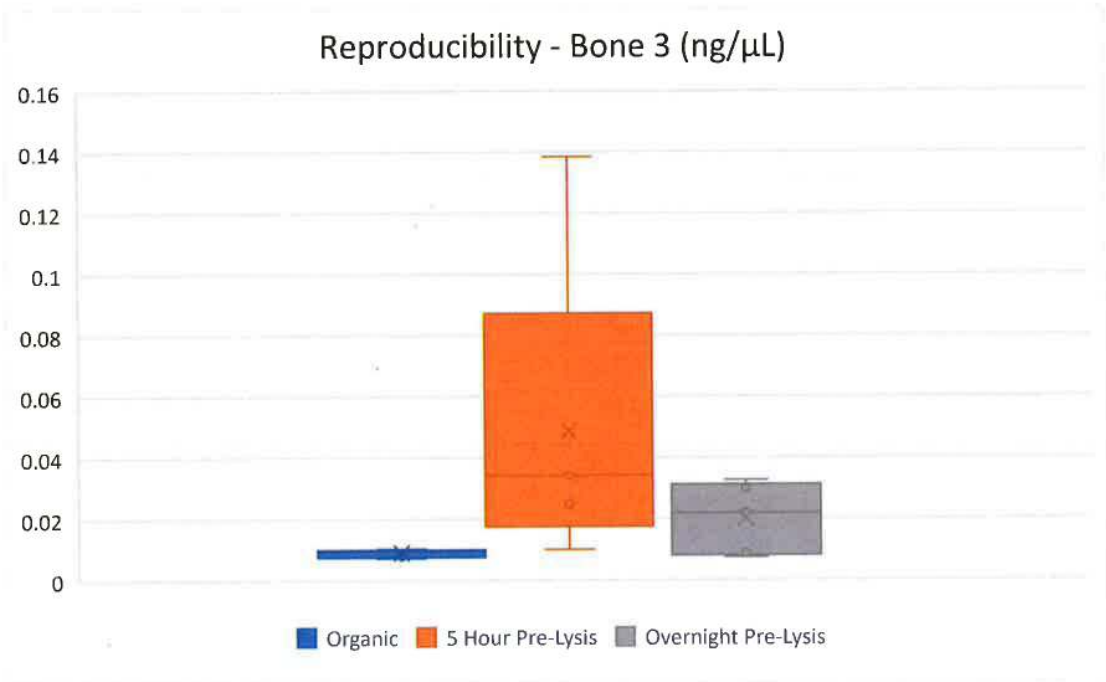

Figure 9: Reproducibility results for Bone 3 using each protocol and quant values

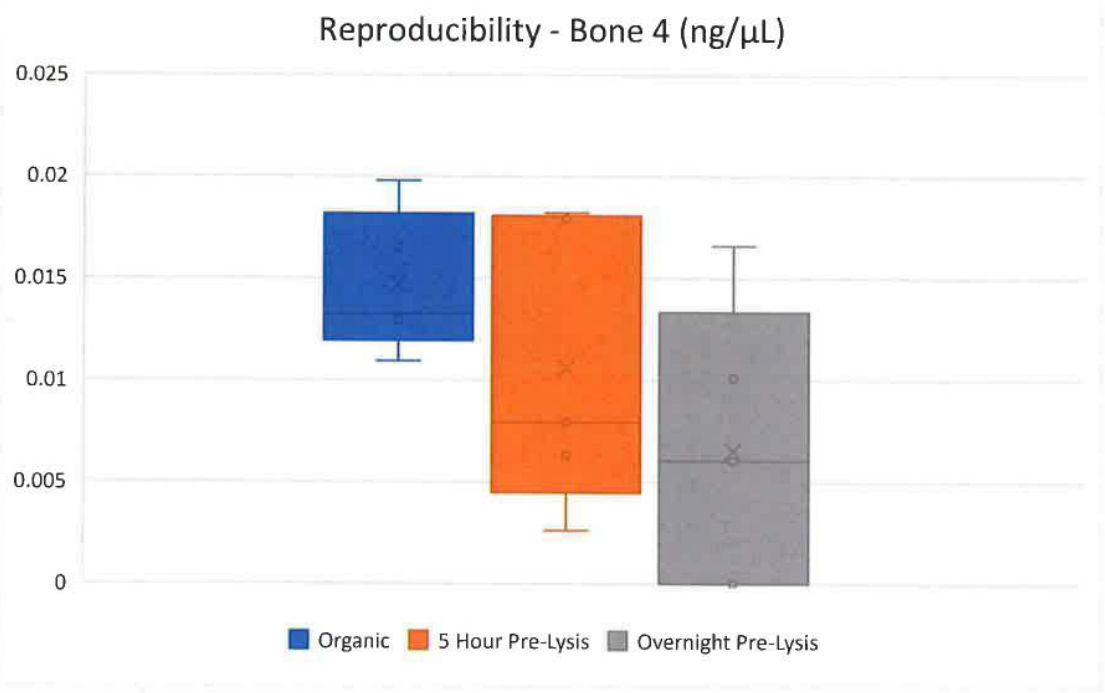

Figure 10: Reproducibility results for Bone 4 using each protocol and quant values

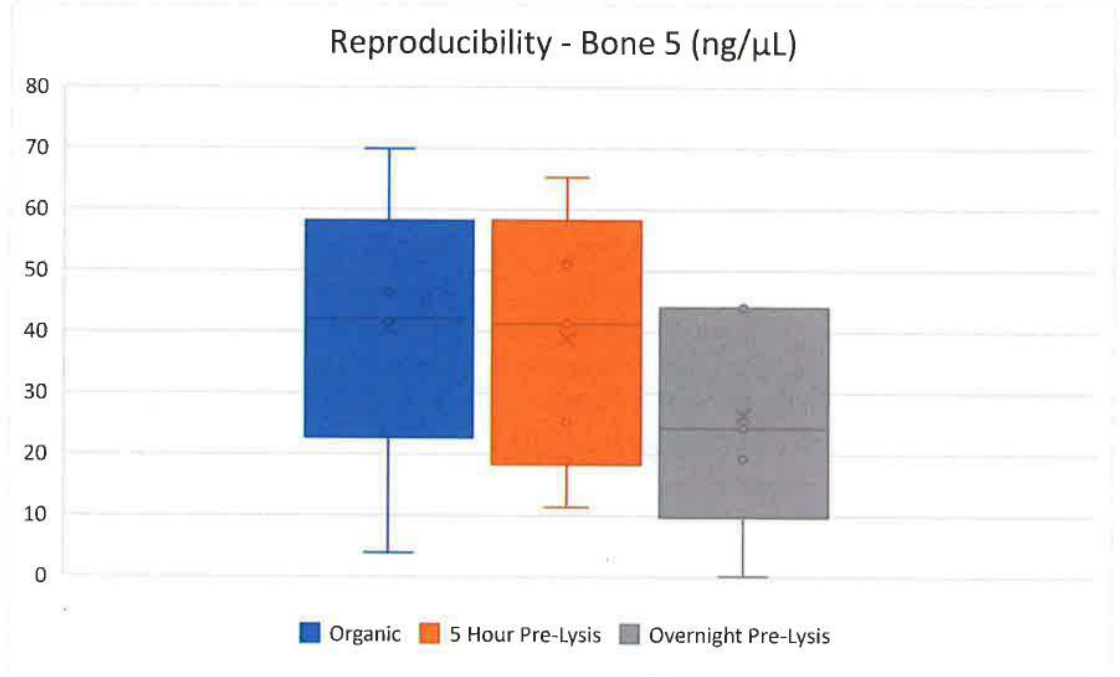

Figure 11: Reproducibility results for Bone 5 using each protocol and quant values

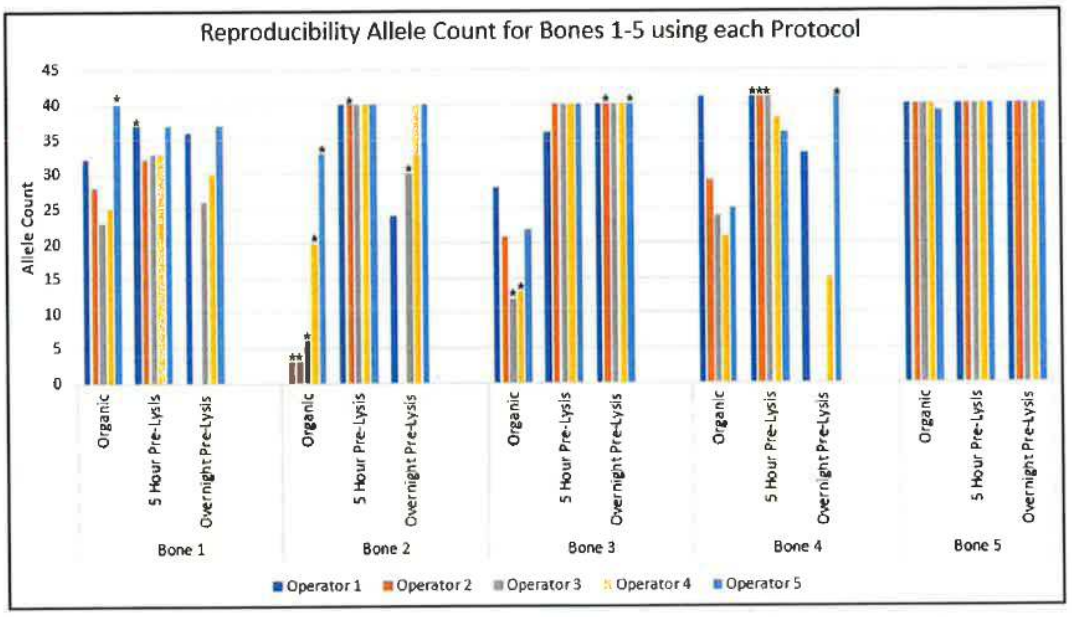

Figure 12: Reproducibility results for Bones 1-5 using allele counts for each protocol. * indicates samples which have undergone microcon concentration.

Discussion

Similar to repeatability, the reproducibility for each extraction protocol varied between samples with no apparent consistency or trend. No one protocol appeared to be more or less reproducible with consistency across the 5 samples. The organic and both QIAsymphony® protocols appeared to have a comparable level of reproducibility.

As with repeatability, the quantification results for bones 1-4 were lower than bone 5 for all extraction protocols. This is likely due to the quality of the bone samples given the consistency across each of the three extraction protocols.

For bones 1, 2 and 3, both QIAsymphony® protocols gave higher mean quantification results (across the 5 operators) than the organic extraction. For Bone 4, the organic protocol gave high average quantification results than both QIAsymphony protocols. The maximum quantification results were comparable across the three protocols protocols (0.01979, 0.01821 and 0.01660 ng/µL for organic, QIAsymphony 5 hour pre-lysis and respectively) however both QIAsymphony protocols gave more samples with lower quantification results (when compared to the Organic protocol). This was particularly evident for the overnight protocol, where two replicates gave a zero quantification result. This may be a sample specific issue as this trend was not replicated in the other bones.

For bone 5, the organic and QIAsymphony® with 5 hour pre-lysis gave comparable results, while the QIAsymphony® overnight pre-lysis extraction gave a lower mean quantification result.

Sample extracts quantified in the range 0.001-0.0088 ng/µL underwent microcon concentration prior to amplification to mimic real processing conditions. Bones 1-4 gave low quantification results which resulted in a number of samples undergoing microcon concentration. Across all samples tested, 7 organic, 5 QIAsymphony® 5 hour pe-lysis and 4 QIAsymphony® overnight lysis samples underwent microcon

concentration (see Appendices 6 - 8 for details of specific samples). Given the final DNA profile results include samples which have and have not undergone microcon concentration, the final profile and allele count results have been used only to assess any negative impact the extraction protocols may have had on profile quality. No negative impact on profile quality was noted for any of the extraction protocols.

Overall this experiment showed the QIAGEN protocols using either the 5 hour or overnight incubations gave DNA quantification results which were comparable to the organic extraction.

Additional Analysis – IPCCT

Purpose

To provide comparative analysis of IPCCT results for the tested bone extraction protocols. The compared methods were:

- The current validated method of extracting DNA from bone and teeth using organic extraction.
- The QIAGEN pre-lysis method with the samples being incubated for 5 hours only and then extracted on the QIAsymphony® SP instrument.
- The QIAGEN pre-lysis method with the samples being incubated overnight and then extracted on the QIAsymphony® SP instrument.

Results

Raw data IPCCT results can be located in the Change Management folder (I:\Change Management\Proposal#192 - QIAsymphony Bone Extraction\Supplementary R&R\Results – Supp R&R.xls). Figures 13-17 below contain the IPCCT results for bones 1-5.

