SECOND STATEMENT OF EMMA-JAYNE CAUNT

I Emma-Jayne Caunt of Queensland Health at the Forensic and Scientific Services, 39 Kessels Road, Coopers Plains, do solemnly and sincerely declare that:

1. This is my second statement provided to the Commission. It deals with scientific and cultural issues that I have concerns about at QHFSS.

Consistency between scientists

2. There are disagreements between reporting scientists in the laboratory about certain topics. I have seen these disagreements escalate into heated arguments. I have also observed heated discussions in meetings where individual scientists dominate the meeting with their opinion causing others to not participate even if they don't agree. As a result of this, there is a reluctance for scientists to engage in respectful scientific debate. This is further exacerbated by a lack of team meetings and management stepping in to address this behaviour.

Stutter threshold

- 3. There are two ways that stutter is assessed during interpretation:
 - a. STRmix assessment
 - b. Reporting scientist assessment.
- 4. STRmix has a built-in variance for stutter. The threshold for determining whether a peak in stutter position could be allelic or stutter will vary dependent upon the height of the parent allele. If the height of the parent allele is large, the stutter variance is lower, and the threshold for stutter will be lower. If the height of the parent allele is small, the stutter variance is higher, and the threshold for stutter will be higher.
- 5. Stutter thresholds used by the reporting scientists for assessing the number of contributors to a DNA profile are calculated from a dataset using the mean of the stutter peaks within the dataset + 3 x standard deviations (SD). This equation (mean+3SD) is used to calculate the stutter threshold for each different allele. For a peak that sits in stutter position, the reporting scientist will calculate the stutter peak height as a percentage of the parent allele peak height. If the calculated percentage is above the stutter threshold the peak in stutter position will be considered an allele, and below, a stutter.





6. In some circumstances, when the peak in stutter position is very close to the stutter threshold, the reporting scientist uses their discretion in determining whether they consider it an allele or stutter. This can affect the assessment of the number of contributors to the profile and therefore can also affect whether a profile is considered a mixture or single source.

Combined stutter

- 7. Stutter peaks can appear before and after an allelic peak, described as back stutter (-1 repeat) and forward (or post) stutter (+1 repeat) respectively. In some circumstances, another stutter peak can appear beside an existing back stutter peak, described as double-back stutter (-2 repeat).
- 8. Currently in Forensic DNA Analysis, STRmix is set up to model back stutter and forward stutter but is not set up to model double-back stutter, so if there is a peak in double-back stutter position, STRmix will model it as an allele, regardless of its height. For reporting scientists, this double-back stutter will also have a threshold of mean + 3SD. If the peak falls below the threshold then it is removed from the profile to prevent STRmix from calling it an allele.
- 9. If two different stutter types fall in the same position, this is called 'combined stutter'. For example, an allele at a locus may have a forward stutter and the other allele may have a back stutter that falls in the same position.
- 10. I have heard that some scientists have stated that they do not believe in combined stutter.
- 11. Not considering combined stutter could lead to an overestimation of the number of contributors to a DNA profile. While the overestimation of the number of contributors may not impact the overall interpretation of a DNA profile; the consideration (or not) of combined stutter can cause results to be changed at the statement preparation stage due to lack of agreement between scientists.
- 12. The journal article Taylor D, Bright J-A, Buckleton J. The interpretation of single source and mixed DNA profiles. Forensic Science International: Genetics. 2013;7:516-28 states that "Expected peak heights are assumed to be additive when there are multiple contributions to a peak, whether from multiple alleles or a combination of alleles and stutter". Furthermore, page 43 of the STRmix v2.8 User's Manual describes how STRmix calculates the expected height of a peak using the additive effects of different stutter types and allele.



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Number of contributors

- 13. There are differing opinions between reporting scientists in the laboratory regarding the determination of the number of contributors in mixed DNA profiles.
- 14. For example, if there are four peaks on an electropherogram, it is possible that there are two people with different information, or there could be four people who share information. When different people share information, this can result in allelic imbalance where there is differing intensity between two alleles at a locus. This is particularly evident when two people share one allele but not the other; the shared allele will have twice the intensity of the non-shared alleles.
- How much allelic imbalance is allowed before it becomes another contributor varies between scientists.

Removing loci

- 16. Sometimes large peaks can cause 'pull-up', which is where light from one dye bleeds into another causing a peak that isn't DNA. Reporting scientists need to remove any pull-up peaks from a profile otherwise STRmix will consider them to be alleles.
- 17. If a pull-up peak corresponds with a stutter peak, then that stutter peak may be inflated such that it is higher than the stutter threshold. This may be referred to as 'pull-up affected stutter'.
- There are a number of ways that pull-up affected stutter can be dealt with, one of those ways involves removing the locus from the STRmix analysis.
- 19. Removing a locus from the STRmix analysis may affect the modelling of the profile, as STRmix considers the profile as a whole. While a whole locus can be removed from the STRmix interpretation, I believe it is a problem if removal is occurring at two or three loci as this reduces the amount of information available for STRmix to consider and could ultimately affect the likelihood ratio and the ability to resolve a DNA profile for upload to NCIDD.
- 20. There are some scientists that are removing two or three loci from their STRmix analyses.
- 21. I wrote a workflow on how to deal with pull-up affected stutter in October 2021 and provided it to Allison Lloyd who was the Acting Team Leader of FRIT at the time. Annexed and marked EC-01 is a copy of this email and workflow.



- 22. On 26 October 2021 I emailed Sharon Johnstone, Kylie Rika, Allison Lloyd and Justin Howes (being the management of FRIT) asking how they would like to proceed with the workflow. I received no response. Annexed and marked EC-02 is a copy of this email.
- 23. On 2 November 2021, and numerous times since, Kylie Rika emailed Justin to follow up on this issue. Annexed and marked EC-03 is a copy of this email.
- 24. I have not been advised of any actions taken regarding my concerns. I do not feel that my voice is being heard on this issue.
- 25. The removal of loci is not recorded in statements provided to the QPS. The removal of loci is only recorded in the case file.

Recent communication

- 26. On 31 May 2022, Kylie Rika sent an email to Justin outlining the minutes from a meeting she and Sharon had the week prior. The email listed issues raised by Angela Adamson, Cassandra James and I about inconsistencies with interpretations, and outlined possible solutions for each one.
- The issues included the inconsistencies between how scientists approach combined stutter and the workflow that I created in October around pull-up in stutter position (including removal of loci).
- 28. In response to this email, Justin stated that he had asked BSAG their opinions of dealing with stutter affected by pull-up and had kept the survey in G:/drive. He did not tell me that he was seeking that information. Annexed and marked EC-04 is a copy of this email chain and spreadsheet.
- 29. I have read the BSAG excel spreadsheet, and every interstate opinion represents the same position as me about when and how many loci to remove. Justin had this information last year and he did not tell me about it. I don't feel like Justin is working with us to make decisions.
- 30. I believe it is a really big concern that scientists are removing loci differently.
- 31. The issues with different scientists removing loci differently presents the following issues:
 - Inconsistency in the reporting of results means that an interpretation of a DNA profile could have a different outcome depending on which scientist reports the results.
 - b. Removing multiple loci from an interpretation reduces the amount of information available to STRmix to model a profile.





c. Any loci removed from an interpretation are not able to be included in the calculation of the likelihood ratio, and therefore any inclusionary/exclusionary information at these loci is lost. This means that the likelihood ratio could be larger or smaller depending on the information available at the dropped loci.

Validations

- 32. The purpose of validation is to ensure that a method is fit for purpose in the laboratory and meets the requirements for its specific intended use.
- 33. Validation is tailored to each instrument or system. For example, the validation of STRmix included assessing DNA profiles with known contributors against the STRmix output to ensure that what STRmix called the profile was reflective of what was in the sample. It also included examining likelihood ratios, anomalous results, and whether the set of diagnostic figures were in range.

STRmix

- 34. A mixed DNA profile can present in different ways on an electropherogram. In some cases, there are larger amounts of DNA from one contributor and smaller amounts from another contributor. As peak heights are proportional to the amount of DNA present, this allows for a visual separation of each contributor's profile by the reporting scientist.
- 35. Mixtures consisting of even contributions of DNA present as peaks the same size. There are multiple ways a person can contribute to those peaks, with multiple combinations of alleles which cannot be distinguished visually by the reporting scientist.
- 36. When data from an electropherogram is entered into STRmix along with the number of contributors, STRmix considers all possible options for contribution and provides probabilities, or weights, for each different allelic combination.
- 37. I have either undertaken or overseen all validations of STRmix in Forensic DNA Analysis at FSS since its implementation in 2012, except Version 2.6.2 due to a period of leave:
 - a. Version 1.05 was validated in 2012 and implemented in December 2012.
 - b. Version 2.0.1 was validated in June 2014 and implemented in July 2014 due to additional functionality.
 - c. Version 2.0.6 was validated in implemented in January 2015 to fix a miscode found in version 2.0.1.



- d. Version 2.6 was validated and implemented in 2019 due to improved programming which allowed profiles to be interpreted where previously computing power was inhibitive.
- e. Version 2.7 was validated in November 2019 and implemented in February 2020 to fix issues found with modelling profiles in version 2.6.
- f. Version 2.8 was validated and implemented in 2021 due to the inclusion of the VeriFiler Plus kit and improved batching capability.
- 38. In some instances, the validation of a new version did not lead to its implementation:
 - a. In version 2.3, there was a change in the biological modelling and the way STRmix modelled stutter peaks. The previous stutter modelling allowed more flexibility in the height of stutter peaks, and the new version reduced that flexibility. This change required a completely new data analysis of stutter peaks, and version 2.3 was not completely validated or implemented.
 - b. STRmix v2.5.11 was validated in conjunction with the 3500xL Genetic Analysers (3500) in 2018. During the early stages of the validation of the 3500, the peak heights were so large they were causing 'pull-up' in the baseline which interfered with interpretation. When the 2018 validation was completed, the pull-up peaks were less significant, but ultimately it was recommended that the 3500 and STRmix v2.5.11 not be implemented.
- 39. I don't believe the samples used for the validation of STRmix with PP21 in 2012 were affected by the 3/5 second issue on the 3130xl Genetic Analyser (3130). It is my understanding that the affected samples were isolated and reprocessed.

ProFlex

- 40. The ProFlex validation was undertaken through Project #199 Verification of ProFlex™96 Well PCR System using PowerPlex®21. The project was completed on 22 December 2021.
- 41. When the experimental design for Project #199 was being reviewed by the management team, Kylie Rika asked me to have a look at it and provide her with any feedback. I recall advising Kylie that we should complete a Model Maker analysis as the ProFlex instruments may cause a change in peak height variability.
- 42. STRmix relies on information that describes how DNA profiles behave in the laboratory such as peak height variation for both allelic and stutter peaks and variation in amplification efficiency between loci to interpret a DNA profile after a sample has been

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amplified. If anything changes in the amplification process, it is important to ensure that the settings within STRmix are appropriate for that change.

Model Maker

- 43. Model Maker is the module of the STRmix software that determines how the variability between samples is accounted for, so if an instrument that affects variability is changed, Model Maker should be considered in the validation/verification of that instrument. For example, any time there is a camera change in the Genetic Analyser, Model Maker is repeated because that change can affect peak heights.
- Following her response from Luke Ryan, Kylie asked me to seek advice from STRmix Support, which I did on 31st March 2021.
- 45. The feedback I received from STRmix Support was a recommendation to re-run Model Maker to determine whether the ProFlex instruments had affected the peak height variance parameters. The second recommendation was to carry out a performance check of STRmix by comparing the results obtained from samples amplified using the old instruments and the new ProFlex instruments. I recall later being told by Justin Howes, who was the Team Leader at the time, that I was not to contact STRmix Support because it costs money and that any requests to STRmix Support were to be made by him.
- 46. Justin Howes asked Angela Adamson, Cassandra James, Allan McNevin and I to look at ProFlex and STRmix and provide advice about the work that should be done; the four of us met to discuss this. At this time, Allan was a member of the management team and the way that he communicated during our meeting gave me the perception that he had some prior background knowledge of how the management team were expecting the Model Maker analysis of the ProFlexes to be performed. I felt like we had to reduce the amount of work that had been suggested by STRmix Support in order to gain the support of the management team. I recall that Allan was of the opinion that Model Maker wasn't something that needed to be done during the validation of the ProFlex instruments but could be done as part of the implementation. I didn't think there was any point in disagreeing, although my view was that we should do the work suggested by STRmix Support.
- 47. Sometime in January 2022, I found out that ProFlex had been implemented and that no Model Maker work had been performed. I recall discussing this with Justin and Kylie, including whether casework on the ProFlex instruments should be stopped until the

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Model maker analysis had been performed. Justin said that since the ProFlexes had only just been implemented and given the size of the work lists in the FR, that it would be a while until any samples processed on the ProFlexes would be analysed and therefore there would be no need to stop using the instruments.

- Model Maker was eventually considered, with results reported on 25 March 2022 in the document titled "Summary – Model Maker results for Project #199".
- 49. At the request of Justin Howes, this report was written on a 'Minor Process Change' form, which indicates that Model Maker was considered to be a minor change in process when it should have been part of the original ProFlex validation. Annexed and marked EC-05 is a copy of the Minor Process Change document.
- 50. The Model Maker analysis showed that there was a difference between the settings currently used in the laboratory and the settings required for the ProFlex instruments.

Results

- 51. The Model Maker module of STRmix calculates four different settings:
 - 1) Allele variance
 - 2) -1 repeat stutter variance
 - 3) +1 repeat stutter variance
 - 4) LSAE variance (locus specific amplification efficiency)
- 52. The +1 repeat stutter variance and the LSAE variance obtained from the ProFlex instruments were found to be significantly different from the current settings. If a DNA profile was analysed with STRmix with the current settings, a peak in the +1 repeat stutter position would be more likely to be considered to be a stutter than if the same profile was analysed in STRmix using the ProFlex settings; using the ProFlex settings the same peak is more likely to be considered an allele. This could cause the likelihood ratio to be different under current and ProFlex settings.
- 53. It is the case that if the ProFlex instruments are validated without running Model Maker, that STRmix's interpretations of peaks may be affected.
- 54. I am of the opinion that the ProFlex instruments were not validated from beginning to end before they were implemented, and Forensic DNA Analysis is still interpreting results with settings that may be incorrect. However, I am of the opinion that the overall impact and risk of obtaining inaccurate results is low, because any difference in likelihood ratio between the two different settings is likely to be small.



OQI

- 55. The new STRmix variances for the ProFlex were calculated as per the report "Summary – Model Maker results for Project #199", however prior to implementation of the new settings I identified an error in the Model Maker analysis.
- 56. Justin told Angela, Cassandra and I to write an OQI to document this error.
- 57. QIS 13965v16 Opportunity for Quality Improvement (OQI) Management Procedure (HSQ) states that "OQIs should not be used for.....minor methodology or QC errors until the problem becomes systemic, calamitous or a regular occurrence..."

58. I recently sent an email to Justin asking why an OQI would be required for this error since implementation had not occurred and therefore there was no risk to casework. Justin's response implied that he still wanted the OQI to be raised. Annexed and marked EC-06 is a copy of this email.

- 59. In this email I also suggested that an OQI should be raised for the implementation of the ProFlex instruments without Model Maker being performed as I considered this to be systemic. Justin did not respond to this suggestion.
- 60. The Model Maker analysis for the ProFlex instruments is ongoing.

VeriFiler Plus

- 61. VeriFiler Plus is an amplification kit.
- 62. The validation of VeriFiler Plus (Project #213) began in mid-2019 and has been ongoing for over 3 years.
- 63. In the early stages of the project, it was decided that the validation of STRmix for VeriFiler Plus would be incorporated into the VeriFiler Plus validation.
- 64. The data from the analytical component (Project Report #213 VeriFiler[™]Plus Full volume Amplification) showed that one locus amplified with much less efficiency than the rest and that the reproducibility indicated that there could be issues with interpretation.
- 65. I raised this as a problem to Kirsten Scott, who is the Project Manager, and she said that the analytical part of the project is only to "categorise the kit", and it was up to the reporters (Cassandra James, Sharon Johnstone and myself) to determine whether the kit was fit for purpose, which it appeared not to be. I was told to keep going with the validation by Kirsten.



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- 66. As part of the validation, Cassandra and I needed to analyse the stutter data in order to calculate stutter thresholds. At that point in time, I considered that the current stutter thresholds were too high and believed this was the perfect time to lower them. Luke Ryan did not agree even though he had previously agreed in principle when I first raised the idea.
- 67. Cassandra and I had to do hours of work to produce data to show that VeriFiler Plus was not suitable for use, even though I had already highlighted the issues before we started our work.
- 68. Whilst struggling with the afore mentioned aspects of the VeriFiler Plus kit, I was asked by Kirsten Scott whether we could use VeriFiler Plus as a backup rather than the primary kit.
- 69. Since Cassandra and I drafted a report to show that VeriFiler Plus was not suitable for use, further work has been performed. On 25th August 2022 I sent an email to Kirsten Scott and Sharon Johnstone explaining my opinion that we not do any further work on the VeriFiler Plus project. Kirsten insisted that the work needed to be completed and that three reports need to be produced by Sharon, Cassandra and I; this is hours of work. Annexed and marked EC-07 is a copy of this email. Of note is that one of the reports is the *VeriFiler Plus Stutter* which has been in draft for over a year as Luke Ryan does not agree with lowering the stutter thresholds (as per paragraph 66). I don't feel like I am part of the decision-making process about how we should progress this project.
- 70. The VeriFiler Plus work is still in progress.

Quantifiler Trio

- 71. Quantifiler® Trio is a quantification kit.
- 72. In my opinion there are issues with the validation of Quantifiler Trio. The validation report (Project #152) investigates how long the quantification standards (used to estimate how much DNA is in a sample) remain stable. Section 6.2 recommends that the standards be used for up to four weeks, the conclusion and recommendations state five weeks, however page 27 of the manufacturers user guide states not exceeding two weeks.
- 73. Section 6.3 of the validation report states that the limit of detection of Quantifiler Trio is 0.001ng/µL, however the experiments did not test any samples with a concentration less than 0.001ng/µL. I do not understand how a limit of detection can be set without testing

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whether the system can detect DNA in samples known to contain less DNA than the proposed limit of detection.

74. The validation did not amplify any samples and therefore provides no information about the ability to obtain a usable DNA profile for samples with known concentrations. Since the limit of detection was then used as a threshold to cease amplification of samples (no DNA detected), the ability to obtain useable DNA profiles from samples with concentrations less than the limit of detection should have been tested.

PowerPlex 21

- 75. Since I validated the use of STRmix for the interpretation of DNA profiles amplified using PowerPlex 21 (PP21), I was involved with the PP21 validation.
- 76. A full volume amplification involves adding 15µL of sample to the amplification reaction; a half volume amplification involves adding 7.5µL of sample to the amplification reaction. A half volume reaction requires less reagents than a full volume reaction and is therefore more cost effective. The PP21 validation involved the validation of both full and half volume amplifications. Following validation, half volume amplifications were implemented into routine casework.
- 77. I recall telling Paula that I didn't think we should be implementing PP21 at half volume because it caused problems with interpretations, Paula told me that we had to implement half volume because Cathie Allen said so.
- 78. The PP21 validation then stated that the optimal input template was 0.5ng. In 2014, Robert Morgan and I undertook an optimisation project (Project #141) that found that 0.5ng may not be the optimum input template but was more likely to be around 0.7ng or 0.8ng. This meant we may not have been adding enough sample to our amplifications and minor DNA profiles may not have been detected. The optimisation project was never completed so no project reports exist, however some presentations were provided to the management team and to staff. Annexed and marked EC-08 is a copy of a presentation dated 8 July 2014. Annexed and marked EC-09 is a copy of a presentation titled 'PP21 Optimisation Phase 1B'. Annexed and marked EC-10 is a copy of a presentation given to the Forensic Reporting and Intelligence Team on 30 October 2014.

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3500xL Genetic Analyser

- 79. The initial validation of the 3500xL Genetic Analyser (3500) for casework samples started in 2015. The DNA profiles generated by the 3500 were unable to be interpreted due to the interference of really high pull-up peaks. The initial project was Project #145 which went on to become Project #186.
- 80. The validation went on for a few years due to the inability to obtain interpretable profiles before Cassandra James, Angela Adamson and I were asked to complete the validation of STRmix for the 3500 in 2021 (Project #219). This project involved the creation of new samples and therefore new DNA profiles. These profiles had good peak heights, clean baseline and little to no interference from pull-up
- 81. On reflection, I believe that the pull-up that interfered with the initial validation could have been caused by an issue with the quantification of the samples at the time of the initial validation. Since nothing had changed, this is the only explanation I can come up with.

Other issues

Forensic Register automation

- 82. The Forensic Register drives the workflow of DNA analysis by automatically putting samples onto the relevant 'lists' depending on the results recorded.
- 83. Prior to implementing the threshold of insufficient DNA between 0.001ng/µL and 0.0088ng/µL in 2018, the Forensic Register would automatically assign all samples with quant values within this range to the Microcon list.
- 84. After the 2018 threshold was implemented and the results between 0.001ng/µL and 0.0088ng/µL were considered 'insufficient DNA for further processing' (DIFP), the samples were automatically added to a list for validation before being reported as DIFP on the FR.
- 85. I believe the validator of that list was Luke Ryan and staff from the Analytical team, not reporting scientists from the Reporting team.
- 86. I believe validation of these samples consists of clicking a checkbox labelled 'validate'. I believe the validation should be done by a reporting scientist and involve reviewing the quant value, the results of any body fluid testing and the details of the sample in relation to the case to determine whether processing of a sample should continue.



87. I am the reviewer of the **Constant Constant** case. I completed my review of the final statement on 23 February 2017. I was on leave when the issues arose around this case. I have had one meeting with Kylie Rika and Rhys Parry about the case since my return, but no one above Kylie has spoken to me about the matter.