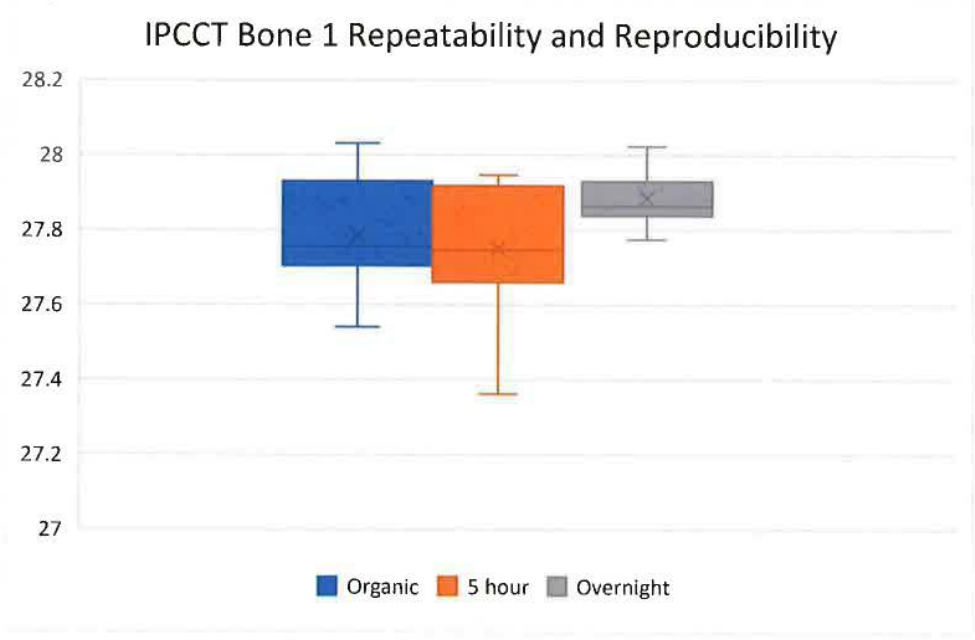

Figure 13: IPCCT results for Bone 1 – Combined Repeatability and Reproducibility data.

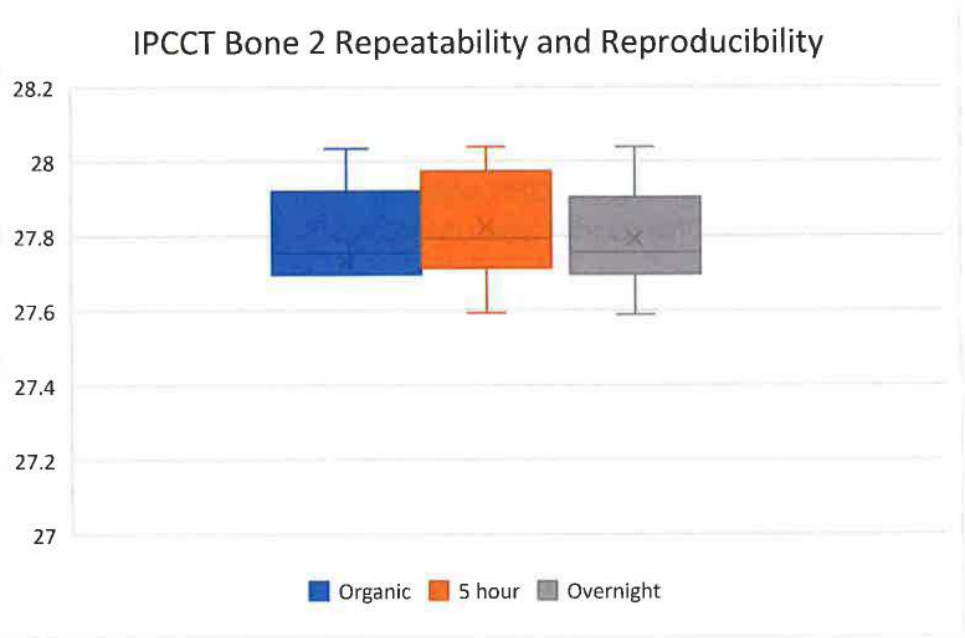

Figure 14: IPCCT results for Bone 2 – Combined Repeatability and Reproducibility data.

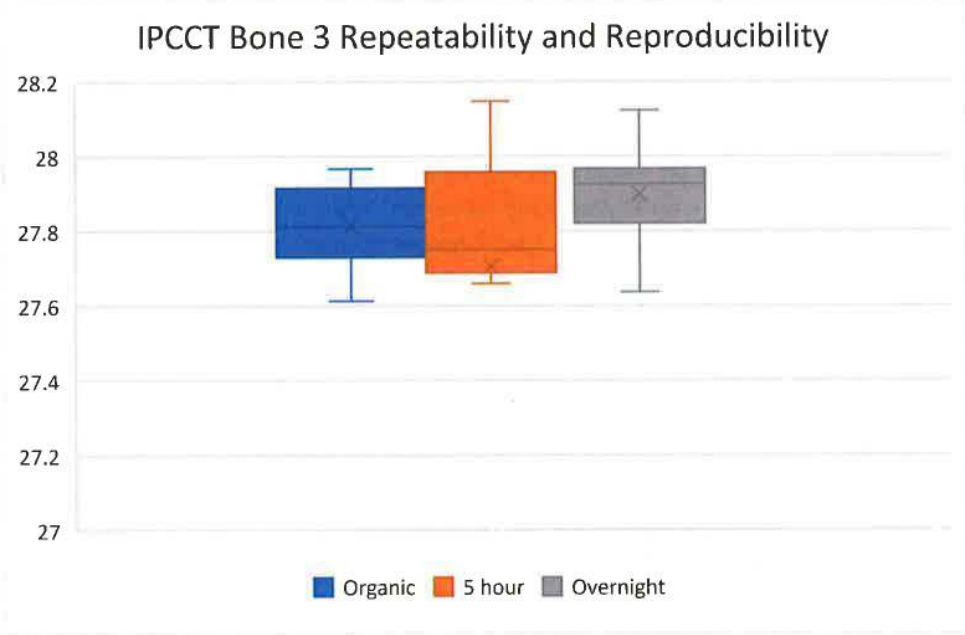

Figure 15: IPCCT results for Bone 3 – Combined Repeatability and Reproducibility data.

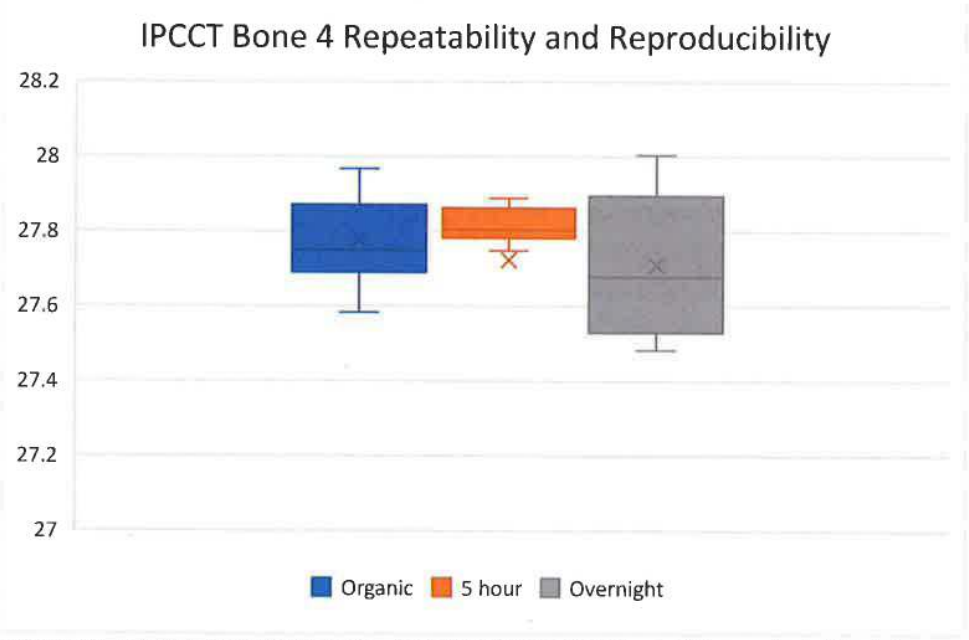

Figure 16: IPCCT results for Bone 4 – Combined Repeatability and Reproducibility data.

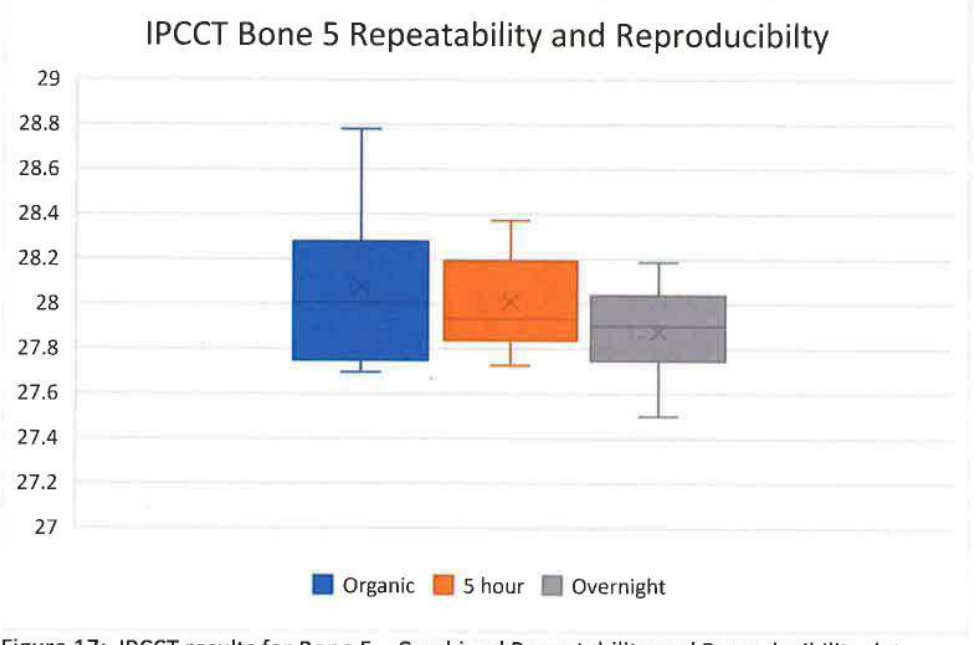

Figure 17: IPCCT results for Bone 5 – Combined Repeatability and Reproducibility data.

Discussion

The combined repeatability and reproducibility IPCCT results for bones 1-5 were compared across the three extraction protocols. Bones 1-5 showed comparable IPCCT results across each of the three tested protocols, with no indication of inhibition.

Conclusion and Recommendations

Overall the results of these additional experiments have shown that the QIAsymphony SP extraction with both 5 hour and overnight pre-lysis produce comparable DNA yields and repeatability and reproducibility to the current organic extraction. There was some evidence that the overnight pre-lysis produced higher DNA yields than the 5 hour pre-lysis and this fits with intuitive expectations given the longer reaction time. 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.

As noted in the discussion, sample extracts quantified in the range 0.001-0.0088 ng/µL underwent microcon concentration. Samples underwent microcon concentration 13 times for the organic extraction, 8 times for the QIAsymphony 5 hour pre-lysis protocol and 4 times for the QIAsymphony overnight pre-lysis protocol. This indicates the QIAsymphony overnight pre-lysis protocol gave extracts with less samples in the 0.001-0.0088ng/µL microcon concentration range.

In addition to workflow efficiency improvements, implementation of the QIAsymphony for bone extraction also improves occupational health and safety for staff by removing the use of phenol chloroform in the organic extraction.

It is therefore recommended that:

- The DNA extraction of bones on the QIAsymphony SP is implemented as a replacement for organic extraction.
- The organic extraction SOP is archived.
- The overnight pre-lysis is used for routine, non-time critical bone processing given the evidence of higher DNA yields.
- The 5 hour pre-lysis protocol is considered for use where
    - there is a large number of samples and/or where time critical processing is required (i.e. for DVIs), or
    - samples are expected to provide good DNA yields and there is sufficient material for retesting if required.

References

Aguilera, M., Micic, B., Acedo, P., Ryan, L. and Allen, C. (2016) Validation of the QIAsymphony6® SP/AS Modules [Final Report].

- QIS 34039 Extracting DNA from Bone and Teeth
- QIS 34045 Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit
- QIS 34052 Amplification of Extracted DNA using the PowerPlex®21 System
- QIS 34112 STR fragment analysis of PowerPlex® 21 profiles using GeneMapper® ID-X software
- QIS 34131 Capillary Electrophoresis Quality (CEQ) Check
- QIS 34132 DNA Extraction and Quantification of samples using the QIAsymphony® SP and AS – FR

Appendix 1 - Table of Results: Bone 1

Bone	Method	Barcode	Quant Value ng/µL	Rework	Allele Count
1	Organic	[redacted]	0.01296		32
1	Organic		0.00032		1
1	Organic		0.00747	Microcon	33
1	Organic		0.01054		29
1	Organic		0.00956		30
1	Pre-Lysis 5 hour		0.00568	Microcon	37
1	Pre-Lysis 5 hour		0.00835		40
1	Pre-Lysis 5 hour		0.00694	Microcon	40
1	Pre-Lysis 5 hour		0.00619	Microcon	40
1	Pre-Lysis 5 hour		0.00754	Microcon	40
1	Pre-Lysis Overnight		0.014		36
1	Pre-Lysis Overnight		0.015		36
1	Pre-Lysis Overnight		0.009		29
1	Pre-Lysis Overnight		0.010		32
1	Pre-Lysis Overnight		0.013		33

Table 3: Repeatability for Bone 1 using the three different methods tested including the Organic Extraction, the QIAsymphony® Pre-Lysis (5 hour) and Pre-Lysis (Overnight incubation) and QIAsymphony® SP Extraction.