CTS testing

- 88. Collaborative Testing Services (CTS) is a US based company that undertakes the proficiency testing for FSS.
- 89. Each reporting scientist is required to undertake at least one interpretation and one review for a normal case each year, and a paternity case each year.
- 90. For a 'normal' case, the CTS test only requires the generation of a DNA profile and the allelic designations, e.g., "in locus **case, the set of the analytical system.**
- 91. Based on our experience, we expect that DNA profiles generated from CTS samples only contain one or two contributors not higher order mixtures containing three and four contributors. This can also affect the interpretation, because if there are indications of an additional contributor, the scientist may explain it away as high stutter as they know what the outcome is likely to be.
- 92. The proficiency tests are not blind; we are emailed by a member of the Quality Team saying there is a case assigned to us. It has an FR number, but not a QP number, which means that CTS tests are not able to be reported in the FR in the same way as a routine case.
- I recently reviewed a CTS case for Angelina Keller where we obtained a three-person mixture which caused Kirsten Scott to raise an OQI.
- 94. I believe that CTS send some kind of report detailing the results of the proficiency tests. We are not provided with the report, but we do get an email from Kirsten or a member of the Quality team detailing the tests performed and whether we met the requirements.
- 95. When I worked at the FSS in the UK, we had proficiency tests that were completely blind that came in as a submission from the police. The scientist had no idea that it was a proficiency test until the results came in.

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Sperm microscopy

- 96. I was Amanda Reeves support person during the time sperm microscopy was raised as an issue (beginning in 2016), and attended all meetings related to her issues. Due to this I tried to remain impartial and didn't raise any issues that I had personally.
- 97. After Amanda raised her concerns with sperm microscopy, she took a period of leave and when she returned, she was moved to the library to do alternative work and not return to her reporting team. Management didn't explain to me why she was moved.
- 98. The way Amanda was treated by management has made me feel more hesitant to raise issues in the laboratory.
- 99. I was involved with Project 181 at the beginning I was asked to work on this with Allan McNevin. Due to an extended period of leave in 2019 I was taken off of the project and Matthew Hunt took over. I haven't had anything to do with Project 181 since before my leave in 2019.
- 100. I have never seen the ESR report and wasn't involved in it being obtained. My understanding is that ESR did not visit the lab and that their review was only a paper exercise.
- 101. As far as I am aware, we did not go back and examine the diff lysis slides from old cases to see if sperm were present that were not originally detected but I believe that we should have done this.

Issues with culture/management

- 102. I don't think we have a good culture at QHFSS. Cathie and Justin don't talk to us about decisions in the lab. We don't have many meetings to discuss science or issues that arise. I don't feel like it is an environment where everybody has the opportunity to share their views.
- 103. The following paragraphs set out some of the cultural and management issues that I have seen and encountered in my years working at FSS.

External contact

104. I was told by Justin that I was not to contact other laboratories but he has not provided me with a reason for the blanket rule. He said that the lab in SA didn't want Duncan Taylor to be contacted all the time by other labs, but Duncan has since told me that I



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can contact him at any time. Being unable to confer with other scientists outside of our laboratory is an issue for areas where we do not have the appropriate expertise.

- 105. I recall in December 2020 that I had a case with a mixed product of conception for paternity determination. I had never done one of these before and there was no information in the standard operating procedure. I derived the formulae myself and compared them with a case that had been done previously by a different reporter. My formulae were different from theirs and I wanted to obtain advice from other labs regarding which way was correct. I asked Justin if I could contact the other labs and he would not allow me to. He told me that he thought that my formulae were wrong but couldn't explain why. It took some time for me to convince him to let me seek external advice, but I had to put my email together and let him check it before it could be sent. The advice received was that my formulae were incorrect, but I was provided with an explanation as to why they were incorrect which gave me the confidence to report the final result.
- 106. I would like to contact people at other laboratories to ask advice about issues we may encounter within the lab. Another lab may have a solution or be experiencing the same issue. There are also many things that we have little knowledge of; there are many experts across Australia that can help us learn. Not being able to contact people at other laboratories is isolating. There is also the risk that our processes may deviate from best practice if we don't keep abreast of what other labs are doing.

Response to issues and OQI's

- 107. Generally, if I raise an issue to Justin Howes or Paula Brisotto, I will provide examples, but I don't feel like my opinion is valued. I don't feel like I am included in making decisions.
- 108. In 2018 after the validation of the 3500's, peak heights were of a reasonable height and profiles were easy to interpret, however now the peaks heights are much larger and show issues with pull-up which affects the interpretation. When I raised this, I was told by Paula that the examples I was giving her were a few months old (as is the case due to the backlog in reporting) and that the samples being processed right now are probably fine.
- 109. I believe that OQIs are an issue because management are very particular, and inconsistent, about what does and does not require an OQI. Something that might meet



the criteria of an OQI may be dismissed by management and recorded in some other way, like an adverse event.

Intelligence reports

- 110. Occasionally it is necessary to change the interpretation of a result that has already been reported to the QPS. This is often due to our workflow of the scientist reporting the original result being different from the scientist writing the statement.
- 111. When a result is changed, the QPS may be advised of this change via an intelligence report.
- 112. Appendix 7 of QIS 34308v3 *Procedure for Intelligence Reports and Interstate/Interpol Requests* provides three options for the reason for changing a result:
 - 1. Change of result due to receiving a reference sample,
 - 2. Case consistency,
 - 3. Unintentional human error.
- 113. For one of my matters I wanted to explain the reason for changing a result as being a difference of opinion between myself and the original scientist as we had a different opinion of how many contributors there might be to the DNA profile. When Cathie Allen reviewed my intelligence report explaining the change in result, she wanted me to use the reason of 'unintentional human error'. I didn't think this was accurate because, when I asked them, the original scientist and reviewer stated "I think this one is borderline for me, I can see why you would call it complex...." and "I'm ok if you want to complex it, I can see it both ways....". Neither of these statements indicated that an error had been made in the original interpretation, more that the opinion of the original scientist and
 - reviewer differed from mine. Annexed and marked EC-11 is a copy of the email strings and draft reports. Annexed and marked EC-12 is a copy of my personal notes in relation to this event.
- 114. Eventually Cathie asked another scientist to write the report even though I had carriage of the case and issued a statement of witness. This report used the wording required by Cathie.

Confidential bin issue

115. On 30 April 2018 I received an email from Cathie asking me to attend a meeting with her and a HR representative. The email provided no agenda for the meeting and



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included a 'lawful direction' to keep this matter confidential. The email also stated that further action may be required. Annexed and marked EC-13 is a copy of this email.

- 116. In the absence of any information surrounding the meeting and the fact that I felt threatened, I engaged an Industrial Advocate who was unable to attend at the time Cathie had arranged for the meeting and requested a change of date. Annexed and marked EC-14 is a copy of the email chain.
- 117. Since my advocate was unable to attend the meeting at the given time, Cathie sent me an email requesting that I find another support person. Annexed and marked EC-15 is a copy of this email.
- 118. I responded to Cathie stating that I would not find another support person and forwarded this email to my advocate stating "I'm terrified". Annexed and marked EC-16 is a copy of this email chain.
- 119. The meeting progressed on Thursday 4th May 2018 where Cathie asked me if I had seen anybody place anything in the confidential bin on 29th March that shouldn't have been put in there, such as diaries. I told her that 29th March was a long time ago so I couldn't remember. She said that items were found in the confidential bin that should have been retained. I asked her if she was concerned that somebody had been through the confidential bin given that it contained confidential documents. Cathie said that she had access to the key. She told me that she would speak to Paul Csoban about our conversation, and she would let me know if there was to be any further action. Cathie has never spoken to me about this incident since.
- 120. I found this whole incident incredibly stressful. I was terrified and physically ill because I had no idea what the meeting was about, and I had been given a lawful direction.
- 121. With hindsight, I believe that this incident was borne out of my friendship with Amanda Reeves as Ingrid Moeller and Kylie Rika, who are also friends with Amanda, received the same meeting request from Cathie. The date in question, 29th March 2018, was Amanda's last day at Forensic DNA Analysis. I recall Amanda attending the lab to clear her desk and I assisted her along with other colleagues.
- 122. I believe that Cathie came into the lab on 30th March 2018 (which was the Good Friday public holiday) and gained access to the confidential bin. Annexed EC-17 is a screenshot from AUSLAB showing that Cathie tracked a casefile from Amanda's desk to the admin area on 30th March 2018.





Approaching the Commission

- 123. I approached the Commission voluntarily as I felt that this was my only chance to be heard, however I did not make this decision lightly. I first met with staff from the Commission at a public library on a weekend because I was concerned about being seen going to the Commission's office.
- 124. I was scared of colleagues and managers finding out that I was speaking with the Commission as I was scared of retribution and how I would be treated by others. I have worked hard to ensure that people outside of my support network did not find out that I have been speaking with the Commission. Although I do not want to, I have also considered the possibility that I may have to leave my job as a result of speaking with the Commission.

Improvements

125. The following paragraphs set out some of my ideas to improve the processes of the laboratory.

QFlags

- 126. Before the interpretation stage, managers use the QFlag system to check contamination against staff, QPS or other known profiles. The QFlag system utilises the FR to check every profile against the staff database and flags any matches with 12 alleles in common. A manager at a HP5 or HP6 level will check the samples that have been flagged to determine whether contamination could have occurred.
- 127. If a profile appears to have been contaminated by a QPS staff member, the information is sent to the QPS for investigation.
- 128. This process is not fit for purpose, it is a like-for-like process with the old AUSLAB system, and it is possible that, even after the QFlag process has been completed, contamination can still be identified by the reporting scientist. This shows that the current QFlag system is flawed.
- 129. This contamination identification can be done by STRmix. Instead of randomly matching alleles and intuitively looking at each possible contributor, STRmix can consider the possible contamination and provide likelihood ratios.
- 130. This is an example of the laboratory not embracing the full capacity of our current technology.

Emma-Jayne Caunt

THE PEACE

1011

Mixture searching for intelligence purposes

- 131. STRmix also has the capability to perform database searches that is not currently being utilised by the laboratory.
- 132. NCIDD only has the ability to search single source profiles to identify matches. STRmix has the ability to search a database of reference samples against mixed DNA profiles to identify possible contributors. This could be particularly useful where a single contributor cannot be resolved from a mixed DNA profile.
- 133. I spoke to Justin about the QFlags and mixture database searching in 2014. He said they were good ideas, but the proposals never progressed even though he wanted 'ideas for improvement'. The matter has not been raised again.

DBLR

- 134. DBLR is an addition to STRmix that has many capabilities, of most interest currently is its kinship calculation ability. The system we currently have for kinship calculations doesn't allow us to calculate likelihood ratios for mutation events or linkage.
- 135. We have had trial licences to evaluate the use of DBLR three times (to establish what it does, how it does it etc.):
 - 1. Project #225 (March 2021),
 - 2. Evaluation in August 2021,
 - 3. Project #238 (current).
- 136. As a part of the ANZFSS Conference held in Brisbane in September there was a DBLR workshop. I asked Justin Howes if the department would fund my attendance at this workshop (\$195) to assist with the completion of the project report for Project #238. I had no response. Annexed and marked EC-18 is a copy of the email string where I requested this funding.

Full time project officer

- 137. I believe that the laboratory would benefit from having a scientist employed to work on projects fulltime. We have so much capability within current software that we could be implementing that we are not.
- 138. Projects currently are piecemeal and generally do not include scientists with the appropriate knowledge, expertise, and experience. Many projects performed in the analytical sections are around instruments and reagents, but analytical staff do not

Emma-Jayne Caunt

necessarily understand the impacts that these instruments have on final results and therefore the projects would benefit from input from a reporting scientist.

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

I make this solemn declaration conscientiously believing the same to be true 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.