Appendix 2 - Table of Results: Bone 2

Bone	Method	Barcode	Quant Value ng/µL	Rework	Allele Count
2	Organic	[redacted]	0.00361	Microcon	16
2	Organic		0.00274	Microcon	2
2	Organic		0.00311	Microcon	9
2	Organic		0.00038		0
2	Organic		0.00444	Microcon	16
2	Pre-Lysis 5 hour		0.01811		40
2	Pre-Lysis 5 hour		0.0177		38
2	Pre-Lysis 5 hour		0.01412		40
2	Pre-Lysis 5 hour		0.01253		40
2	Pre-Lysis 5 hour		0.01984		40
2	Pre-Lysis Overnight		0.01112		24
2	Pre-Lysis Overnight		0.02394		38
2	Pre-Lysis Overnight		0.02606		40
2	Pre-Lysis Overnight		0.02558		39
2	Pre-Lysis Overnight		0.02697		40

Table 4: Repeatability for Bone 2 using the three different methods tested including the Organic Extraction, the QIAsymphony® Pre-Lysis (5 hour) and Pre-Lysis (Overnight incubation) and QIAsymphony® SP Extraction

Appendix 3 - Table of Results: Bone 3

Bone	Method	Barcode	Quant Value ng/µL	Rework	Allele Count
3	Organic	[redacted]	0.00965		28
3	Organic		0.00520	Microcon	18
3	Organic		0.01075		27
3	Organic		0.01485		32
3	Organic		0.01017		27
3	Pre-Lysis 5 hour		0.00996		36
3	Pre-Lysis 5 hour		0.00840		40
3	Pre-Lysis 5 hour		0.02136		40
3	Pre-Lysis 5 hour		0.01434		40
3	Pre-Lysis 5 hour		0.01998		40
3	Pre-Lysis Overnight		0.02185		40
3	Pre-Lysis Overnight		0.01757		40
3	Pre-Lysis Overnight		0.02838		40
3	Pre-Lysis Overnight		0.02348		40
3	Pre-Lysis Overnight		0.01603		40

Table 5: Repeatability for Bone 3 using the three different methods tested including the Organic Extraction, the QIAsymphony® Pre-Lysis (5 hour) and Pre-Lysis (Overnight incubation) and QIAsymphony® SP Extraction

Appendix 4 - Table of Results: Bone 4

Bone	Method	Barcode	Quant Value ng/µL	Rework	Allele Count
4	Organic	[redacted]	0.01979		41
4	Organic		0.01077		41
4	Organic		0.00044		0
4	Organic		0.0000		0
4	Organic		0.00999		30
4	Pre-Lysis 5 hour		0.00792		31
4	Pre-Lysis 5 hour		0.00643	Microcon	41
4	Pre-Lysis 5 hour		0.00787	Microcon	41
4	Pre-Lysis 5 hour		0.00591	Microcon	33
4	Pre-Lysis 5 hour		0.00643	Microcon	41
4	Pre-Lysis Overnight		0.01660		33
4	Pre-Lysis Overnight		0.01300		33
4	Pre-Lysis Overnight		0.01471		33
4	Pre-Lysis Overnight		0.01193		32
4	Pre-Lysis Overnight		0.01296		32

Table 6: Repeatability for Bone 4 using the three different methods tested including the Organic Extraction, the QIAsymphony® Pre-Lysis (5 hour) and Pre-Lysis (Overnight incubation) and QIAsymphony® SP Extraction

Appendix 5 - Table of Results: Bone 5

Bone	Method	Barcode	Quant Value ng/µL	Rework	Rework Barcode	Allele Count
5	Organic	[redacted]	3.90037			40
5	Organic		7.82573	Dilution	[redacted]	40
5	Organic		2.79068			40
5	Organic		3.52519			40
5	Organic		6.41383	Dilution		40
5	Pre-Lysis 5 hour		25.25375	Dilution		40
5	Pre-Lysis 5 hour		27.41910	Dilution		40
5	Pre-Lysis 5 hour		45.80926	Dilution		40
5	Pre-Lysis 5 hour		41.76097	Dilution		40
5	Pre-Lysis 5 hour		34.41719	Dilution		40
5	Pre-Lysis Overnight		24.29558	Dilution		40
5	Pre-Lysis Overnight		31.82610	Dilution		40
5	Pre-Lysis Overnight		31.86150	Dilution		40
5	Pre-Lysis Overnight		32.42724	Dilution		40
5	Pre-Lysis Overnight		36.27103	Dilution		40

Table 7: Repeatability for Bone 5 using the three different methods tested including the Organic Extraction, the QIAsymphony® Pre-Lysis (5 hour) and Pre-Lysis (Overnight incubation) and QIAsymphony® SP Extraction

Appendix 6 - Reproducibility Table of Results for Organic Extraction

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	Allele Count
Day 1	Scientist 1	1	[redacted]	0.01296		32
Day 2	Scientist 2	1		0.00999		28
Day 3	Scientist 3	1		0.00990		23
Day 4	Scientist 4	1		0.00974		25
Day 5	Scientist 5	1		0.00647	Microcon	40

Table 8: Reproducibility results for the Current Organic Extraction for Bone 1

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	Allele Count
Day 1	Scientist 1	2	[redacted]	0.00361	Microcon	3
Day 2	Scientist 2	2		0.00196	Microcon	3
Day 3	Scientist 3	2		0.00458	Microcon	6
Day 4	Scientist 4	2		0.00713	Microcon	20
Day 5	Scientist 5	2		0.00692	Microcon	33

Table 9: Reproducibility results for the Current Organic Extraction for Bone 2

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	Allele Count
Day 1	Scientist 1	3	[redacted]	0.00965		28
Day 2	Scientist 2	3		0.00953		21
Day 3	Scientist 3	3		0.00785	Microcon	12
Day 4	Scientist 4	3		0.00723	Microcon	13
Day 5	Scientist 5	3		0.01027		22

Table 10: Reproducibility results for the Current Organic Extraction for Bone 3

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	Allele Count
Day 1	Scientist 1	4	[redacted]	0.01979		41
Day 2	Scientist 2	4		0.01093		29
Day 3	Scientist 3	4		0.01657		24
Day 4	Scientist 4	4		0.01294		21
Day 5	Scientist 5	4		0.01324		25

Table 11: Reproducibility results for the Current Organic Extraction for Bone 4

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	Rework Barcode	Allele Count
Day 1	Scientist 1	5	[redacted]	3.90037			40
Day 2	Scientist 2	5		69.92214	Dilution	714140860	40
Day 3	Scientist 3	5		42.16170	Dilution	720478242	40
Day 4	Scientist 4	5		46.41128	Dilution	714153578	40
Day 5	Scientist 5	5		41.47680	Dilution	714153589	39

Table 12: Reproducibility results for the Current Organic Extraction for Bone 5

Appendix 7 - Reproducibility Table of Results for the QIAsymphony Pre-Lysis (5 hour incubation)

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	Rework Barcode	Allele Count
Day 1	Scientist 1	1	[redacted]	0.00568	Microcon		37
Day 2	Scientist 2	1		0.00922			32
Day 3	Scientist 3	1		0.01038			33
Day 4	Scientist 4	1		0.01321			33
Day 5	Scientist 5	1		0.01056			37

Table 13: Reproducibility results for the QIAsymphony® Pre-Lysis (5 hour incubation) and QIAsymphony® SP Extraction for Bone 1

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	Rework Barcode	Allele Count
Day 1	Scientist 1	2	[redacted]	0.01811			40
Day 2	Scientist 2	2		0.00614	Microcon		40
Day 3	Scientist 3	2		0.02598			40
Day 4	Scientist 4	2		0.02227			40
Day 5	Scientist 5	2		0.01705			40

Table 14: Reproducibility results for the QIAsymphony® Pre-Lysis (5 hour incubation) and QIAsymphony® SP Extraction for Bone 2

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	Rework Barcode	Allele Count
Day 1	Scientist 1	3	[redacted]	0.00996			36
Day 2	Scientist 2	3		0.1384			40
Day 3	Scientist 3	3		0.02495			40
Day 4	Scientist 4	3		0.03412			40
Day 5	Scientist 5	3		0.03583			40

Table 15: Reproducibility results for the QIAsymphony® Pre-Lysis (5 hour incubation) and QIAsymphony® SP Extraction for Bone 3

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	Rework Barcode	Allele Count
Day 1	Scientist 1	4	[redacted]	0.00792	Microcon		41
Day 2	Scientist 2	4		0.00594	Microcon		41
Day 3	Scientist 3	4		0.00632	Microcon		41
Day 4	Scientist 4	4		0.01793			38
Day 5	Scientist 5	4		0.01821			36

Table 16: Reproducibility results for the QIAsymphony® Pre-Lysis (5 hour incubation) and QIAsymphony® SP Extraction for Bone 4

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	Rework Barcode	Allele count
Day 1	Scientist 1	5	[redacted]	25.25375	Dilution	714153523	40
Day 2	Scientist 2	5		11.38112	Dilution	718880513	40
Day 3	Scientist 3	5		41.33673	Dilution	718880491	40
Day 4	Scientist 4	5		51.12676	Dilution	718880541	39
Day 5	Scientist 5	5		65.29295	Dilution	718880530	40

Table 17: Reproducibility results for the QIAsymphony® Pre-Lysis (5 hour incubation) and QIAsymphony® SP Extraction for Bone 5

Appendix 8 - Reproducibility Table of Results for the QIAsymphony Pre-Lysis (Overnight incubation)

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	Allele count
Day 1	Scientist 1	1	[redacted]	0.01409		36
Day 2	Scientist 2	1		0.00000		0
Day 3	Scientist 3	1		0.01114		26
Day 4	Scientist 4	1		0.01741		30
Day 5	Scientist 5	1		0.01084		37

Table 18: Reproducibility results for the QIAsymphony® Pre-Lysis (Overnight incubation) and QIAsymphony® SP Extraction for Bone 1

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	
Day 1	Scientist 1	2	[redacted]	0.01112		24
Day 2	Scientist 2	2		0.00013		0
Day 3	Scientist 3	2		0.00521	Microcon	30
Day 4	Scientist 4	2		0.02773		40
Day 5	Scientist 5	2		0.01552		40

Table 19: Reproducibility results for the QIAsymphony® Pre-Lysis (Overnight incubation) and QIAsymphony® SP Extraction for Bone 2

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	Allele count
Day 1	Scientist 1	3	[redacted]	0.02185		40
Day 2	Scientist 2	3		0.00865	Microcon	40
Day 3	Scientist 3	3		0.02976		40
Day 4	Scientist 4	3		0.03259		40
Day 5	Scientist 5	3		0.00743	Microcon	40

Table 20: Reproducibility results for the QIAsymphony® Pre-Lysis (Overnight incubation) and QIAsymphony® SP Extraction for Bone 3

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	Allele count
Day 1	Scientist 1	4	[redacted]	0.01660		33
Day 2	Scientist 2	4		0.0000		0
Day 3	Scientist 3	4		0.0000		0
Day 4	Scientist 4	4		0.01010		15
Day 5	Scientist 5	4		0.00604	Microcon	41

Table 21: Reproducibility results for the QIAsymphony® Pre-Lysis (Overnight incubation) and QIAsymphony® SP Extraction for Bone 4

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	Rework Barcode	Allele Count
Day 1	Scientist 1	5	[redacted]	24.29558	Dilution	[redacted]	40
Day 2	Scientist 2	5		0.11813			40
Day 3	Scientist 3	5		44.00444	Dilution		40
Day 4	Scientist 4	5		44.07124	Dilution		40
Day 5	Scientist 5	5		19.29339	Dilution		40

Table 22: Reproducibility results for the QIAsymphony® Pre-Lysis (Overnight incubation) and QIAsymphony® SP Extraction for Bone 5

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1.1 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 should devise and submit a comprehensive implementation plan for management review. Once complete, the checklist should be submitted to the quality team for filing with the signed project documents.

Task	Details	Date Completed
Staff Training	All current QIAsymphony trainers to be assessed at CTT using RCC given similarity of bone and other substrate protocols.	24/03/2020
Staff Training	All current QIAsymphony operators (assessed as competent) will be assessed as competent using RCC given similarity of bone and other substrate protocols.	24/03/2020
Add to minor change register	Ensure that implementation has been added to the minor changes register	24/03/2020
Communication	Communicate to staff and other stakeholders – by meetings and emails.	24/03/2020
SOP	Archive Organic extraction SOP (QIS# 34039)	24/03/2020
SOP	Add bone extraction protocols to QIS # 34132 DNA Extraction and Quantification of Samples Using the QIAsymphony® SP and AS Modules	23/03/2020

AK-27

Queensland Health

HealthSupport Queensland

Project Report#192: Teeth Extraction

Verification of QIAsymphony® SP for Teeth Extraction

Melissa Cipollone, Angelina Keller, Luke Ryan and Cathie Allen

October 2020

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: Luke Ryan
Title: Senior Scientist – Analytical
Phone:
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Version history

Version	Date	Changed by	Description
1.0	July 2020	M Cipollone	Document Created.