	 		ICE OF THE PEACE (QUALIE)
Emma-Jayne Caunt	Witness	Rene The kov (JP. Qual)	Reg.No.: 123143





Schedule of Exhibits

EC-01	Email from Allison Lloyd to Allan McNevin, Cassandra James, Angela	
	Adamson, Emma Caunt, 'Proposed Flowchart for dealing with Pull up	
	peaks in Stutter position, 15.10.2021.	
EC-01-1	Workflow for pull-up affected stutter peaks.	
EC-02	Email from Emma Caunt to Allan McNevin, Cassandra James, Allison	
	Lloyd, Sharon Johnstone, Kylie Rika, Angela Adamson, Justin Howes,	
	'RE: Something to think about - Workflow for pull up affected stutter	
	peaks, 20. 10.2021.	
EC-03	Email from Kylie Rika to Angela Adamson, Emma Caunt, Allan McNevin,	
	Cassandra James, Allison Lloyd, Sharon Johnstone, Justin Howes, 'RE:	
	Something to think about - Workflow for pull up affected stutter peaks', 02.11.2021.	
EC-04	Email chain between Kylie Rika, Justin Howes and Emma Caunt, 'RE:	
	PIM agenda meeting and actions', 22.08.2022.	
EC-04-1	Excel spreadsheet: Feedback from jurisdiction 'Pullup and ignore locus_	
EC-05	Minor Process Change, 'Summary - Model Maker results for Project #199', 25.03.2022	
EC-06	Email from Justin Howes to Emma Caunt, 'RE: Model Maker for	
	Proflexes', 12.08.2022	
EC-07	Email from Kirsten Scott to Emma Caunt, Sharon Johnstone, Cassandra	
	James, 'Verifiler next steps for reporting sub-team', 25.08.2022	
EC-08	Presentation slides: PP21 Optimisation Update, 08.07.2014	
EC-09	Presentation slides: PP21 Optimisation Phase 1B, undated.	
EC-10	Presentation slides: PowerPlex Observations, October 2014.	

Emma-Jayne Caunt

Witness

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EC-11	Email chain between Emma Caunt and multiple scientists, 'RE: Intel report, 08.06.2022.
EC-12	Handwritten notes: 'Incorrect result', 25.05.2022 - 17.06.2022.
EC-13	Email from Cathie Allen to Emma Caunt, 'Meeting', 30.04.2018.
EC-14	Email from Brian Newman to Cathie Allen, 'Re: Proposed meeting with Emma Caunt and Kylie Rika', 02.05.2018.
EC-15	Email from Cathie Allen to Emma Caunt, 'Meeting', 01.05.2018.
EC-16	Email from Emma Caunt to Brian Newman, 'RE: Meeting', 01.05.2018.
EC-17	Screenshot: AUSLAB 'Specimen Audit History'.
EC-18	Email from Emma Caunt to Justin Howes and Sharon Johnstone, 'RE: DBLR workshop', 25.08.2022.



Witness



EC-01

Emma Caunt

From:	Allison Lloyd
Sent:	Friday, 15 October 2021 4:33 PM
To:	Allan McNevin; Cassandra James; Angela Adamson; Emma Caunt
Cc:	Kylie Rika; Sharon Johnstone
Subject:	Proposed Flowchart for dealing with Pull up peaks in Stutter position
Attachments:	Pull up in stutter position Flowchart.pdf

Hi Strmix group,

During the last Profile Interpretation Meeting, the issue with what to do with pull up peaks when they are in stutter position was raised.

Emma has prepared a proposed workflow for dealing with these pull up peaks which has been reviewed by the FRIT seniors. We would like your opinions as Strmix SMEs as to your opinions on this workflow in case there are other things to consider that haven't been thought of, etc. Could you please take a look at provide any feedback to Kylie, Sharon or myself sometime next week? The aim is to then send the flow chart to the rest of the case managers for their feedback.

Thanks in advance,

AL



Allison Lloyd A/Team Leader - Forensic Reporting and Intelligence Team

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

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If a peak is affected by pull-up it is always best to attempt to eliminate the pull-up, this may involve amping at a lower template or re-CE. If the pull-up is unable to be eliminated then this workflow describes how to deal with peaks that may ordinarily be stutter but are elevated in height due to the effects of pull-up.

EC-02

Emma Caunt

From:	Emma Caunt
Sent:	Tuesday, 26 October 2021 12:39 PM
То:	Allan McNevin; Cassandra James; Allison Lloyd; Sharon Johnstone; Kylie Rika; Angela Adamson
Cc:	Justin Howes
Subject:	RE: Something to think about - Workflow for pull up affected stutter peaks

Hi all

In order to address some of the questions that have come up I contacted Pam Fietz at FSSA. Her response was:

"If a stutter peak is affected by pull up we would remove the stutter peak. STRmix will note that a stutter is missing but can run anyway. The stutter variance may be increased but you know why and can explain why. Increased stutter due to pull up would not be dealt with by ignoring the locus.

Regarding ignoring a locus. If a locus needs to be ignored (because of masked peaks) we would only ignore maximum of 2 loci in any one decon. Any more than 2 loci and the profile is becoming non-interpretable because of missing/masking of information."

This information may or may not change how we wish to proceed with this workflow.

We know through troubleshooting that a 'missing' stutter peak has the potential to significantly effect a STRmix decon so I'm not sure whether a blanket 'remove the peak' guideline should be used. My preference would always be to run the decon first to see what effect the pull-up has and to only attempt to rectify if there is an issue.

Currently some scientists are choosing to drop a locus in the case of a pull-up affected stutter based on advice provided by FSSA some years ago, since this advice appears to have changed we should probably reassess whether this is still an appropriate course of action.

I am seeking advice from the reporting seniors about how you would like to proceed.

Thanks

Emma



Hi all,

A few thoughts:

- Maybe we should make a better definition of what is considered a pull-up peak / pull-up affected peak first.
- Thanks for your example Cass, I think the NOC might be a bigger issue with that one (I didn't look at the whole profile, just went off your picture).
- With respect to the workflow, it could be simplified by replacing the red circle with the yellow highlighted text (see below) and cut out a loop



If a peak is affected by pulk-up it is always bent to attempt to elemente the pulk-up, this may involve among at a lower temptate or re-CT. If the pulk-up is unable attempted than this workflow describes how to deal with reads that may preferantly be studied to begin a pulk-up to the effects of pulk-up.

- Last thought, is "a maximum of one locus can be dropped per interpretation" a hard and fast rule? Is there something we can refer to that will guide us in this? Without looking at all of the profile Cass refers to below, let's assume there is two loci like the one attached where it is decided the locus should be dropped, would we want to make the profile "complex unsuitable" because we have a rule that says only one locus can be dropped?

Cheers

Al



Allan McNevin Senior Scientist - Evidence Recovery Team

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

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From: Emma Caunt <		
Sent: Monday, 18 October 2021 2:45 PM		
To: Cassandra James	>; Allison Lloyd <	
Sharon Johnstone 🖌	Kylie Rika <	-
Cc: Angela Adamson	>; Allan McNevin	>
C. Line DE Committe and this has a Monthle	for all a ffort data to the	

Subject: RE: Something to think about - Workflow for pull up affected stutter peaks

Thanks Cass.

So a question we might want to add to the workflow is "would the affected peak be able to be considered allelic under the assigned number of contributors" thinking that the pull-up affected stutter might actually add a contributor.



Cc: Emma Caunt <

McNevin •

; Angela Adamson <

>; Allan

Subject: Something to think about - Workflow for pull up affected stutter peaks

Hello 😊

I just wanted to let you know that I came across a sample that made me think about the workflow. I have case managed sample **sample methods** as a 2P mix, but it actually turns out that maybe this sample is 3P, the 8 is over to the side so I'm not sure it is pull up. However it did show that depending on what we call the sample, will influence how often STRmix can call this peak an allele. As a 2P mix it is unlikely to pair with the 9 peak and therefore STRmix is calling this peak drop in almost all of the time. We may also need to consider this when assessing the profile that there is the option for STRmix to model it both ways. It might be something we need to note in the workflow **(c)**

Thanks Cassie



Cassandra James Scientist – 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.



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EC-03

Emma Caunt

From:	Kylie Rika
Sent:	Tuesday, 2 November 2021 11:24 AM
То:	Angela Adamson; Emma Caunt; Allan McNevin; Cassandra James; Allison Lloyd;
	Sharon Johnstone
Cc:	Justin Howes
Subject:	RE: Something to think about - Workflow for pull up affected stutter peaks

Thanks all

Justin, how would you like this to proceed? This was in progress when Allison was acting T/Leader FRIT.

Thanks Kylie

From: Angela Adamson <		
Sent: Monday, 1 November 2021	8:08 AM	
To: Emma Caunt <	>; Allan McNevin	>; Cassandra
James 🖣 👘	>; Allison Lloyd	>; Sharon Johnstone
<	. >; Kylie Rika <	•
Cc: Justin Howes <		
Subject: RE: Something to think a	bout - Workflow for pull up affected stutter peak	٢S

Thanks for getting this info Emma 😇 Are we still waiting on advice from seniors?

From: Emma Caunt <		
Sent: Tuesday, 26 October	2021 12:39 PM	
To: Allan McNevin <	>; Cassandra James	;
Allison Lloyd <	>; Sharon Johnstone <	>; Kylie
Rika -	>; Angela Adamson <	
Cc: Justin Howes <	>	

Subject: RE: Something to think about - Workflow for pull up affected stutter peaks

Hi all

In order to address some of the questions that have come up I contacted Pam Fietz at FSSA. Her response was:

"If a stutter peak is affected by pull up we would remove the stutter peak. STRmix will note that a stutter is missing but can run anyway. The stutter variance may be increased but you know why and can explain why. Increased stutter due to pull up would not be dealt with by ignoring the locus.

Regarding ignoring a locus. If a locus needs to be ignored (because of masked peaks) we would only ignore maximum of 2 loci in any one decon. Any more than 2 loci and the profile is becoming non-interpretable because of missing/masking of information."

This information may or may not change how we wish to proceed with this workflow.

We know through troubleshooting that a 'missing' stutter peak has the potential to significantly effect a STRmix decon so I'm not sure whether a blanket 'remove the peak' guideline should be used. My preference would always be to run the decon first to see what effect the pull-up has and to only attempt to rectify if there is an issue.

Currently some scientists are choosing to drop a locus in the case of a pull-up affected stutter based on advice provided by FSSA some years ago, since this advice appears to have changed we should probably reassess whether this is still an appropriate course of action.

I am seeking advice from the reporting seniors about how you would like to proceed.

Thanks

Emma

From: Allon McNovin			
FIOID: Allan MICNEVIII			
Sent: Tuesday, 19 October 2	021 8:08 AM		
To: Emma Cau <u>nt <</u>	; Cassandra James <		>;
Allison Lloyd <	; Sharon Johnstone <		>; Kylie
Rika 🗸	; Angela Adamson <	>	
Subject: RE: Something to th	ink about - Workflow for pull up affected stutter peaks		

Hi all,

A few thoughts:

- Maybe we should make a better definition of what is considered a pull-up peak / pull-up affected peak first.
- Thanks for your example Cass, I think the NOC might be a bigger issue with that one (I didn't look at the whole profile, just went off your picture).
- With respect to the workflow, it could be simplified by replacing the red circle with the yellow highlighted text (see below) and cut out a loop



If a peak is affected by pulling it is always best to attempt to elemente the pulling. His may involve among at a lower temptate or re-CL. If the pulling is unable to be attempted in the workflow describes how to deal with seals that non-selected to the temptate of the DL.

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Cheers Al



Allan McNevin Senior Scientist - Evidence Recovery Team

Forensic DNA Analysis, Forensic and Scientific Services

Prevention Division, Queensland Health

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From: Emma Caunt <	
Sent: Monday, 18 October 2021 2:45 PM	
To: Cassandra James <	; Allison Lloyd <
Sharon Johnstone <	Kylie Rika 🗸
Cc: Angela Adamson <	; Allan McNevin
Subject: RE: Something to think about - Workflow	for pull up affected stutter peaks

Thanks Cass.

So a question we might want to add to the workflow is "would the affected peak be able to be considered allelic under the assigned number of contributors" thinking that the pull-up affected stutter might actually add a contributor.

From: Cassandra James <		
Sent: Monday, 18 October 2021 2:37 Pl	M	
To: Allison Lloyd <	Sharon Johnstone	>;
Kylie Rika <		
Cc: Emma Caunt <	>; Angela Adamson <	>; Allan
McNevin <		

Subject: Something to think about - Workflow for pull up affected stutter peaks

Hello 😊

I just wanted to let you know that I came across a sample that made me think about the workflow. I have case managed sample **sample methods** as a 2P mix, but it actually turns out that maybe this sample is 3P, the 8 is over to the side so I'm not sure it is pull up. However it did show that depending on what we call the sample, will influence how often STRmix can call this peak an allele. As a 2P mix it is unlikely to pair with the 9 peak and therefore STRmix is calling this peak drop in almost all of the time. We may also need to consider this when assessing the profile that there is the option for STRmix to model it both ways. It might be something we need to note in the workflow 😊

Thanks Cassie



Cassandra James Scientist – 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.



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EC-04

From:Kylie RikaSent:Tue, 23 Aug 2022 08:44:35 +1000To:Jess WellardCc:Emma CauntSubject:FW: PIM agenda meeting and actionsAttachments:Pullup and ignore locus_____xlsx

Hi Jess

Please see below and attached. Emma and I were hoping to have a quick teams meeting with Susan to talk through all of this as there are some quite concerning points. We are available until 3pm today if Susan has a spare 30mins?

Thanks Kylie and Emma



Subject: RE: PIM agenda meeting and actions

Hi,

I had asked BSAG and kept the survey in G: drive normal location. I don't recall anything from literature.

Re SS, I had asked if you both feel any more discussion is needed on this. I am sure staff would want to have a scientific discussion on many things and this could be one. I am not sure what staff would want so could be worth more discussion? The SOP has the guidelines and perhaps there are further considerations that could be discussed at the PIM that might improve the SOP? If there is an action item from a meeting already held that shows appetite for a discussion, then I think that would be good for staff to continue discussing as a group.

Justin



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Did you follow up on:

7. I will dig around. I had asked Angela for some lit searching and I know nothing came through. I think I asked BSAG as a survey item and sent finding through, but will double check.

Also, Justin, are you OK with the SS guidelines being the default position, unless really good reason to deviate?

Thanks Kylie

From: Justin Howes	>	
Sent: Monday, 22 August 2022 2:40 PM		
To: Kylie Rika	>; Sharon Johnstone	

Subject: RE: PIM agenda meeting and actions

Hi, I think you can both get together and move forward on the PIM. I would interested to hear how it goes.

Justin

е



Team Leader - Forensic Reporting and Intelligence Team Forensic DNA Analysis, Police Services Stream, Forensic & Scientific Services Prevention Division, Queensland Health p _____ m

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Subject: RE: PIM agenda meeting and actions

Hi both

Just touching base on this. Justin, did you have any final thoughts before we start moving? I have just had a training meeting with Tegan (nearly finished her mix rev training). She let me know that she is encountering different ways that people are doing things and I let her know that we are trying to get some of the interpretation issues resolved.

Thanks Kylie



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Wash your hands regularly to stop the spread of germs.

From: Kylie Rika <
Sent: Tuesday, 31 May 2022 1:12 PM
To: Justin Howes
Cc: Sharon Johnstone <
Subject: RE: PIM agenda meeting and actions

Thanks Justin

3. I don't think plate reading is affected here. In thinking on this point again, another option would be to consider modelling -2 rpt stutter in STRmix. This would remove all ambiguity. We would need to re-do model maker, but maybe this option could be the best?

8. I will chat with you Justin on this point

Any final thoughts before we start actioning?

Thanks Kylie

From: Justin Howes		
Sent: Tuesday, 31 Ma	y <u>2</u> 022 12:59 PM	
To: Kylie Rika <		
Cc: Sharon Johnstone	<	
Subject: RE: PIM agen	da meeting and actions	

Hi

I have some general points re below to consider:

3. Is there something here that needs to be communicated with plate readers? I don't think so as the points mostly relate to leaving the peak labelled. Just checking that there is no impact on plate readers.

4. I would think that stoch effects for 4p profiles would be a reasonable expectation given amped at 0.5ng or less, and split between at least 4 contributors in various ways. Would a reamp really assist low level ones like this as dropin values would come into effect a bit more too?

5. This is an interesting point for CMers discussion and could be good for one/two to put guidelines together on. Essentially, it is a reasonable assumption based on info so we could potentially condition on more that we currently do. I have had this point on my whiteboard for some time and would be interesting what comes out of it.

6. Another good discussion point. This could be a BSAG survey point.

7. I will dig around. I had asked Angela for some lit searching and I know nothing came through. I think I asked BSAG as a survey item and sent finding through, but will double check.

8. Does this need more discussion at a PIM? It is a comment against and I don't think it is particularly controversial (in my opinion), so as a guideline to assist opinions, do you think it needs any more fleshing out?

9. This could be a minor change request and would need some consultation with QPS if the default changes. It would be an interesting discussion point, esp alongside point 4 which considers a poss benefit in reamping samples. A Mic to full presents only one shot at it without knowing how many conts could be in the sample and pushes the case manager to make a decision not too dissimilar to approaching a P3 sample. I know some staff are amping without MIC at all for some of these low level samples so it would be interesting how the discussion goes.

Overall, excellent and looks like a pretty full agenda.

Justin



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From: Kylie Rika < Sent: Tuesday, 31 May 2022 8:23 AM To: Justin Howes < Cc: Sharon Johnstone < Subject: PIM agenda meeting and actions

Hi Justin

Here are the meeting minutes (and action items) from a meeting Sharon and I had last week. Let me know if you are OK with us starting on the action items and/or if you would like to discuss.

Kylie and Sharon met to discuss the email from Emma, Angela and Cassie [Inconsistencies with interpretations, 01 April 2022]

1. Unresolved D8

We are seeing higher peak heights in our amps at the moment which means that the D8 issue is being seen again. This issue is where D8 amps higher than the rest of the profile and STRmix is

unable to resolve the major peaks. There seems to be some inconsistency in the way people are handling this. Our previous advice was to try to resolve the issue where possible. The method for this is to re-decon at double accepts and if this doesn't work to amp down to try to reduce the peaks heights at D8. Not all people are doing this. Additionally we do not have an agreed solution if amping down doesn't help.

How would you like to progress this?

• KDR to check if staff have been informed of the pathway of double iterations and amping down to resolve over-amped D8. If yes, KDR to send an email from FRIT seniors as a reminder. KDR to ask STRmix trainers to keep an eye out for times when this pathway doesn't resolve so that a discussion can occur with line manager, reporter and STRmix trainer

2. Saturation point

Since we are seeing larger peak heights, sometimes we reach saturation (30,000rfu). This is being missed because people are not used to seeing it. A reminder needs to be sent out. Additionally the question is asked whether peak heights >30,000rfu are ok for reference samples – the answer to this is yes because STRmix doesn't use the peak heights of the ref.

• KDR to send an email from FRIT seniors as a reminder.

3. <u>-2 rpt stutter</u>

There are inconsistencies with how people approach potential -2 rpt stutter peaks that sit in a +1 rpt stutter position. Some people remove them, some people leave them labelled. Our advice is as follows:

- If the peak is below the +1 rpt stutter threshold leave it labelled
- If the peak is above the combined +1 and -2 rpt stutter threshold leave it labelled
- If the peak is above the +1 rpt threshold but below the -2 rpt threshold run STRmix and see if it is modelled as stutter some of the time. If it won't falsely exclude then leave it labelled. This requires some discussion about % weighting of the peak being designated as allelic.

How would you like to progress this?

• KDR to send an email from FRIT seniors on recommending the first two points. The third point can also be included in the email with recommendation that if anyone has this scenario occur, let line manager know so a discussion can occur with line manager, reporter and STRmix trainer.

4. <u>*Ap mixtures an the use of ratios in determining NoC*</u>

Low level 4p mixtures can be difficult to assign NoC due to AI and inconsistent ratios. Should we be amping twice to assist with identifying stochastic effects versus true peak heights of the contributors?

• SMJ to get more information from STRmix trainers

5. Inconsistent conditioning

This is still causing issues and needs some guidelines.

• Discussion point at PIM to get views. Then perhaps task someone with putting some guidelines together.

6. <u>Mutations</u>

There has been some discussion around whether our guidelines for dropping the locus when a mutation is present is appropriate/too strict. We had implemented the current process based on advice from Duncan Taylor many years ago. It is not known whether this advice still stands or whether it has been reconsidered.

How would you like to progress this?

- Discussion point at PIM to get views. If large split in views then seek advice from statspwg or bsag
- 7. Pull-up in stutter position

In October last year I put together a workflow for dealing with pull-up in stutter position. This workflow still has not been finalised and the issue continues to occur.

- JAH, KDR and SMJ to search emails, diaries to find where this was last left. Is workflow ready to go to case managers for feedback?
- 8. <u>Use of s/s guidelines & inclusion in the SOP so that everyone is interpreting these profiles in the same way</u>
 - KDR to speak with JAH re guidelines being default position, unless really good reason to deviate
- 9. Can we change the DIFP process so instead of mic to 30ul, they are mic to full?
 - JAH checking with Steve Foxover if QPS are mass ordering further processing on a set of cases.
 - SMJ to send an email from FRIT seniors asking if case managers want mic to 30ul or mic to full as default
 - KDR to write up PIM agenda and send appointment etc..