Document sign off

This document has been **approved** by:

Name	Position	Signature	Date
Cathie Allen	Managing Scientist	[redacted]	Digitally signed by Cathie Allen, Managing Scientist - Police Services Stream Date: 2020.10.20 15:00:19 +10'00'

The following officers have **endorsed** this document

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Allison Lloyd	A/Senior Scientist Intel		Digitally signed by Allison Lloyd - A/Senior Scientist - Intel Team Date: 2020.10.13 14:11:48 +10'00'

Contents

1. Introduction

Forensic DNA Analysis currently performs automated DNA extractions on a range of sample types and substrates using a QIAGEN® QIAsymphony® SP/AS instrument. The QIAsymphony® SP/AS instrument is a modular automated system which enables the processing of up to 96 samples on a single run. The QIAsymphony® SP module is used for the extraction and purification of DNA from forensic casework samples. It uses pre-programmed optimized protocols and the QIAGEN® cartridge-based magnetic-particle chemistry kit, the QIAsymphony® DNA Investigator Kit. The SP module was the only module tested in this validation.

The original validation of the QIAsymphony® SP/AS did not include teeth extraction (refer to the Final Project Report#192: Validation of QIAsymphony SP for Bone Extraction). Forensic DNA Analysis currently have two QIAsymphony® SP/AS instruments and the use of these instruments for teeth extraction would be particularly beneficial in the event of a large scale DVI as it will dramatically increase the efficiency and processing capacity of teeth DNA extractions. The purpose of this project was to verify the extraction of teeth as an additional substrate type on the QIAsymphony® SP.

The results of this project have shown that the extraction of teeth on the QIAsymphony® SP instrument gave good DNA quantification results and DNA profiles with no adverse issues. DNA quantification results from the QIAsymphony® SP were consistent with DNA quantification results obtained from teeth previously extracted using the organic extraction protocol. This project has successfully verified teeth as a suitable sample type for QIAsymphony® SP extraction.

2. Purpose and Scope

2.1 Purpose

The purpose of this project was to verify the extraction of teeth on the QIAsymphony® SP instrument, as this would dramatically increase the efficiency and processing capacity of extracting DNA from teeth samples (when compared to the current organic extraction protocol). Furthermore, organic extraction involves the use of phenol chloroform isoamyl alcohol, which is a chemical hazard, therefore implementing an alternative protocol would remove this hazard.

2.2 Scope

The scope of this project is limited to the extraction of teeth using the QIAsymphony® SP.

3. Governance

Project Personnel

- Project Manager: Luke Ryan
- Project Officers: Melissa Cipollone

Decision Making Group

The Management Team were the Decision Making Group for this project.

4. Samples and Methods

4.1 Samples

Five adult teeth samples were donated by Forensic DNA Analysis staff or their immediate family and processed as per the approved experimental design (Project Proposal #192: Part 2 Validation of QIAsymphony SP for teeth extraction (July 2020)). Each Donor completed a consent form which was submitted to the Quality Team. Table 1 contains the teeth selected for this project (for simplicity in the remainder of this document, these samples will be referred to using their Sample Number rather than Lab Number).

Table 1: Teeth samples used in this validation

Lab Number	Sample Number
[redacted]	Donor 1
	Donor 2
	Donor 3
	Donor 4
	Donor 5

4.2 Methods

All reagents, materials and equipment used in this project were as specified in the approved in-house experimental design Project Proposal #192: Part 2 Validation of QIAsymphony SP for teeth extraction (July 2020).

5. Experiment 1 - Results

5.1 Purpose

The purpose of this experiment was to extract human genomic DNA from powdered adult teeth using the current validated method of extracting DNA from bone using the QIAsymphony® SP with the overnight pre-lysis incubation.

5.2 Results

Table 2 provides a summary of results for controls processed in this experiment.

Table 2: Quantification results for extraction controls and equipment controls

Barcode	Control	First Quant Result (ng/µL)	Rework	Second Quant Result (ng/µL)	Profile
[redacted]	Positive Extraction Control	0.625522	No	NA	OK
	Negative Extraction Control	0.000	No	NA	OK NSD
	Equipment control (Donor 4 crush)	0.000	No	NA	OK PP (<3pks)
	Equipment control (Donor 5 crush)	0.000	No	NA	OK NSD
	Equipment control (Donor 2 crush)	0.000	No	NA	OK NSD
	Equipment control (Donor 3 crush)	0.000	No	NA	OK NSD
	Equipment control (Donor 1 crush)	0.003832	Microcon	0.00598	NOT OK MIX

The positive extraction control (blood) gave the full expected profile and the negative extraction control gave a 0.000 ng/µL quantification result and NSD profile.

Equipment controls [redacted] gave a 0.000 ng/µL quantification result and NSD profiles as expected. Equipment control [redacted] gave a 0.000 ng/µL quant value and an OK PP DNA profile (<3pks) which is acceptable as per QIS 34131 Capillary Electrophoresis Quality (CEQ) Check.

However, equipment control [redacted] (Donor 1 crush) gave a 0.003832 ng/µL quantification value and was sent for a microcon rework, which concentrated the sample to 0.00598 ng/µL. This sample gave a mixed DNA profile (NOT OK MIX) which was then forwarded to the Quality Team for a quality search. It was found that the major contributor to the mixture was consistent with Donor 1. It was also concluded that there was a possible minor contributor from Donor 3, however a peak at D8[16] could not be attributed to either Donor.

Equipment control samples are taken to monitor the effectiveness of the freezer mill cleaning protocols, therefore the results of this control may be due to ineffective cleaning or contamination during crushing. Table 3 shows the order in which the teeth were crushed using the Freezer Mill. Donor 3 and 1 were crushed sequentially which supports the idea that contamination was due to ineffective cleaning between crushes (i.e. bone residue from the Bone 3 crush was still present on crushing equipment when the Bone 1 equipment control swab was taken for the Bone 1 crush). The Evidence Recovery Team were notified of the findings and will investigate their current procedures to avoid any future contaminations.

Table 3: Teeth crushing order

Donor	Crushing date/time
Donor 4	17th July at 10am
Donor 5	17th July at 11.30am
Donor 2	27th July at 9am
Donor 3	27th July at 11am
Donor 1	28th of July at 8am

Note: Crushing was done by the Freezer Mill in Evidence Recovery.

With the exception of Donor 2, all samples underwent a dilution and were then re-quantified and analysed (Table 4). All samples gave full single source profiles.

Table 4: Quantification results for Experiment 1

Sample	Donor	Quant Result (ng/µL)	Rework?	Dilution Rework Barcode	Dilution Factor	Final Quant Result (ng/µL)	Allele Count
[redacted]	1	54.306931	Dilution	[redacted]	1:110	0.35749	40
	2	0.032868	No	N/A	N/A	0.032868	40
	3	60.011963	Dilution	[redacted]	1:120	0.30581	40
	4	23.16795	Dilution		1:50	0.38172	40
	5	17.290394	Dilution		1:35	0.37946	40

6. Discussion

The availability of teeth samples for validation was limited, therefore validation teeth samples could not be extracted using both the organic and QIAsymphony® SP protocols for a direct comparison. To facilitate an indirect comparison of extraction performance, teeth samples previously extracted using the organic protocol were identified and DNA quantification and DNA profile results tabulated (see Table 5). Given that different samples were extracted using organic and QIAsymphony® SP direct comparison of quantification results (or yield) are not possible. The comparison of these results is intended as an indication of overall performance only.

Table 5: Teeth previously extracted by Organic Extraction

Lab Number	Initial Quant Result (ng/µL)	Rework	Final Quant Result after rework (ng/µL)	Allele Count
[redacted]	149.92284	Dilution 1:300	0.82000	40
	148.40590	Dilution 1:300	0.25807	40
	20.30151	Dilution 2:100	0.32509	40

Results were comparable between previously extracted teeth by organic extraction (Table 5) and teeth extracted using the QIAsymphony® SP (Table 4). Samples processed on both protocols gave high DNA quantification results and the majority required dilution. All samples processed using both the organic and QIAsymphony® SP

gave full DNA profiles. This experiment has demonstrated that the QIAsymphony® SP with overnight pre-lysis incubation effectively extracts DNA from teeth.

The 3 teeth extracted by Organic Extraction and the 5 teeth extracted by the QIAsymphony® SP were reviewed by a reporting scientist with experience in DVI reporting to assess profile quality and ability to report (see Tables 6 and 7). All samples were assessed as reportable with no adverse finding for samples extracted on the QIAsymphony® SP.

Table 6: DVI Reporting Scientist Findings for Teeth Extracted by Organic Extraction

Sample Barcode	Quant Value (ng/µL)	Result	Morphology	Comments	Reportable
[redacted]	0.820	Full PP21 single source DNA profile	Acceptable	Nil	Reportable
	0.258	Full PP21 single source DNA profile	Acceptable	Nil	Reportable
	0.325	Full single source profile, possible sub-threshold information (D1 – sub 14)	Acceptable	Alleles noted in lower stochastic range at Penta E – 7,19; CSF – 10,11; Penta D – 12,13; D5 – 11,13; TPOX – 8,8; D19 – 15.2; FGA – 25	Reportable

Table 7: DVI Reporting Scientist Findings for Teeth Extracted by QIAsymphony® SP

Sample	Quant Value (ng/µL)	Result	Morphology	Comments	Reportable
[redacted]	0.033	Full PP21 single source DNA profile	Acceptable	Alleles noted in lower stochastic range at Penta E – 14 & Penta D - 12	Reportable
	0.379	Full PP21 single source DNA profile	Acceptable	Allele noted in lower stochastic range at TPOX – 8	Reportable
	0.357	Full PP21 single source DNA profile	Acceptable	Nil	Reportable
	0.382	Full PP21 single source DNA profile	Acceptable	Nil	Reportable
	0.306	Full PP21 single source DNA profile	Acceptable	Nil	Reportable

In addition to the above, it should be noted that for the majority of samples, DNA concentrations were very high, well above the required input for PCR amplification, and likely nearing saturation of the magnetic beads used for the extraction. It should also be noted that very high quantification results (i.e. above 10 ng/µL) are known from routine processing to be unreliable and outside the normal performance range for quantification processes.

The extraction of teeth using the QIAGEN pre-lysis with overnight incubation and QIAsymphony® SP extraction was successful in yielding good DNA quantification values and full DNA profiles. A DVI experienced reporting scientist has assessed all results as reportable with no adverse findings. These results have shown that the QIAsymphony® SP is suitable for the extraction of teeth and should be implemented as the routine extraction protocol.

7. Recommendations

It is recommended that:

- The extraction of teeth, using the QIAGEN pre-lysis with overnight incubation and QIAsymphony® SP Extraction, be implemented.
- Organic extractions are ceased, QIS# 34039 archived and phenol chloroform isoamyl alcohol is disposed.

- Further validation is conducted to assess the ability to store lysates for up to 8 days.

8. References

Aguilera, M., Micic, B., Acedo, P., Ryan, L. and Allen, C. (2016) Validation of the QIAsymhpony SP/AS Modules [Final Report].

Cipollone, M., Ryan, L., Mathieson, M. and Allen, C. (2018) Validation of QIASymphony SP for Bone Extraction [Final Report].

AK-28

Angelina Keller

From: Luke Ryan
Sent: Friday, 28 August 2020 11:37 AM
To: Angelina Keller
Cc: Melissa Cipollone
Subject: RE: Project# 192 QIAsymphony extraction of Teeth

Hi Ange
I spoke to Kirsten and she said really liked the way you assessed and reviewed. For the final report, I think we should include your assessment as you have written as it is very thorough. I would like to add after your assessment something similar to "The above assessment by a DVI experienced reporting scientist has assessed each result from the QIAsymphony as reportable. No adverse findings were noted for the QIAsymphony extracted teeth". Are you happy with that??

Happy for you to change that wording if you want.

hanks
Luke

From: Angelina Keller <[redacted]>
Sent: Friday, 28 August 2020 10:58 AM
To: Luke Ryan <[redacted]>
Subject: RE: Project# 192 QIAsymphony extraction of Teeth

Hi Luke,

You're welcome and sounds good!

Thanks,
Ange

From: Luke Ryan <[redacted]>
nt: Friday, 28 August 2020 10:53 AM
To: Angelina Keller <[redacted]>; Melissa Cipollone <[redacted]>
Subject: RE: Project# 192 QIAsymphony extraction of Teeth

Hi Ange
Perfect thank you!! Let me just check with Kirsten to see if she is happy as well.

Thanks
Luke

From: Angelina Keller <[redacted]>
Sent: Thursday, 27 August 2020 10:50 AM
To: Melissa Cipollone <[redacted]>; Luke Ryan <[redacted]>
Subject: FW: Project# 192 QIAsymphony extraction of Teeth

Hi Mel and Luke,

I have assessed the eight samples listed below within the scope of the project; that is, assessing peak morphology and height, however not comparing each sample to a reference profile or a sample extracted at the same time using an alternative extraction technique, as requested.

Five samples extracted on the QIAsymphony (plate [redacted]

- Sample [redacted] (parent barcode – [redacted], Quant 0.033 ng/uL
  Full PP21 single source DNA profile
  Morphology acceptable
  Alleles noted in lower stochastic range at Penta E – 14 and Penta D – 12
  Reportable

- Sample [redacted] (dilution – [redacted]), Quant 0.379 ng/uL
  Full single source profile
  Morphology acceptable
  Allele noted in lower stochastic range at TPOX – 8
  Reportable

- Sample [redacted] (dilution – [redacted], Quant 0.357 ng/uL
  Full single source profile
  Morphology acceptable
  Reportable

- Sample [redacted] (dilution – [redacted]), Quant 0.382 ng/uL
  Full single source profile
  Morphology acceptable
  Reportable

- Sample [redacted] (dilution – [redacted]), Quant 0.306 ng/uL
  Full single source profile
  Morphology acceptable
  Reportable

Three samples extracted using organic extraction (case work)

- Sample [redacted] (dilution – [redacted]), Quant 0.258 ng/uL
  Full single source profile
  Morphology acceptable
  Reportable

- Sample [redacted] (dilution – [redacted]), Quant 0.820 ng/uL
  Full single source profile
  Morphology acceptable
  Reportable

- Sample [redacted] (dilution – [redacted]), Quant 0.325 ng/uL
  Full single source profile, possible sub-threshold information (D1 – sub 14)
  Morphology acceptable
  Alleles noted in lower stochastic range at Penta E – 7,19; CSF – 10,11; Penta D – 12,13; D5 – 11,13; TPOX – 8,8; D19 – 15.2; FGA – 25
  Reportable

Please let me know if more detailed assessment or further clarification is required.

Kind regards,
Angelina

From: Melissa Cipollone <[redacted]
Sent: Friday, 21 August 2020 8:39 AM

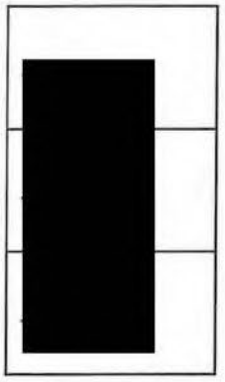
To: Angelina Keller <[redacted]
Subject: Re: Project# 192 QIAsymphony extraction of Teeth

Hi Angelina

Apologies that I am only responding now as I have been away for most of this week.

At this stage, if you could look at those 5 samples in isolation, that would be great. We have no way of doing a direct comparison unfortunately.

There are also another three teeth samples that need to be reviewed and these were extracted a while ago using the Organic Method. I think we are just wanting a reporter to agree that the same results can be obtained when teeth are extracted on the QIAsymphony as opposed to the Organic Method. In this case, I guess we are just looking at peak morphology and peak sizes to say that yes, the profiles are SS and have good peak heights etc. The lab numbers are:



Mel

From: Angelina Keller <[redacted]
Sent: Tuesday, 18 August 2020 2:44 PM
To: Melissa Cipollone <[redacted]
Subject: FW: Project# 192 QIAsymphony extraction of Teeth

Hi Mel,

I have located 5 x samples on plate [redacted] and [redacted] that have been extracted on the QIAsymphony. For clarity, am I looking at these profiles in isolation or comparing them to known reference samples or equivalent organic profiles.

Kind regards,
Angelina

From: Justin Howes <[redacted]
Sent: Tuesday, 11 August 2020 12:11 PM
To: Angelina Keller [redacted]>
Cc: Paula Brisotto <[redacted], Luke Ryan <[redacted]; Kylie Rika <[redacted]>
Subject: FW: Project# 192 QIAsymphony extraction of Teeth

Hi Angelina,
Kylie nominated you to assist where requested in the Expntl Design for #192.

Please have a look at the batch below and please note the information from Luke below the table.

Please provide your assessment to Melissa and Luke.

Thanking you

Justin

Justin Howes
Team Leader - Forensic Reporting and Intelligence Team
Forensic DNA Analysis, Police Services Stream
Forensic & Scientific Services, Health Support Queensland, Queensland Health
Please note that I may be working from a different location during the COVID-19 Pandemic. The best contact method is via email.
p [redacted] m [redacted]
a 39 Kessels Road, Coopers Plains, QLD 4108
e [redacted] w www.health.qld.gov.au/healthsupport/businesses/forensic-and-scientific-services

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From: Luke Ryan [redacted]
Sent: Tuesday, 11 August 2020 12:03 PM
To: Justin Howes [redacted]
Cc: Paula Brisotto [redacted]; Melissa Cipollone [redacted]
Subject: Project# 192 QIAsymphony extraction of Teeth

Hi Justin
The Experimental Design for this project included that a DVI experienced reporter would compare final results for the teeth extracted on the QIAsymphony and a selection of teeth previously extracted using the Organic protocol.

Melissa has identified three teeth extracted using Organic:

Lab Number	Final Quant Result (ng/µL)	Rework	Final Quant Result after reworked (ng/µL)	Allele Count
[redacted]	149.92284	Dilution 1:300	0.82000	40
	148.40590	Dilution 1:300	0.25807	40
	20.30151	Dilution 2:100	0.32509	40

The teeth extracted using the QIAsymphony for Project# 192 are contained in [redacted] Can you please arrange for a DVI experienced reporting scientist to look at these samples and advise if any adverse findings? These are not the same samples, and DNA concentrations are normalised in the amp – so direct peak height comparison is not useful.

Thanks
Luke

Luke Ryan
Senior Scientist – Analytical Team
Forensic DNA Analysis, Forensic and Scientific Services
Health Support Queensland, Queensland Health
p [redacted] m [redacted]
a 39 Kessels Rd, Coopers Plains, QLD 4108
e [redacted] w www.health.qld.gov.au/healthsupport/businesses/forensic-and-scientific-services

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AK-29

Angelina Keller

From: Angelina Keller
Sent: Tuesday, 9 July 2019 7:58 AM
To: Justin Howes
Cc: Kylie Rika
Subject: FW: Coronial meetings

Hi Justin,

Thank you for your e-mail following our meeting on Thursday 20 June. I acknowledge the direction that has been given.

As per my most recent CSP, I have found attendance at the Coronial meetings during the past 12 years to be invaluable for streamlining the DNA identification process. I feel it is in alignment with all of the ICARE principles for the organisation as a whole as well as for personal in attendance. I believe inclusion of the reporters with ER/Quality would be ideal for all parties involved in this sometimes complicated process.

Thanks,
Angelina

From: Justin Howes
Sent: Thursday, 20 June 2019 10:21 AM
To: Angelina Keller <[redacted]>; Jacqui Wilson <[redacted]
Cc: Paula Brisotto <[redacted]>; Kylie Rika <[redacted]>; Sharon Johnstone <[redacted]
Subject: Coronial meetings

Hi,
Just summarising our conversation that I have been given a direction to work towards aligning tasks and roles appropriately within Forensic DNA Analysis. One direction is that attendance of FRIT staff (as a standard approach) to Coronial meetings is no longer required; this will be for ERQ Team to attend. This will be for Team Leader of ERQ to attend or delegate. We get notified of items for DNA through SSLU and we will continue to encourage wider use of the FR to assist the communication process.

Just confirming that this conversation was not about cancellation of the Coronial meeting as this is not within my responsibilities.

I am happy for you to attend the next meeting set for 26 June, then we can transition this time for the meeting to ERQ.

I acknowledge there is work to do between For Path, CSU, Sample Mgt and FDNA – we will continue to strive for solutions, which are being worked through at Mging Scientist and ED levels.

Thanks
Justin

Justin Howes
Team Leader - Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream
Forensic & Scientific Services, Health Support Queensland, Queensland Health

p [redacted] m [redacted]
a 39 Kessels Road, Coopers Plains, QLD 4108
e [redacted] w www.health.qld.gov.au/healthsupport/businesses/forensic-and-scientific-services

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AK-30

Angelina Keller

From:	Angelina Keller
Sent:	Thursday, 19 May 2022 10:05 AM
To:	Lara Keller
Subject:	Coronial Meetings
Attachments:	Coronial meetings 2019.pdf
Follow Up Flag:	Follow up
Flag Status:	Flagged

Hi Lara,

Thank you for your time. I really appreciate you listening to my rant. I wish I was much more detached from this situation but I am distressed for the families we are continually letting down (even a week can mean a lot to a grieving family) and it has been gradually getting worse since 2019. Attached is the e-mail chain directing that all reporters stop attending Coronial ID meetings (Allison is the DNA representative / manager allowed to attend).

Polly Williams is in SSLU and is a fantastic chair who genuinely cares for the impacted families.

Kind regards,
Angelina

Angelina Keller

From: Angelina Keller
Sent: Tuesday, 9 July 2019 7:58 AM
To: Justin Howes
Cc: Kylie Rika
Subject: FW: Coronial meetings

Hi Justin,

Thank you for your e-mail following our meeting on Thursday 20 June. I acknowledge the direction that has been given.

As per my most recent CSP, I have found attendance at the Coronial meetings during the past 12 years to be invaluable for streamlining the DNA identification process. I feel it is in alignment with all of the ICARE principles for the organisation as a whole as well as for personal in attendance. I believe inclusion of the reporters with ER/Quality would be ideal for all parties involved in this sometimes complicated process.

Thanks,
Angelina

From: Justin Howes
Sent: Thursday, 20 June 2019 10:21 AM
To: Angelina Keller <[redacted]>; Jacqui Wilson <[redacted]>
Cc: Paula Brisotto <[redacted]>; Kylie Rika <[redacted]>; Sharon Johnstone [redacted]
Subject: Coronial meetings

Hi,
Just summarising our conversation that I have been given a direction to work towards aligning tasks and roles appropriately within Forensic DNA Analysis. One direction is that attendance of FRIT staff (as a standard approach) to Coronial meetings is no longer required; this will be for ERQ Team to attend. This will be for Team Leader of ERQ to attend or delegate. We get notified of items for DNA through SSLU and we will continue to encourage wider use of the FR to assist the communication process.

Just confirming that this conversation was not about cancellation of the Coronial meeting as this is not within my responsibilities.

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I acknowledge there is work to do between For Path, CSU, Sample Mgt and FDNA – we will continue to strive for solutions, which are being worked through at Mging Scientist and ED levels.

Thanks
Justin

Justin Howes
Team Leader - Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream
Forensic & Scientific Services, Health Support Queensland, Queensland Health

p [redacted] m [redacted]
a 39 Kessels Road, Coopers Plains, QLD 4108
e [redacted] w www.health.qld.gov.au/healthsupport/businesses/forensic-and-scientific-services

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AK-31

Angelina Keller

From: Kylie Rika
Sent: Tuesday, 30 March 2021 11:21 AM
To: Angelina Keller
Subject: RE: Information regarding Risk Assessments undertaken in Forensic DNA Analysis

Hi Ange – for your eyes only:

Cathie send this doc to mgmt. team before sending to all of DNA. I had a question:

Hi Kylie

Thanks for your email. If additional assistance is required, staff members with competency in this area will be called upon. Sometimes, it's limited when it comes to sample selection, due to the incidence that has occurred (ie there's a large amount of fragmentation).

Cheers
Cathie

Cathie Allen
Managing Scientist

Social Chair, Organising Committee for 25th International Symposium of the
Australian and New Zealand Forensic Science Society (ANZFSS), Brisbane, 11 – 15 Sept 2022

Police Services Stream, Forensic & Scientific Services
Health Support Queensland, Queensland Health

p [redacted] m [redacted]
a 39 Kessels Road, Coopers Plains, QLD 4108
e [redacted] w www.health.qld.gov.au/healthsupport

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From: Kylie Rika <[redacted]>
Sent: Friday, 19 March 2021 5:12 PM
To: Cathie Allen <[redacted]>
Subject: Can I please ask a clarifying question: Correspondence re: risk assessments undertaken.

Sorry Cathie for my late reply.

I was just wondering if any of our DVI/coronial reporters have been or would be called upon to aid in the training of the ER senior and pathologists with regards to sample selection, triage and follow through into the case management and reporting?

I think we could all learn a lot from those who have pulled the results together at the end (in terms of sample selection, triage etc...)

Thanks
Kylie

From: Angelina Keller <[redacted]>
Sent: Tuesday, 30 March 2021 11:16 AM
To: Kylie Rika <[redacted]>
Subject: FW: Information regarding Risk Assessments undertaken in Forensic DNA Analysis

Hi Kylie,

FYI quietly - I'll have a talk to John about this when he returns.