Thanks Kylie and Sharon Kylie Rika Senior Scientist, Forensic Reporting and Intelligence Team Forensic DNA Analysis, Police Services Stream, Forensic & Scientific Services Prevention Division, Queensland Health p a 39 Kessels Road, Coopers Plains, QLD 4108 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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EC-04-1

Date	Lab	Question/ Response
		Hi there,
		We have some discussion points going around reporting scientists at the moment, and I was curious on processes within your labs:
		1 Be you have criteria for when a peak is removed from STRMIX consideration? For example, if a neak istuiter or allele) is thought to be affected by null-up do
		Led you have citere to the appendix to deconvolution, or do you run with STRmix (and not remove the queried peak) and assess whether the peak inclusion is affecting
		the deconvolution and then consider removing the peak? ** PIs note, this is after a rework is performed
	3	2. Dther than in a suspected mutation/triallele situation, do you have criteria that describe when a locus may be ignored? For example, is there a maximum
		number of loci that may be ignored and is this number affected by the MW location of the loci that are being ignored?
		Thanks
15/12/2021	QLD	Justin
		Below are some excerpts from our procedures. Basically we allow the two typers to remove any potential artefacts at the typing stage, before STRmix, and we do allow people to ignore a locus if they think something is not right with that particular locus and they think it is affecting the decon. To my knowledge we have only ever had one locus in a particular profile at a time needing to be ignored. We have not set a maximum number allowable.
		Likely artefact peaks should not be typed
		A probable stutter peak should be ignored if its height is equal to or less than the locus specific stutter cut-off listed in the table below
		The 'Kit Settings' button at the top of this screen allows loci to be ignored by adding a tick in the check box next to the locus or loci to be ignored during the deconvolution. This should only be used rarely to address issues like known triallelic contributors, or other locus specific issues with the profile.
		If you are not satisfied with the results of a deconvolution due to the checks given above there are reveral possible pathe-
		If you are not satisfied with the results of a deconvolution due to the checks given above, there are several possible parts.
	_	applying user informed priors or ignoring an anomalous locus. A locus can be ignored for deconvolution or LR generation. However, it is still necessary to ensure that the reference sample is manually compared to the ignored locus on the electropherogram and that two scientists are satisfied that the reference profile is not
		excluded at the ignored locus.
		Hope this helps.
		Cheers,
		Pam
15/12/2021	FSSI	
		We don't have specific criteria for when a peak that combines pull up is removed from the deconvolution. We actually don't see this very often as if pull up is extreme and affecting peaks at other loci it usually means the sample is overamped and likely to display peaks beyond our saturation threshold. These samples would normally be wiped (as in not typed) and the sample would be re-amped at a dilution. If this happens then the first amp doesn't get considered. It's quite rare that pull up aligns directly with an allele, but if we believe pull up is contributing to a peak but it isn't having a big affect then we would leave the peak labelled, if it is contributing to stutter then we may consider removing the peak but only if STRmix can cope with the absence of the stutter peak. We do have the ability to drop a locus if pull up is affecting peaks but this would be very rare.
		As for dropping a locus, this is generally only done for trisomies, D12 allele shifts or unintutive results due to unresolved (absorbed) stutter peaks:
		Absorbed peaks, OL peaks, trisomies and primer binding mutations – dropping loci
		STRmix cannot model unlabelled peaks, nor does it consider trisomies within the proposed contributor genotypes. Thus if a peak is absorbed or OL (refer to 8.5.4
		Off ladder (OL) peaks at D12 for further information), or if an extra peak is present that is believed to be from a trisomy, or if the Mx will be affected by a contributor with a suspected primer binding mutation, then it might be necessary to omit the affected loci from the deconvolution.
		STRmix can account for the absence of peaks through dropout if the absorbed or unlabelled peaks are of a relatively small peak height. In this situation it might not
		be necessary to drop the locus from the deconvolution. If, however the peak in question is of a sufficient height that genotype combinations, mixture proportions and weights given to genotypes across the profile would be incorrect then the locus should be omitted.
		It is not always necessary to redeconvolute the sample with a dropped locus. If the issue only affects the comparison of a particular POI and genotypes are intuitive then the locus can be omitted during the calculation of the LR.
		We don't have rules around how many loci can be dropped from the one sample, however, I don't know of any situation where we have had to drop more than one.
		Cheers
16/12/2021	Viceol	Cheers Lisa

		Hi Justin,
		This is the feedback I received from one of my reporting staff. Apologies it is a bit lengthy:
		1)Bo you have criteria for when a peak is removed from STRmix consideration? For example, if a peak (stutter or allele) is thought to be affected by pull-up, do you remove from the data prior to deconvolution, or do you run with STRmix (and not remove the queried peak) and assess whether the peak inclusion is affecting the deconvolution and then consider removing the peak? ** PIs note, this is after a rework is performed
		The most common reason for removal of a peak from the STRmix input file is if it appears to be double back stutter (minus 2 repeat units). Double back stutter peaks are retained by the DNA Laboratory during profile analysis and are therefore present in the STRmix input file. If the profile proceeds to interpretation in STRmix, these may be manually removed from the input file if certain criteria are met (parent peak >10,000 rfu and stutter ratio of the double back stutter peak <2.3%).
	×	We cannot recall any specific instances where we have encountered a stutter or allelic peak that was perhaps affected by underlying pull-up. Given that profile analysis and interpretation are undertaken by different staff in different work units, it is likely that we would not identify this unless unintuitive results were observed following STRmix interpretation. In this circumstance, the likely course of action would be to address the issue biologically (e.g., re-amplification with a smaller amount of target DNA). If this still failed to correct the issue, we would consider ignoring the locus during STRmix interpretation.
		2)Other than in a suspected mutation/triallele situation, do you have criteria that describe when a locus may be ignored? For example, is there a maximum number of loci that may be ignored and is this number affected by the MW location of the loci that are being ignored?
		The most common reason for ignoring a locus is where there is a suspected 1 base pair resolution issue (i.e., closely sized peaks that differ in size by 1 bp). While such peaks are typically able to be resolved if their peak heights are similar, they may fail to be resolved where one peak is substantially shorter than the other and falls on the shoulder of the taller peak. In extreme cases, this may lead to the false exclusion of a minor donor. This is readily identifiable by assessment of the primary and secondary diagnostics in STRmix (an exclusion at a single locus but inclusionary LRs at all/most other loci). Stutter peaks may also fail to be resolved however this usually only affects the stutter variance parameter. Provided that all other diagnostics were intuitive/acceptable, we would not ignore the locus for such a result (we would be comfortable to explain why the stutter variance was elevated). Newer versions of STRmix can assist with this issue by Identifying possible evidence peak issues (i.e., missing stutter peaks) prior to interpretation. Usually the peak morphology can indicate an apparent unresolved peak. If this peak appears to be fairly high in height, we would generally ignore the locus in the initial deconvolution. Otherwise, we would closely assess the STRmix results at the affected locus and consider re-interpretation with the locus ignored if unintuitive results were produced.
		Hope this helps. Get back to me if you have any follow up questions.
		Regards,
24/12/2021	FASS	Clint Cochrane Laboratory Manager, Forensic Biology/DNA I Forensic & Analytical Science Service
		Response below from one of our reporting FS:
		1.Do you have criteria for when a peak is removed from STRmix consideration? For example, if a peak (stutter or allele) is thought to be affected by pull-up, do you remove from the data prior to deconvolution, or do you run with STRmix (and not remove the queried peak) and assess whether the peak inclusion is affecting the deconvolution and then consider removing the peak? ** Pls note, this is after a rework is performed
		Where a peak is clearly affected by pull-up it will be removed prior to deconvolution. Often the preference is to ignore the locus as it becomes difficult to assess whether there is a true peak present given the masking. This is also the case where a microvariant is present and has not resolved (eg where you have peaks at 18.3 and 19 that are assigned as a single 19 peak) or if there is a reproducible artefact (generally associated with animal products). Ideally we would re-amp to try and resolve the problem biologically before we consider it statistically.
		Generally if following comparison there is an unintuitive LR (either exclusion or elevated non-exclusion) we will re-amp the sample (potentially increasing the DNA input where possible) to determine whether the issue can be replicated. Generally, unintuitive LRs are either due to an additional contributor that is present at trace levels, stutter peaks that are incorrectly modelled as allelic or an unresolved peak. If there is no capacity to fix the issue biologically and we can justify our decision making scientifically, we will ignore the locus.
		2.@ther than in a suspected mutation/triallele situation, do you have criteria that describe when a locus may be ignored? For example, is there a maximum number of loci that may be ignored and is this number affected by the MW location of the loci that are being ignored?
		Other than tri-alleles (which are observed at multiple loci for some genetic conditions) and sometimes cross over between D7 and D21 that cannot be accurately assigned, we don't ignore multiple loci. If there is a requirement to ignore multiple loci, I would suggest that the profile has systemic issues and should not be interpreted. However, we do not have strict guidelines as to the maximum number of loci or molecular weight of the loci that may be ignored. If there is clear justification to ignore a locus (that can be supported scientifically), I would consider potentially ignoring multiple loci.
21/12/2021		Webling all a very hanny new year!
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EC-05

Forensic and Scientific Services

Minor Process Change

Stage Z			
		Project #:	199
Proposed by :	Angela Adamson, Emma Caunt, Cassandra James, Rhys Parry	Date:	25/03/2022
Title:	Summary – Model Maker results for	Project #199	
Comment to be added to SOP:	☐ Yes QIS# ☐ No	Completed date:	v
Email communication sent:	Yes Team/s No	Completed date:	
Add to minor change register	Yes	Completed date:	
Outline of Minor Chang	je:		

Introduction

The ProFlex[™] 96-well PCR System (ProFlex) thermal cyclers were implemented in Forensic DNA Analysis on the 10th January 2022, replacing the end of life GeneAmp[®] PCR System (9700) thermal cyclers.

Advice from the STRmix[™] support group recommended re-running Model Maker to see whether the new thermal cyclers have affected the peak height [1]. If there were no substantial changes to the variances determined by Model Maker then it would be acceptable to keep using the existing STRmix[™] parameters.

Summary of work undertaken

Results from single source samples that were analysed during the validation of the Proflex thermal cyclers as part of Project #199 were used.

A batch of 42 single source samples run once at a template of 0.5ng, and 6 samples run as a serial dilution at templates of 0.001ng, 0.005ng, 0.025ng, 0.125ng,0.25ng, 0.5ng and 0.7ng was created. This batch of 78 samples was amplified on each of the 6 Proflex instruments and once on a 9700 instrument. Samples were read at 80 rfu with -1 rpt Stutter and +1 rpt Stutter left labelled as per standard operating procedures.

Data obtained from each of the 6 Proflex thermal cyclers were combined into one single source (casework) input file and reference profile information was collated into a separate input file. The data obtained from the 9700 was kept in a separate single source (casework) input file. These files were analysed using the Model Maker function of STRmixTM v2.8.0.

The variances obtained from the Proflex instruments and the 9700 were compared with those used currently in casework assessment using STRmix[™].



Study findings

A summary of each variance value calculated by Model Maker is included in Table 1 below, along with the values currently in place for routine analysis (sourced from Project#219 - Verification STRmix[™] 2.7 for 3500xL).

Table 1 Summary of Model Maker output

	Current Settings			Proflex Model Maker		9700 Model Maker			
	α	β	MODE	α	β	MODE	α	β	MODE
Allele Variance C ²	10.197	1.801	16.564	14.095	1.366	17.888	10.327	1.663	15.511
Back (-1rpt) Stutter Variance k ²	1.703	14.134	9.936	2.082	8.192	8.864	3.399	4.194	10.061
+1rpt stutter Variance k ²	5.519	28.11	127.029	2.908	31.797	60.669	4.626	17.636	63.948
	λ	MEAN		λ	MEAN		λ	MEAN	
LSAE Variance	103.756*	0.01		69.312	0.014		57.382	0.017	

*Note: Current setting used in STRmix[™] v2.8 is 100.00 due to rounding by STRmix

Comparisons of the current values with those obtained from the Proflexes and 9700 showed that there were differences between them.

In order to visualise the above data, graphical representations comparing the STRmix[™] settings with those generated from two different Model Maker runs (Proflexes and 9700), are shown in Figures 1-4.





Figure 1 shows that the allele variances between the current settings, Proflexes and 9700 were all similar.





Figure 2 - Back (-1 rpt) stutter variance

The -1 rpt stutter (back stutter) variance values (Figure 2) have a similar mode however the distribution for Proflex and 9700 variance is considerably narrower than the existing distribution. This could result in more stutters being designated as allelic more so than the current settings being used. It therefore could be considered that the current settings would be more lenient than Proflex model maker settings.







Figure 3 - +1 rpt stutter variance

The +1rpt stutter variance values (Figure 3) are very different with respect to mode and the shape of the distribution. This could result in more +1pt stutters being designated as allelic under the Proflex settings than under the current settings being used. It therefore could be considered that the current settings for +1 rpt stutters are more lenient than Proflex model maker settings.





Figure 4 – LSAE variance

The LSAE variance value for the Proflexes is higher than that of the current LSAE variance. This difference could have a significant effect on profile modelling as it may allow for more profile variations than the current settings. It was also considered that this observation could be due to the lower quality of samples in Proflex model maker dataset.

The 9700 variances and the Proflex variances differed from each other and from the current settings. The graphs demonstrated that there are differences observed with the back stutter variance and +1rpt stutter variance. These differences may have also resulted from the limitations of the data used or could indicate a drift in the settings over time. In order assess these differences further a decision was made to conduct further experimentation using a full sample set.

Summary of further work undertaken:

A batch of 10 single source samples were amplified at input templates of 0.025ng, 0.078ng, 0.131ng, 0.183ng, 0.236ng, 0.289ng, 0.342ng, 0.394ng, 0.447ng, 0.125ng,0.25ng, 0.5ng, 0.6ng and 0.7ng across two Proflex instruments. Samples were read at 80 rfu with -1 rpt stutter and +1 rpt stutter left labelled as per standard operating procedures. The resulting files were analysed using the Model Maker function of STRmix[™] v2.8.0.



Summary of findings

A summary of each variance value calculated by Model Maker is included in Table 2 below, along with the values currently in place for routine analysis (sourced from Project#219 - Verification STRmix[™] 2.7 for 3500xL).

Table 2 – Model Maker output comparison

		Current Settings	Proposed Settings
Allele Variance c ²	α	10.197	9.288
	β	1.801	1.974
	MODE	16.564	16.361
Back (-1rpt) Stutter Variance k ²	α	1.703	1.875
	β	14.134	12.316
	MODE	9.936	10.777
+1rpt stutter Variance k ²	α	5.519	4.780
	β	28.11	24.405
	MODE	127.029	92.251
LSAE Variance	λ	103.756*	54.096
	MEAN	0.010	0.018

*Note: Current setting used in STRmixTM v2.8 is 100.00

In order to visualise the above data, graphical representations comparing the current values to those generated from the full Model Maker analysis are shown in Figures 5-8 below.

















The input data from the full Proflex Model Maker analysis described above was entered into the Model Maker check spreadsheet (provided by STRmix[™] technical support), this showed that the data provided a 98.5% coverage which is above the required 95%. This is represented in Figure 9 below.







Hypothesis testing

The proposed Model Maker data was compared with the Model Maker data used in the current STRmixTM settings. Differences were noted within the comparisons of the two sets of data. Hypothesis tests were conducted to assess the significance of the differences found.

The hypothesis tests involved conducting an analysis of data obtained from the 9700 (current settings) and Proflex (proposed settings) systems to determine if there was a significant difference between the variances observed for the allele height, the +1 rpt stutter, the -1 rpt stutter, and the LSAE. Testing was undertaken using a process known as bootstrapping. In this process, a simulated sampling is undertaken from an estimated distribution to simulate real data when that data is not available for analysis. In this case, the distributions and their defining parameters (rate and shape) have been obtained from Model Maker. Data was modelled for each of the allele height, +1 rpt stutter, -1 rpt stutter, and the LSAE using n=100, 200, 300, and 500. The Model Maker data is based on a 10x12 matrix (120 samples), and so will have at most 4800 alleles, -1 rpt stutter peaks, +1 rpt stutter peaks, and 2400 loci upon which the data is based. Allowing that at lower dilutions many peaks will not be observed and hence the true number of peaks in the original Model Maker analysis will be much lower. As such, the values for n used are considered to be conservative and would likely be much higher. It should be noted that as n increases, the probability of a significant difference being observed between two groups increases.

The distributions from Model Maker were modelled in R at the various n-values using standard sampling methodology (Crawley, 2007). Hypothesis testing (examining the distributions obtained from the two analysers for significant differences) was undertaken using the following three tests:

Welch Two Sample t-test: this test is the least ideal as it requires an assumption of normality in the distributions. However, it can be employed as an indicator because it will work asymptotically due to the constraints of the Central Limit Theorem. That is, essentially, that if a distribution is sampled enough times



the mean of the means will tend towards the true mean of the distribution. Though, it should be stated, that the t-test is not ideal for highly skewed distributions (Crawley, 2007).

Wilcoxon rank sum test: The unpaired two-samples Wilcoxon test (also known as Wilcoxon rank sum test or Mann-Whitney test) can be used to compare two independent groups that are non-parametric (ie. is not normally distributed). This is the most ideal test for this analysis (Crawley, 2007).

Kolmogorov-Smirnov Test - this test makes no assumption about the distributions. This test is most ideal when it is not known what distributions are involved. Even though we are using gamma and exponential distributions, it must be noted that there is no definitive "distribution" for a set of data, and so while data might fit a particular distribution it is possible for it to also fit other types of distribution (Crawley, 2007).

The null hypothesis for all these tests is that the data is all from the same population. The alternative hypothesis is that the data likely comes from two different populations. The significance level was set at $p \le 0.05$.

Discussion

The significantly different results for the -1 rpt stutter at the n=100 and n=200 levels were unexpected. However, as these groups were not significantly different at n=300 and n=500, the effect is likely to be due to the small sample size not reflecting the full gamut of the respective distributions. This is supported by the observation that when reanalysed using a different seed for the modelling, the results for the n=100 and n=200 analyses were not significantly different (p>0.05).

The results obtained are illustrated in Table 3. The values of p≤0.05 have been highlighted in orange.

	n	t-test	Wilcoxon	KS
	100	0.60917	0.585	0.81275
Allele	200	0.95202	0.90876	0.99719
Variance	300	0.63267	0.83527	0.78704
	500	0.37253	0.19716	0.25743
	100	0.11885	0.015298	0.054103
n-1	200	0.037185	0.21057	0.3275
Stutter	300	0.40401	0.39405	0.51755
	500	0.15476	0.24195	0.41315
	100	0.00019131	0.000791	0.015814
n+1 Stuttor	200	8.6668E-08	7.95E-08	9.91E-08
Stutter	300	1.0302E-11	1.83E-11	8.7E-09
	500	2.22E-16	2.22E-16	2.22E-16
	100	8.1796E-05	5.45E-05	3.73E-05
ISAE	200	4.0082E-06	7.18E-07	1.22E-05
LOAL	300	5.365E-11	4.42E-12	5.22E-09
	500	2.22E-16	3.83E-16	3.84E-13

Table 3 Hypothesis Tests



The hypothesis tests indicated a significant difference in the data obtained. In order to determine the impact of changing the STRmixTM variance settings for casework, a comparison between the two sets of variances was made. For this comparison seven mixed DNA profiles consisting of two- and three-contributors from *Project #219 – Verification of STRmixTM v2.7.0 for 3500xL Part B* were used.

Comparison of LR of current settings vs proposed settings

The seven mixtures were deconvoluted in STRmix[™] v2.8.0 using both the current Model Maker settings and the proposed Model Maker settings and LRs calculated for the true contributors. The LRs obtained using both sets were compared to each other to assess the differences between them. 21 sets of LRs obtained were all within the same order of magnitude indicating little impact on the LRs with the proposed settings additionally the result lines used to report these samples would not change. One LR set did change by one order of magnitude but the result would still be reported within the greater than 100 billion result line so it would not change the final result line. The comparison of the log₁₀(LR) is represented in Figure 10 below.



The number of alleles resolved to \geq 99% were also compared to detemine whether there are any differences in the number of uploads to NCIDD using the proposed settings. The results (Figure 11) show that there is little difference in the number of resolved alleles between the current and proposed settings.





Figure 11 – Comparison of resolved alleles to ≥99%

Conclusion:

Based on the findings of the further testing and comparisons made using the current and proposed model maker settings it demonstrates that there would be minimal risk with the introduction of the model maker settings created using the latest sample set. The result lines for all samples compared would not have changed, this indicates that STRmix[™] can be updated to the proposed model maker settings and continue with casework without having to reanalyse samples already processed using the current settings.

Recommendations:

- It is recommended that all computers with STRmix[™] v2.8 be updated with the new model maker settings by the STRmix[™] team members.
- It is acceptable to have a mix of model maker settings in one case.
- Samples run on Proflex using 9700 settings do not require re-analysis with Proflex settings.

Acknowledgment:

We would like to acknowledge Allan McNevin for his assistance with data analysis.



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 STRmix[™] support - Crawley, Michael J 	ticket 3422 (2007) The R Book. John Wiley &	& Sons Ltd. p293	-4; p317-8.	
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EC-06

Emma Caunt

From: Sent: To: Subject: Justin Howes Friday, 12 August 2022 12:10 PM Emma Caunt RE: Model Maker for Proflexes

Hi Emma

I had consulted Paula before asking for an OQI to be raised and we agreed that it is the best way to document what we found, and what we have done about it. This was important given we had received some advice on any risks to samples reported, and we were then organising implementation when the issue was detected.

If you have any further clarifications on the OQI and what could be in/out of scope, please discuss with Kirsten. She will be back to the office next week I believe.

Thanks Justin



Justin Howes

Team Leader - Forensic Reporting and Intelligence Team

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



a 39 Kessels Road, Coopers Plains, QLD 4108

e 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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Hi Justin

I am following up on your email below regarding Model Maker for the Proflexes. Angela and I met with Sharon to discuss this and Sharon stated that she was going to follow up with you. I am conscious of the extended timeline for this and so wanted to follow up with you directly.

Your first point was about external technical verification. Of course I am all for review outside of the project staff, my concern is who this would be. The error that was made with the Model Maker analysis could only have been picked up by somebody with intimate knowledge of STRmix. Currently any tech reviewer assigned would need to be directed by Allan (who ran the MM analyses in this instance), Angela, Cassie or myself; we would not have directed a tech reviewer to check the drop-in settings for Model maker as we had missed it ourselves.

Your second point regarded raising an OQI for this error. Since the new Model Maker settings had not been implemented when the error was identified then there was no risk to casework. I have consulted the OQI SOP (QIS 13965) which states that an OQI should not be raised for minor methodology errors until the problem becomes systemic – I would consider that this event comes under this.

Since the Proflexes were implemented without Model Maker being run and new STRmix settings applied, I would like to suggest that this incident be documented in an OQI especially since advice from STRmix Support was that Model Maker needed to be included in the verification of the Proflexes. I suspect that this may also come under "Minor methodology or QC errors until the problem becomes systemic" since all profiles from the Proflexes are using old STRmix settings it could be considered systemic.

Could you please advise how you would like to progress with respect to a tech reviewer and the OQI?