Ange

From: Cathie Allen <[redacted]
Sent: Tuesday, 30 March 2021 10:54 AM
To: Abigail Ryan <[redacted]>; Adam Kaity <[redacted]>; Adrian Pippia <[redacted]>; Alanna Darmanin <[redacted]>; Alicia Quartermain <[redacted]>; Allan McNevin <[redacted]>; Allison Lloyd <[redacted]>; Amy Cheng <[redacted]>; Amy Cleaver <[redacted]>; Amy Morgan <[redacted]>; Angela Adamson <[redacted]>; Angelina Keller <[redacted]>; Anne Finch <[redacted]>; Belinda Andersen <[redacted]>; Biljana Micic <[redacted]>; Cassandra James <[redacted]>; Cecilia Flanagan <[redacted]>; Chantal Angus <[redacted]>; Chelsea Savage <[redacted]>; Cindy Chang <[redacted]>; Claire Gallagher <[redacted]>; Deborah Nicoletti <[redacted]>; Emma Caunt <[redacted]>; Generosa Lundie <[redacted]>; Helen Williams <[redacted]>; Ingrid Moeller <[redacted]>; Jacqui Wilson <[redacted]>; Janine Seymour-Murray <[redacted]>; Josie Entwistle <[redacted]>; Julie Brooks <[redacted]>; Justin Howes <[redacted]>; Karryn Rogers <[redacted]>; Kerry-Anne Lancaster [redacted]; Kevin Avdic <[redacted]>; Kim Estreich <[redacted]>; Kirsten Scott <[redacted]>; Kristina Morton <[redacted]>; Kylie Rika <[redacted]>; Kynan Frei <[redacted]>; Lai-Wan Le <[redacted]>; Lisa Farrelly <[redacted]>; Luke Ryan <[redacted]>; Maria Aguilera <[redacted]>; Matthew Hunt <[redacted]>; Megan Mathieson <[redacted]>; Melissa Cipollone <[redacted]>; Michael Goodrich <[redacted]>; Michael Hart <[redacted]>; Michelle

Margetts <[redacted]>; Naomi French <[redacted]>; Nicole Roselt <[redacted]>; Paula Brisotto <[redacted]>; Penelope Taylor <[redacted]>; Phillip McIndoe <[redacted]>; Pierre Acedo <[redacted]>; Rhys Parry <[redacted]>; Ryu Eba <[redacted]>; Sandra McKean <[redacted]>; Sharelle Nydam <[redacted]>; Sharon Byrne <[redacted]>; Sharon Johnstone <[redacted]>; Suzanne Sanderson <[redacted]>; Tara Prowse <[redacted]>; Tegan Dwyer <[redacted]>; Thomas Nurthen <[redacted]>; Valerie Caldwell <[redacted]>; Vicki Pendlebury-Jones <[redacted]>; Wendy Harmer <[redacted]>; Yvonne Connolly <[redacted]>

Subject: Information regarding Risk Assessments undertaken in Forensic DNA Analysis

Hi Everyone

Please find attached some information regarding risk assessments that have been undertaken with respect to Forensic DNA Analysis processes.

Please let me know if you have any questions regarding the information attached.

Cheers
Cathie

Cathie Allen BSc, MSc (Forensic Science)
Managing Scientist

Social Chair, Organising Committee for 25th International Symposium of the
Australian and New Zealand Forensic Science Society (ANZFSS), Brisbane, 11 – 15 Sept 2022

Police Services Stream, Forensic & Scientific Services
Health Support Queensland, Queensland Health

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Risk Assessments undertaken in Forensic DNA Analysis
In response to an issue raised.

HealthSupport
Queensland
Forensic and Scientific Services

An issue had been raised regarding the access to the Mortuary and the Scientific Skills Development Unit (SSDU) approached all relevant areas including Forensic DNA Analysis. All staff that had entered the Mortuary were required to supply FSS – Infection Control with further evidence of their Vaccine Preventable Diseases status, which included Measles Mumps Rubella (MMR), Pertussis, Varicella, Tuberculosis (TB), Meningococcal ACWY and B. All staff who had entered the Mortuary were also to undergo additional mortuary specific training and a gradual exposure program. Many areas across the campus were non-compliant as each of these criteria hadn't been satisfied.

An initial meeting was held with Mortuary staff, Forensic DNA Analysis staff and Casey Gardener (HSQ Safety) to discuss the risks to Forensic DNA Analysis staff entering the Mortuary. HSQ Safety advised the meeting attendees to undertake Risk Assessments to account for the level of exposure and determine appropriate controls with consideration on not only the biological but also the psychological risks.

Damien Cass, Mortuary Manager preferred Forensic DNA Analysis limit the number of people entering the Mortuary. To ensure continued access to this sensitive area, a clear purpose for the work conducted would be required. The meeting attendees agreed that undertaking a Risk Assessment would identify the need for Forensic DNA Analysis staff to enter the mortuary and ensure that only essential business was being conducted.

Deborah Whelan, Managing Scientist, Coronial Services Stream, supported this as a reduction in the number of staff entering the Mortuary area reduces the overall risk for FSS. Staff/personnel required to enter the Mortuary must be inducted, vaccinated and trained in Infection Prevention control practices specific to the Mortuary and undergo a gradual exposure program.

Samantha Granato, SSDU, recommended a consistent approach be used by all areas in completing a Risk Assessment for each method/procedure, which ensures all risks were captured, including the ones raised by HSQ safety, due to the increase of VPD and training requirements. In conducting the risk assessments on each task, the hierarchy of controls were considered, including (but not limited to) elimination, substitution, engineering controls, administration, Personal Protective Equipment, etc.

Four risk assessments were completed on Forensic DNA Analysis' Evidence Recovery processes: Examination of Items (general), Processing In-tube samples; Examination of Sexual Assault Cases, and Examination of Post-mortem and other samples from deceased persons. All completed risks assessments are in the Risk Assessment Folder on I:\Quality and Projects\Risk assessments.

Elimination or substitution were considered the preferred control mechanism where possible. Casey Gardner and Damien Cass were involved in the discussion regarding the impact of eliminating Forensic DNA Analysis staff from entering the Mortuary. Substitution controls such as updating the training with respect to Forensic Pathologists taking the most appropriate samples and phoning a line manager for consultation were considered the best controls in ensuring staff safety.

The Executive Director and Managing Scientists for Police Services Stream and Coronial Services Stream approved the elimination of Forensic DNA Analysis staff from entering the mortuary as the preferred control for VPD and psychological risks. A new process will be implemented for Forensic Pathologists and Mortuary staff to direct enquiries to the Evidence Recovery Senior Scientist in the first instance, and if the issue can't be resolved over the phone, further consultation will be subsequently coordinated by this role.

NB: FSS staff are permitted to enter a meeting room for meetings with QPS Coronial Support Unit, SSLU or Coronial Support Services, held outside of the mortuary theatre or mortuary loading bay. FSS staff can enter the mortuary loading bay for the intent to deliver or retrieve waste bins.

AK-32

Angelina Keller

From: John Doherty
Sent: Friday, 28 May 2021 9:06 AM
To: Angelina Keller
Subject: RE: A follow up talk

Hi Angelina

Yes, that works. See you then.

Thanks
John

From: Angelina Keller <[redacted]
Sent: Friday, 28 May 2021 9:04 AM
To: John Doherty [redacted]
Subject: RE: A follow up talk

Hi John,

Good to hear back. I will be brave and meet in your office with Alison.

Would 9:30 am this Monday suit?

Kind regards,
Angelina

From: John Doherty <[redacted]>
Sent: Friday, 28 May 2021 8:56 AM
To: Angelina Keller [redacted]
Subject: RE: A follow up talk

Hi Angelina

Apologies, I missed that one. Happy to meet, although I've been advised by HR to now only meet staff in my office and with an independent person present (Alison, my Advisor, sits in on staff meetings now). If you're okay to meet in my office early next week, then I'm sure I can arrange that. Monday is pretty clear at the moment...

Thanks
John

From: Angelina Keller <[redacted]>
Sent: Friday, 28 May 2021 8:16 AM
To: John Doherty <[redacted]
Subject: FW: A follow up talk

Hi John,

Happy Friday!

Hopefully you saw my original e-mail? Would it be possible to catchup with you soon.

Looking forward to your hearing back.

Kind regards,
Angelina

From: Angelina Keller
Sent: Monday, 17 May 2021 10:43 AM
To: John Doherty <[redacted]
Subject: A follow up talk

Hi John,

Welcome back.

I would appreciate an opportunity to talk with you regarding an e-mail and attachment that Cathie distributed to Forensic DNA Analysis during your absence.

Thank you,
Angelina

Angelina Keller
Reporting Scientist – Forensic Reporting and Intelligence Team
Forensic DNA Analysis, Forensic & Scientific Services,
Health Support Queensland, **Department of Health**

p | [redacted]
a | 39 Kessels Road, Coopers Plains, QLD 4108
w | Queensland Health e | [redacted]

31/05/2021

office and witness necessary - HR

Me - Positive, DVI celebration and complicated bone case, Jodie Ward

Me - Document = Door shut to going downstairs

John - Focus on where possible

Stop ER people from having a look

VPD - NA on viewing platform, police

If a pathologist requests someone to come down -

AOK, tricky Coronial or DVIs

Sampling in mortuary on list to be addressed by Management Team

Nominated person(s) who can go down if requested for Coronial or DVI, critical for DVI and TATs!

Me - Happy to be vaccinated if approached

Me - Followup?

John - Back in a few months

AK-33

Angelina Keller

From: Paula Brisotto
Sent: Friday, 9 September 2022 8:57 AM
To: Nathan Milne; Ricky Truong; Angelina Keller; Justin Howes
Cc: Jack Garland
Subject: RE: Assistance with sampling of skeletal remains

Hi all,

My apologies, I assumed this request for assistance was related to the current DVI.

Ricky, can I please ask you to contact Allison Lloyd, the Evidence Recovery Team Supervisor, to discuss requirements relating to this assistance.

Many t hanks,
Paula

From: Nathan Milne <[redacted]>
Sent: Friday, 9 September 2022 8:05 AM
To: Ricky Truong <[redacted]>; Paula Brisotto <[redacted]>; Angelina Keller <[redacted]>; Justin Howes <[redacted]>
Cc: Jack Garland <[redacted]>
Subject: RE: Assistance with sampling of skeletal remains

Hi All

These are decades old museum specimens consisting of partial skulls. Very clean unlike a DVI scenario.

Nathan

From: Ricky Truong <[redacted]>
Sent: Thursday, 8 September 2022 4:36 PM
To: Paula Brisotto <[redacted]>; Angelina Keller <[redacted]>; Justin Howes <[redacted]>
Cc: Jack Garland <[redacted]>; Nathan Milne <[redacted]>
Subject: RE: Assistance with sampling of skeletal remains

Hi Paula,

Thanks for your advice. I will speak to the pathologist tomorrow and we will proceed as required.

Kind regards

Ricky Truong
A/Mortuary Manager
Coronial Services
Prevention Division, Queensland Health
p [redacted]
a Forensic and Scientifc Services, 39 Kessels Rd, Coopers Plains QLD 4108
e [redacted] w www.health.qld.gov.au/fss

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From: Paula Brisotto <[redacted]>
Sent: Thursday, 8 September 2022 4:35 PM
To: Ricky Truong <[redacted]>; Angelina Keller <[redacted]>; Justin Howes <[redacted]>
Cc: Jack Garland <[redacted]>; Nathan Milne <[redacted]>
Subject: RE: Assistance with sampling of skeletal remains

Hi Ricky,

The first preference would be to use photos if possible.

If this is not possible, then attendance at the examination of the remains away from other mortuary activities is possible as per Lara's previous endorsement below:

Attendance for Forensic DNA Scientists at the DVI autopsy is endorsed on the following grounds:

- *Risk assessment is in place, and the risks including WHS and vicarious trauma will be managed, and*
- *That in-person attendance is deemed necessary by the Forensic Pathologist*

Thanks,
Paula

From: Ricky Truong <[redacted]>
Sent: Thursday, 8 September 2022 4:10 PM
To: Angelina Keller <[redacted]>; Justin Howes <[redacted]>; Paula Brisotto <[redacted]>
Cc: Jack Garland <[redacted]>; Nathan Milne <[redacted]>
Subject: Assistance with sampling of skeletal remains

Good afternoon Angelina, Justin and Paula,

As we spoke about earlier this afternoon Angelina, we may require assistance with sampling requirements of skeletal remains that are being examined tomorrow by Dr. Jack Garland.

Please do let us know if this is possible. We are able to examine the remains away from other mortuary activities if required. Using photos may also be possible.

Thanks very much

Ricky Truong
A/Mortuary Manager
Coronial Services
Prevention Division, Queensland Health
p [redacted]
a Forensic and Scientifc Services, 39 Kessels Rd, Coopers Plains QLD 4108
e [redacted] **w** www.health.qld.gov.au/fss

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AK-34

Angelina Keller

From: Justin Howes
Sent: Thursday, 1 September 2022 8:55 AM
To: Angelina Keller; Kylie Rika
Subject: RE: DVI

Follow Up Flag: Follow up
Flag Status: Flagged

Thanks Angelina
Damien Cass is ascertaining if assistance can best be provided (if ultimately required) via telephone which would be in line with any health and safety risks involved. We will wait to hear later on what assistance might be required.

Regards
Justin

Justin Howes
Team Leader - Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream, Forensic & Scientific Services
Prevention Division, Queensland Health

p [redacted] m [redacted]
a 39 Kessels Road, Coopers Plains, QLD 4108
e [redacted] w www.health.qld.gov.au/fss

Please note that I may be working from a different location during the COVID-19 Pandemic. The best contact method is via email.

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From: Angelina Keller <[redacted]>
Sent: Thursday, 1 September 2022 8:38 AM
To: Kylie Rika <[redacted]>; Justin Howes <[redacted]>
Subject: DVI

Morning Both,

The Mortuary Manager and Lead DVI Pathologist have requested the assistance of myself and Valerie during todays DVI PMs. They will contact us as we are needed.

Kind regards,
Angelina

Angelina Keller
Reporting Scientist

Forensic DNA Analysis, Forensic & Scientific Services
Prevention Division, Queensland Health

p [redacted]
a 39 Kessels Road, Coopers Plains, Qld, 4108
e [redacted] **w** www.health.qld.gov.au/fss

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AK-35

Angelina Keller

From: Angelina Keller
Sent: Monday, 30 May 2022 9:06 AM
To: Lara Keller
Subject: An update on bone sampling

Follow Up Flag: Follow up
Flag Status: Flagged

Hi Lara,

I have become aware of more information around my involvement in sampling bones. It is clear that Cathie will challenge my involvement as part of a business case for change for DNA that is not yet implemented (ie a reporter should not sample bones – she has not said this me but to the managers). Justin and Paula are in agreement (Justin says Queensland bones can be sent interstate for a very large DVI and Paula says if a pathologist calls for my advice then I should re-direct the phone call to Allison in Evidence recovery). Could I please see you early one morning to talk to you about this. I am definitely in no doubt now that my expertise in this specialised area is being targeted for a re-structure.

Kind regards,
Angelina

AK-36

Angelina Keller

From: Angelina Keller
Sent: Thursday, 28 November 2019 2:58 PM
To: Cathie Allen
Subject: RE: Permission to rework sample [redacted]

Hi Cathie,

Thanks for your response. I will prepare an Intel report detailing the amended result.

Kind regards,
Angelina

From: Cathie Allen <[redacted]
Sent: Thursday, 28 November 2019 2:53 PM
To: Angelina Keller <[redacted]
Cc: Kylie Rika [redacted]; Justin Howes <[redacted]
Subject: RE: Permission to rework sample [redacted]

Hi Angelina

Thanks for the update on the rework – much appreciated.

We gave an undertaking to the QPS that any time we amended a result, we would advise them as the reasons why and we'd provide them with the result prior to or at the time of peer review. Whilst the QPS have provided some concessions to us about Intel Reports not being required for minor changes in LRs, given this interpretation is amended to 3 persons, we should honour our commitment by providing them with an Intel Report.

Cheers
Cathie

Cathie Allen
Managing Scientist

Police Services Stream, Forensic & Scientific Services
Health Support Queensland, Queensland Health

p [redacted] m [redacted]
a 39 Kessels Road, Coopers Plains, QLD 4108
e [redacted] w www.health.qld.gov.au/healthsupport

From: Angelina Keller [redacted]
Sent: Thursday, 28 November 2019 2:03 PM
To: Cathie Allen <[redacted]>

Cc: Kylie Rika <[redacted]
Subject: RE: Permission to rework sample [redacted]

Hi Cathie,

After a reamp at 15 uL, this sample has been assessed as a 3 contributor mixture with no additional uploadable profiles. The LR for the associated reference sample is >100 billion.

I have added 'This sample has undergone further processing' and have updated the result lines (review pending). Given the change in result (2 to 3), is this sufficient or would you like an Intel report to be issued to the QPS.

Kind regards,
Angelina

From: Cathie Allen <[redacted]
Sent: Friday, 15 November 2019 11:21 AM
To: Angelina Keller <[redacted]
Cc: Kylie Rika <[redacted]
Subject: RE: Permission to rework sample [redacted]

Hi Angelina

Yes, I authorise a reamp for this sample.

Please let me know how it turns out.

Cheers
Cathie

Cathie Allen
Managing Scientist

Police Services Stream, Forensic & Scientific Services
Health Support Queensland, Queensland Health

p [redacted] m [redacted]
a 39 Kessels Road, Coopers Plains, QLD 4108
e [redacted] w www.health.qld.gov.au/healthsupport

From: Angelina Keller <[redacted]
Sent: Thursday, 14 November 2019 12:29 PM
To: Cathie Allen <[redacted]
Cc: Kylie Rika <[redacted]
Subject: Permission to rework sample [redacted]

Hi Cathie,

I have had some discussion with Kylie regarding sample [redacted] and I am seeking your approval to rework this sample prior to issuing a statement for court in December.

This sample was initially reviewed as a 2p mixture (P2) but was only amped at 10.8 uL following a microcon. There are 11 peaks present for contributor 2 (many just below the reporting threshold), and a significant possibility that reamping this sample at 15 uL may result in an uploadable profile for contributor 2 and an improved profile for contributor 1 (the LR for this sample and the associated reference sample is >100 billion).

Kind regards,
Angelina

Angelina Keller
Reporting Scientist – Forensic Reporting and Intelligence Team
Forensic DNA Analysis, Forensic & Scientific Services,
Health Support Queensland, **Department of Health**

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w | Queensland Health e | [redacted]

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AK-37

Go Live FDNA: Sample not amplified at the correct input DNA volume according to the quantitation information ([redacted]

Go-Live FDNA: Dashboard not linking to correct place for "Result -NWQPS"

Go-Live FDNA: Not authorised to access samples on NWQPS review worklist

GO LIVE - FC Allocation Date on Worksheets is displaying Submission Date

Go Live FDNA: Profile Record to display updated profile interpretation by greying our previous entry ([redacted]

Go-Live FDNA: Environmental samples are not displaying as green circles as expected

Go Live FDNA: Examination Record no longer visible under the Exhibit Testing/Examination table (sample [redacted]

Go Live DNA: Ability to view and hyperlink to the sample from a ref sample within the CPT which resulted in an upload

GO LIVE - FC The icon to indicate an electronic file is attached does not display on the 'Result' record for an exhibit when viewed from the Exhibit record display

Go Live: FDNA - Page number on worklists/Exhibit list - not clear as to what page you are on

Go Live: FDNA - FR download extensions for pdf

Go Live: FDNA - NCIDD modify and delete ordering

Go Live: FDNA - Images - explicit images are viewable and not hidden (e.g. [redacted])

Go Live FDNA: Exhibit testing table not displaying correctly.

Go Live: FDNA - Presumptive, Result, Micro result lines containing all result information

Go-Live FDNA: Samples are not populating 'NWQPS' result worklist

Go Live: FDNA - Duplicate entry in profile record table [redacted]

Go Live: FDNA - Two STRmix files appear on the one sample

Go-Live FDNA: Profile Data Analysis Worklist - Ref appears to be capped at 100 samples

Go Live: FDNA - PDA worklists

Go Live: FDNA - Sample not populating filtered PDA review list ([redacted]

Incorrect amplification volumes being calculated by the FR. This example is at the lower quant range which should default in an input DNA volume of 15uL
The "Result - NWQPS" on the dashboard is not linked to the appropriately filtered list (see red arrow). It is linking to all samples on the review list, it should link to the filtered NWQPS list only
There are three samples on the NWQPS review worklist. We are not able to access the record on any of the three barcodes as shown in the screen shot. We get an access error on all of them - see below. It addition the table says there are 5 samples, however only 3 records are contained on this list
The Allocation Date should be the date that the Request / Task was allocated to an Action Officer. On the worksheets, it is currently displaying the Submission Date instead. This seems to only effect cases allocated since GO LIVE.

The initial resolved profile within the Profile Record table has not been greyed out upon validation of the result as a result of an update to the profile assessment, mixture to conditioned mixture, where a matching profile has been added to the Profile Record table. The FR is to acknowledge all matching profile in the Profile Record table and not just profiles that match that have a similar suffix.
Samples that come from Environmental cases (Job type=Environmental) in Forensic DNA Analysis should be displaying with a green circle
The Examination Summary, now named Examination Record is no longer able to be accessed from the Exhibit Testing/Examinations table and can be accessed via the 'Examinations' tab - 'Unit Exams' then find the exhibit which take a long time if there mny exhibits for the case.
Have the ability to hover on the 'upload' triangle associated to a reference sample within the CPT and gain info as to the sample which resulted in an upload. There would also be the ability to hyperlink to that sample.
The icon to indicate an electronic file is attached does not display on the 'Result' record for an exhibit when viewed from the Exhibit record display.

However, when the Result entry is viewed (click on result below) the icon appears!. So it is in the background but only displays when the Result page is viewed.
When clicking through worklists/exhibit lists that have multiple pages, it can be difficult to tell which page you are on as all pages stay a similar colour (a slight difference can be seen - but not by all). It may be helpful to bold the number of the current page. Examples added.
When PDFs have been downloaded from the FR - they are being labelled with .pdf twice. Is this a result of the FR or the browser?
When ordering an NCIDD modify or delete, we used to get a little pop-up reminder to add the relevant suffix to the barcode. This no longer appears. Can this please be returned.

Explicit images being immediately visible, and non-explicit being marked as explicit and hidden. E.g. [redacted]- When printing case file able to view multiple explicit images (deceased person) (eg exhibit [redacted] is one of many) when attempting to view and print envelope images - but images of envelope and a tap (exhibit number [redacted] are marked as explicit. Should this be the other way around.

Some samples are showing full examination notes in exhibit testing table and some samples are not, but also not showing a preview of the notes when hovering over the '...' (blank text box appears). Examples provided. added 23/05/2022: microscopic result line also showing added information.

As per issue [redacted] - Below are several examples where the microscopic and DNA result lines are containing all results for the same samples. The result lines should only contain the result for that test. It is going back and doing this for all samples. Also when you hover over the "....." no extra information is appearing.
When the QPS tick the 'No testing required' box, a result line should appear and the sample should populate the 'NWQPS' result worklist. Instead of the 'No further work required as per advice from QPS' result line - as above, we are now getting the result line 'NTR' - see below (example [redacted]. We have found that samples are no longer populating our 'NWQPS' result worklist (Review Result WL). The samples still appear to be populating the generic result worklist, and are all viewable when filtering the list by 'NWDNA'. As well as the issue of the samples not populating the 'NWQPS' result worklist, we are also concerned that this new result line may not be getting fed back to the QPS.
Could we please have all samples with the 'no testing required' box ticked directed to the 'NWQPS' result worklist (Review Result WL), and also ensure that a no further testing result line is being fed back to the QPS?
Sample [redacted] resulted in a dropped down profile that was designated as UKM6. UKM6 now matches to a DNAIntel sample. when the user dropped the profile down again to get the new designation - it coped the profile down in the profile record twice. Is this because it was UKM6 and now is something else? it should only appear in the table once when the result is dropped down.

Also, UKM6 has dropped out of the case profiles record table. I know this has happened and has bene raised in issue [redacted] - but in those instance, it removed as it was being associated to a reference sample. In this instance, nothing has been associated (as we cannot associate UK to DNAIntels) - so am unsure if these are related.

Sample [redacted] - two STRmix PDF files were uploaded against this sample and both were viewable on the PDA screen at the same time. I've checked the STRmix files and while they have the same sample name, they have different case numbers. Just wanted to check what the FR is reading when they are uploading and storing STRmix files to the PDA page. Is it reading the file inside and just checking the sample number - or is it reading the file name and again just reading the sample number - or does it also check the case number. In this instance it looks like only the sample number is checked, and that both copies were in the FR and viewable at the same time because they didn't contain the same case number.

We wouldn't expect to see two copies of the STRmix file there - usually if the case number and sample number are the same it just overwrites the file.
We have noticed that when the Profile Data Analysis Worklist - Ref (Profile Data Analysis WL) goes above 100 samples, it will only show 100 samples. The batch dashboard (DNA Dashboard) will show the correct number of samples on the list.

Some users are getting an error when trying to access the P2 and P3 PDA worklists (some can get into 1 and not the other, some can't get into either - varies among users - however, I was able to access them both this morning, and now can't access P3 worklist). Also, it appears as though all samples have been allocated - they should not be. These are ones that have just the persons name and not their id and name. There should only be a PDA Analyst when the sample has been allocated

Sample [redacted] has a complex result - however it does not appear on the filtered P2 "complex" review list. It is there on the review list - but not when it is filtered

AK-38

Angelina Keller

From: Claire Gallagher
Sent: Thursday, 29 April 2021 2:42 PM
To: Alicia Quartermain; Deborah Nicoletti; Ingrid Moeller; Penelope Taylor; Angelina Keller; Emma Caunt; Kylie Rika; Tegan Dwyer; Josie Entwistle
Subject: Thanks for being a wonderful team

Follow Up Flag: Follow up
Flag Status: Completed

Hi All

I have put everyone's comments together in the format (sort of) of the questions in the survey. I have literally copied and pasted as I didn't want to change anyone's thoughts/words/intentions. There may be some duplication in areas, but all the info is there. Please pick and choose what is important to you and complete the survey by COB tomorrow.

Its very important that we all get this in. We need to be heard and if our thoughts align with people in other teams or even departments (Chemistry), then its even better. It is also important to focus on certain behaviours that have been on going and detrimental over the years despite having several culture change experts.

Thank you all for your input. I know we are all on the same page so it definitely helps with themes!

Here it is:

1. **Are there any improvement suggestions you think your unit could adopt to improve your services?**

- Maintaining skillsets that have been acquired through training

- Research and development team with collaboration with a Uni eg Griffith or UQ. R&D opportunities by EOI for papers/projects non routine work associated. E.g. MPS, NIFA, mRNA etc
- Increase our services to the standards of other labs across the country. YSTR's (in progress) minifiler etc

- **Research and development**
- HP5 Science advisor
- HP4/5 R&D coordinator
- Staff apply via EOI (or project idea) from all sub teams

2. **What successful improvements have been made by your unit that other teams could adopt or build upon?**

None really (Ingrid 😊)

I think about what we have in R2 and I would consider including in the response things such as:
- Team meetings
- Full transparency from manager
- Genuine consultation of staff and provision of information by manager in decision making
- Active commitment to positive morale and staff wellbeing eg supporting general flexible working arrangements, supporting formal FWAs, meeting activities (gratefulness, meditation, videos)
- EOIs for additional tasks/projects

- 'Safe space' for queries/ideas/concerns

All of these points require active positive modelling and active management/intervention by a manager.

3. What services or activities should FSS do more of or less of? Why?

- Staff rotation
- Reintroduce whole reporting team meetings – consistency in interps and therefore less incorrects to QPS
- Remove/refine proposed workflow for scientific disagreement workflow
- Allow and encourage staff to consult and communicate with external labs

- Increase all team cross-skilling and collaboration.
- Training for Senior management in transparency and accountability
- Consult/invite experts outside of our lab, also to do talks such as previous years by Andrew Selenicks from SA, chats about unusual paternity cases with stats experts such a Duncan Taylor.
- Change our focus back to the integrity and improvement of quality of Forensic Biology and DNA analysis, rather than QPS numbers.
- Brain storming sessions and brown paper
- Finalise all aspects of new process **before** implementing eg. Quants/insufficients with 3500.
- Manage with a plan, not with reactive management.
- Our process in terms of getting results out on time is efficient. But making staff feel valued, happy, listened to, not scared to speak up, and want to be here, would improve everything.

4. Do you have any other suggestions or comments related to providing forensic and scientific services effectively and efficiently in the future?

- Collaboration within and between teams
- Utilising SMEs in the interests of good science and making good scientific decisions

- To be only one reporting team.
- There are more efficiencies that we could make with reporting our results and statements in the FR by making the result lines simple, decrease the amount of intel letters, statement writing made more automatic.
- There are efficiencies we could make by not double handling results at PDA and then statement stage by allocating cases from the beginning.
- Also efficiencies by being able to triage samples – if we receive multiple samples from an item, test a couple and if the results address the allegation, put the rest on hold.

- Kylies initiated workflow change – implement - to be like P3
- Teams to have an expert led board/advisor role with regards to the science
- Involve staff in decision making/genuine consultation prior to a process being implemented.
- Managers to actively deal with difficult staff and those resistant to interp guidelines
- Prioritise fixes (FR)
- Restructure management/department

I think about not just regaining and retaining skills within the lab for individuals, and streamlining our reporting processes (Intel & evidential workflow proposal), but also a broader and more future focussed view on our capabilities and opportunities (not just immediate client needs) and I would consider including in the response:

- Consider emerging and existing (but not in use in lab) technologies and actively seeking to implement these (not just as instruments break down or on request of existing client)
- Actively seek and offer services for alternative/additional clients, including within government

- Cross sub-team skills development and retention

Actioning these points could:

- Attract more clients to the lab, bolstering our contribution to the community and reducing reliance on public funds
- Provide a more comprehensive service to existing clients, reducing delays in having external labs provide additional services, some of which may be limited in application with different statistical interpretation and/or different intelligence databases
- Provide more opportunities for skills development for staff, increasing morale and job satisfaction
- Assist in staff retention, especially where succession planning may not be in place
-

- Reallocation of resources – if a restructure is on the cards, perhaps re-instating the Intel Team with dedicated HP3s for Intel duties/PDA OR training a few HP3s (based on meritorious EOI) in PDA/floating FRIT HP3
- Examination of items - reporting consultation for large cases/unusual items
- Scientific board for laboratory process decisions to free up management for higher-level jobs
- Future planning – what other services could we provide QPS? Could we be finding other clients (paternity, immigration, ancestry etc)?

- Increase all team cross-skilling and collaboration.
- Research and development team with collaboration with a Uni eg Griffith or UQ. R&D opportunities by EOI for papers/projects non routine work associated. E.g. MPS, NIFA, mRNA etc
- Increase our services to the standards of other labs across the country. YSTR's (in progress) minifiler etc
- Team of expert scientists that manage projects with the oversight of quality. Once the project is completed, the management team should be approving/rejecting this. They should not be so actively involved in the actual running of projects eg implementation plans etc. This should be separate to the R&D team.
- Changes need to be made to the authoritarian structure and culture to be more cohesive and collaborative. 1st Call consultancy has made the relationship between staff and management worse. However, it has made the relationship between teams better and we are on more of a united front.
- Focus on staff – they are our biggest asset. Our process in terms of getting results out on time is efficient. But making staff feel valued, happy, listened to, not scared to speak up, and want to be here, would improve everything.

Thanks again for your great ideas. I will elaborate on some ideas in there in my survey.

Claire

From: FSS Comms <[redacted]>
Sent: Thursday, 29 April 2021 10:51 AM
To: DL-FSS-Campus-All-Staff <[redacted]>
Subject: Reminder: Invitation to participate in FSS staff survey

Good morning everyone,

I would like to thank those of you who have participated in our current internal analysis survey, and for the honest feedback that has already been received through this process. Please note that while the survey requires staff to log in with their email address, I can assure you that the data captured is absolutely anonymous and responses will be reviewed by the Executive Director before being more broadly summarised under themes for any further use. The first-step survey log in simply ensures that FSS staff are the only participants, and that only one submission per staff member is made.

Remember, sharing your experiences and insights is an important part of this internal analysis process, so again I encourage you to participate and share your ideas, to give the widest range of views across FSS.

The survey is available **here** and will remain open until COB tomorrow, Friday 30 April 2021.

A reminder also that we will be organising a small number of group forums to discuss suggestions and improvements within FSS. If you would like to participate in one of these discussions with your peers and colleagues, please use the voting buttons in this email and select "Yes, I would like to participate in a group discussion".

Thank you, and we look forward to hearing your views.

Regards
Malcolm

Malcolm Stringer
A/Executive Director

Executive Directorate, Forensic and Scientific Services
Health Support Queensland, Queensland Health

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AK-39

Angelina Keller

From: John Doherty
Sent: Friday, 3 September 2021 2:13 PM
To: Angelina Keller
Subject: RE: Catch up

Follow Up Flag: Follow up
Flag Status: Completed

Hi Angelina

Thank you for putting your trust in me. I'm sorry I couldn't see everything through for you, but I do hope that the future works out for you and your colleagues.

All the best

Regards

John Doherty (He/Him*)
Executive Director

Forensic & Scientific Services
Prevention Division, Queensland Health

p [redacted]
a 39 Kessels Road, Coopers Plains, Qld, 4108
e [redacted] w www.health.qld.gov.au/fss

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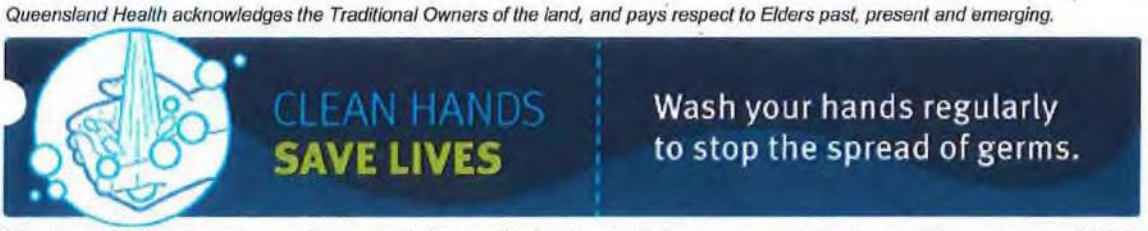

*if you're wondering about the use of pronouns He/Him on this signature block, I encourage you to read some of the resources available here

From: Angelina Keller <[redacted]>
Sent: Thursday, 2 September 2021 9:42 AM
To: John Doherty <[redacted]>
Subject: RE: Catch up

Hi John,

Well it seems that catchup is destined for another time / place / chapter.

All the very best for your next appointment. I hope I will be lucky enough to work with you in the future (have to put it out there).

I am very grateful for the time you have spent talking to me over your years here. It has been very valuable to be able to reach out to someone who cares and will listen with compassion. It has had a great impact in my working world.

Kind regards,
Angelina

From: John Doherty <[redacted]>
Sent: Friday, 30 July 2021 2:28 PM
To: Angelina Keller <[redacted]>
Subject: RE: Catch up

Hi Angelina

Yes, happy to do so. I'm back on 30th August. Please reach out again then and we can set a time.

Regards

John Doherty (He/Him*)
Executive Director

Forensic & Scientific Services
Prevention Division, Queensland Health

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*if you're wondering about the use of pronouns He/Him on this signature block, I encourage you to read some of the resources available here

From: Angelina Keller <[redacted]>
Sent: Friday, 30 July 2021 1:59 PM
To: John Doherty <[redacted]>
Subject: Catch up

Hi John,

I am aware that you are going on leave soon. When you return, would it please be possible to see you confidentially about Coronial cases and some clarity around permissions and training. I hope you are still prepared to talk to me (with Alison of course).

Kind regards,
Angelina

Angelina Keller
Reporting Scientist

Forensic DNA Analysis, Forensic & Scientific Services
Prevention Division, Queensland Health

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AK-40

Meeting was about Allan and Amanda not project #181
AK 20/07/2022

Enquiries To: Mr Jade Franklin
Manager Human Resources and Business Relationships
People Performance and Excellence

Telephone: [redacted]

Queensland Government
Health Support Queensland

Department of Health

Ms Angelina Keller
15 Dorsey Chase
Brassall Qld 4305

Dear Ms Keller

HSQ has appointed Livingstones to conduct an independent investigation into current practices relating to the Sensitivity of Spermatozoa Microscopy project, commonly known as project #181. I understand you work within the Reporting team and as such have been identified as someone who may be able to provide further information to assist with the investigation.

The investigator has been appointed under section 190 of the *Hospital and Health Boards Act 2011* (Qld).

Requirement to attend interview
As part of the investigation process, I invite you to attend an interview to be conducted by Mr Mark Brady from Livingstones. Mr Brady will contact you in the near future to arrange a convenient time to interview you.

You are entitled to have a support person present during the interview. If you wish to bring a support person, please advise Mr Jade Franklin, Manager Human Resources and Business Relationships, on telephone [redacted] who the support person will be at least 24 hours prior to the interview.

Lawful directions
Confidentiality
You are directed to keep the details of this matter confidential as far as possible. You may however discuss the matter with your support person, union or legal representative or Employee Assistance. If you need to discuss this matter with any other staff member you should make this request through Mr Franklin, Manager Human Resources and Business Relationships on telephone number [redacted]

Code of Conduct for the Queensland Public Service
You are directed to behave appropriately towards any person involved in this investigation. You are reminded the *Code of Conduct for the Queensland Public Service* clearly sets out the obligations that apply to you as a Queensland Health employee.

Should you fail to follow these lawful directions, you may be liable for disciplinary action that may lead to dismissal.

Other persons involved in the investigation have an obligation to behave appropriately towards you. If you feel someone is acting inappropriately towards you as a result of your involvement in this matter, I encourage you to immediately report your concerns to me.

Employee Assistance
Employee Assistance offers a confidential counselling service which is free of charge to all employees of Queensland Health for up to six sessions per calendar year. Access to this service is by self-referral. Please contact OPTUM on 1800 604 640. More information on Employee Assistance can be found at: http://qheps.health.qld.gov.au/eap.

Questions
If you have any questions in relation to the matters raised in this letter, please contact Mr Franklin on telephone number [redacted]

Yours sincerely

[redacted]
Sharon Kelly
General Manager
Community and Scientific Support
Health Support Queensland
16/1/17

AK-41

Angelina Keller

From: Cathie Allen
Sent: Friday, 1 December 2017 10:33 AM
To: Angelina Keller
Subject: FW: Appointment of Workplace Edge

Hi Angelina

My sincere apologies for missing you off the below email, its was an oversight on my behalf. I will schedule a discussion time for you on Tuesday.

Cheers
Cathie

Cathie Allen
Managing Scientist – Police Services Stream

Forensic & Scientific Services,
Health Support Queensland, **Department of Health**

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From: Cathie Allen
Sent: Friday, 1 December 2017 9:33 AM
To: Adrian Pippia; Allison Lloyd; Angela Adamson; Cassandra James; Matthew Hunt; Rhys Parry; Thomas Nurthen; Alicia Quartermain; Anne Finch; Deborah Nicoletti; Hannah Pattison; Ingrid Moeller; Josie Entwistle; Jacqui Wilson
Cc: Paula Brisotto; Kylie Rika; Amanda Reeves; Justin Howes; Wendy Harmer
Subject: Appointment of Workplace Edge

Hi Everyone

Michel Lok, General Manager for Strategy, Community and Scientific Support, along with the previous CEO Gary Uhlmann appointed Workplace Edge, specialist consultants, to provide guidance and support to improve our workplace and support the effective delivery of critical services. As part of this, Allan Holz from Workplace Edge would like to have a one on one discussion with you, which is scheduled for Monday and Tuesday next week. Shortly an appointment will be sent to you regarding the time that has been arranged for your discussion with Allan.

If you have any queries regarding this, please don't hesitate to contact me.

Cheers
Cathie

Cathie Allen
Managing Scientist – Police Services Stream

Forensic & Scientific Services,
Health Support Queensland, **Department of Health**

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w | www.health.qld.gov.au e | [redacted]

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