Thanks

Emma

From: Justin Howes <		
Sent: Thursday, 26 May 2022 12:06 PM		
To: Emma Caunt <	: Angela Adamson ·	;
Cassandra James <		
Cc: Sharon Johnstone	; Paula Brisotto <	
Subject: RE: Model Maker for Proflexes		

Hi

I have spoken to Sharon about this and some actions surrounding.

Yes, please move to comparing the decons with the newest settings vs minor change settings. Before that, in light of the VFP data not being too distinct between runs, yet PP21 appears to be, I have chatted to Paula and Kirsten and we would like to see someone external to the project staff verify the information going in to MM for PP21. I don't mind who does it and Sharon can work on finding someone for this external tech verification.

I would like one of the Project staff for MM to raise an OQI to Sharon as line manager for you all. As with all OQIs, it will document the occurrence, corrective and preventative actions for this process. I don't mind who raises it in QIS, but this will be required here.

I will leave the VFP decisions for the Project group there; this is to move forward with the PP21 work.

Thankyou

Justin



Justin Howes Team Leader - Forensic Reporting and Intelligence Team

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

p m a 39 Kessels Road, Coopers Plains, QLD 4108 e 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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Thanks for this.

I will get back to you later as there are some points I wish to meet with Sharon on first.

Justin



Justin Howes Team Leader - Forensic Reporting and Intelligence Team

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

p m m a 39 Kessels Road, Coopers Plains, QLD 4108 e 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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Hi

Overnight Cassie ran the VFP Model Maker with no drop-in settings applied. Attached are the overlaid distributions so you can see the differences. In summary, all of the stutter variances and the LSAE variances are about the same. The allele variance however is quite different.

The allele variances with both drop-in applied and no drop-in have a similar mode, however the no drop-in variance distribution is a lot narrower. This means that, if the no drop-in settings had been used for the VFP STRmix analyses, the results would have been either the same or worse. By worse I mean that STRmix would have been less tolerant of the AI. This means that the outcome of the VFP STRmix analysis would not have changed. If you would like, Cassie and I can run some of the VFP profiles through STRmix with the 'no drop-in' settings to see what (if any) the differences are. Please let us know if you would like us to do this.

Thanks

Emma

To: Emma Caunt +Cassandra James Cc: Angela Adamson <cassandra james<br="">Subject: RE: Model Maker for Proflexes</cassandra>	
Cc: Angela Adamson < Cassandra James Cc: Angela Adamson < Cassandra James Coubject: RE: Model Maker for Proflexes	
Subject: RE: Model Maker for Proflexes	
Subject: RE: Model Maker for Proflexes	
Thankyou	
ustin	

Justin Howes Team Leader - Forensic Reporting and Intelligence Team Forensic DNA Analysis, Police Services Stream, Forensic & Scientific Services Prevention Division, Queensland Health



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CLEAN HANDS SAVE LIVES	Wash your hands regularly to stop the spread of germs.
From: Emma Caunt -	>
Sent: Wednesday, 25 May 2022 4:23 PM	
To: Justin Howes <	s
Cc: Angela Adamson <	; Cassandra James
; Sharon John	istone <
Subject: RE: Model Maker for Proflexes	

Hi

It doesn't say why, but there was a change to the drop-in modelling for stutter peaks in the upgrade from v2.7 to v2.8 so I would suspect that it has something to do with that.

Yes, this does affect Model Maker for VFP. We have done a trial run with the VFP Model Maker data and the drop-in settings changed to zero and it doesn't seem to have made much difference to the variances. We are running another one overnight tonight to double check. We're not sure why the VFP MM doesn't seem to be affected when there is such a large change to the PP21 variances.

Thanks

Emma

From: Justin Howes			
Sent: Wednesday, 25 May 2022 3:4	7 PM		
To: Emma Caunt <	>		
Cc: Angela Adamson <	>;	; Cassandra James	
	>; Sharon Johnstone <		>
C. L. D. D. M. L. M. L. M. L. C. D. C.			

Subject: RE: Model Maker for Proflexes

Hi

Thanks for this information. I will need to consider this further with Paula. To help with that, does the manual describe why the drop-in parameters should be set to zero for this version, as opposed to previous? I would guess to allow MM to assess more information, but interested in why this would be different. Nevertheless, if it is stated to do this, then that is the process we would follow; unfortunate to find out after the work you have all done.

I guess there was a MM for VFP as well. If so, will that component need further work too?

Justin



Justin Howes

Team Leader - Forensic Reporting and Intelligence Team

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

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	CLEAN HANDS SAVE LIVES	Wash your hands regularly to stop the spread of germs.
From: Emma Caunt <		
Sent: Wednesday, 25 N	lay 2022 3:12 PM	
To: Justin Howes <		
Cc: Angela Adamson <		; Cassandra James
<	>; Sharon Johns	tone
Subject: Model Maker f	or Proflexes	

Hi Justin

I understand that there were some issues with the implementation of the new Model Maker settings last week. There was a discrepancy between the value for λ in the Model Maker output and the value that is output in a STRmix deconvolution. The source of this discrepancy has been identified and relates to the rounding of the mean LSAE variance once Model Maker is imported into STRmix. This is not something that we have the option to correct as STRmix calculates the value for λ internally from the mean rather than taking it from the Model Maker output. We are satisfied that this discrepancy is acceptable.

Unfortunately, during the investigation of this issue another issue was identified with the Model Maker analysis performed for the proposed settings following the implementation of the Proflexes.

It was identified that the STRmix manual states that the drop-in parameters should be set to zero when running Model Maker in STRmix v2.8; this was not done for this Model Maker analysis and is not something that has been required for past Model Maker analyses (therefore current settings are not affected). We attempted to re-run Model Maker with the drop-in parameters set to zero however STRmix found issues with the input data – there were peaks that were labelled within the Model Maker data that should not have been but were accepted by STRmix when the drop-in settings were applied. We have re-read the Model Maker plates and removed the anomalous peaks and re-run Model Maker with the resulting data. Unfortunately this has resulted in a change to the settings that were originally calculated.

The proposed settings were:

PROBABILITY DISTRIBUTION

α	β	MODE
9.288	1.974	16.361
1.875	12.316	10.777
4.780	24.405	92.251
0.018		
	α 9.288 1.875 4.780 0.018	α β 9.288 1.974 1.875 12.316 4.780 24.405 0.018 10.018

With drop-in set at zero, the settings are:

PROBABILITY DISTRIBUTION

	α	β	MODE
Allele Variance c ²	9.712	1.861	16.213
Back Stutter Variance k ²	1.508	66.756	33.912
+1 rpt stutter Variance k ²	9.154	45.233	368.83
LSAE Variance	0.026		

Given the large differences in the results, we would like to suggest that we not implement the Model Maker settings that are the subject of the minor change but investigate the Model Maker analysis with drop-in set at zero. This will require the comparison of the deconvolutions with the current casework settings with the deconvolutions with these new settings to determine the risk of implementing the new settings (drop-in set at zero) and additional significance testing.

Please advise whether you would like us to go ahead with this testing.

Thanks

Emma, Angela and Cassie



Emma Caunt Scientist

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

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8

EC-07

Emma Caunt

From:	Kirsten Scott	
Sent:	Thursday, 25 August 2022 10:37 AM	
То:	Emma Caunt; Sharon Johnstone; Cassandra James	
Cc:	Paula Brisotto; Justin Howes	
Subject:	Verifiler next steps for reporting sub-team	

Sharon, Emma and Cassie,

Please proceed with the STRmix analysis, of everything done on the main VF lot number, and on the newer lot number.

I understand your concerns, I now need the data, the analysis and the assessment/discussion committed to draft reports for consideration.

We need the data on how it functions: as the evidence we need to either find this kit suitable for primary use, or not suitable for primary - but suitable for emergency back-up, or not suitable at all.

All kits will have analysis complexities and artefacts, so we need to document them in full, and the implications of such.

The Verifiler team, and the management team is expecting three reports to come from this team, and that does need to be soon.

It is not possible just to cease, we have to justify/report the experimental findings, the analysis, the thinking and the decisions = the project final reports for stutter, mixtures and STRmix.

The progress (or not) of implementation is the optional component.

Please put all available energy and resources into producing these three reports so that the Lab can decide what happens next in terms of kit usage.

When each is drafted it will first need to go to the Verifiler team for review, as happened with each of the analytical reports.

Thanks Kirsten



Hi Kirsten and Sharon

Cassie and I have looked at all samples on the new VFP batch that was amped using the new kit. Overall the interlocus balance looks better than the previous batches of kits but there is still some imbalance present. We will not be able to assess the impacts of this until we run the samples through STRmix.

Of concern is the number of artefacts that we have observed across the batch. Of the 87 samples on the batch, 47 of them had artefacts present. These artefacts are predominantly at D18 and would calculate as 14.3, 15.3, 16.3, 19/19.1 and 20. Additionally one of the positive controls had OLs at D8 and D19. These artefacts are often above LOR and look like peaks and therefore have the potential to interfere with interpretation.

We did not see any of these artefacts with the previous kit lots.

Our concern is that this particular kit lot has its own issues that are different from the issues observed with the other kit lots. This then provides a lack of confidence in what we are going to observe between lots of kits if we were to implement.

Given what we have observed we have not progressed with STRmix analysis as the presence of these artefacts suggests that maybe we should not move any further forward with this validation. We may be better putting our efforts into a another kit such as GlobalFiler.

Please let us know how you would like us to proceed.

Thanks

Emma



Sharon, Cassie and Emma,

Lets get this VF done, I will send out a new batch of appointments for the 4 of us every 2 weeks. Is there a best day and time ?

Kirsten

From: Kirsten Scott		
Sent: Thursday, 25 August 2	022 8·38 AM	
To: Abigail Ryan	; Adam Kaity <	; Allan McNevin
	>; Cassandra James <	>; Chelsea Savage
	>; Emma Caunt <	; Lisa Farrelly
	uke Ryan <l< td=""><td>>; Maria Aguilera</td></l<>	>; Maria Aguilera
	>; Sharon Johnstone <	
Cc: Paula Brisotto <	>; Justin Howes <	
Subject: Verifiler Analytical	program complete	

Verifiler team and Team Leaders,

The analytical component of the Verifiler project is now complete. All reports have been completed and signed, with the finalisation of the EREF report. Thanks Maria and analytical team

The focus will now need to be the FRIT component of the project.

Kirsten

Kirsten Scott Senior Scientist Quality and Projects

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

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

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PP21 Optimisation Update

Robert Morgan and Emma Caunt 8 July 2014


- Different people have been involved in the analysis of different parts of the data
- We have results from Phase 1 and Phase 1B
- Some problem samples have been identified which may skew the data
- Some of the data is not as expected
- The PP21 Optimisation Project has moved away from the original project plan prepared in November 2013
- There is conflicting feedback on the way to move forward
- There are new parameters to address based on the results obtained to date
- PP21 casework interpretation issues still exist and need to be addressed
- JAH and PMB have asked us to put this presentation together and propose a way forward



Recap of Experimental Design



- Phase 1 Optimal PCR cycle number
 - This phase was designed to identify cycle numbers which may not progress to subsequent phases based on the quality of single source profiles obtained using the following assessment/acceptance criteria
 - Heterozygous peak height and linearity
 - Peak height ratio
 - Artefacts
 - Degradation slope
 - Inter locus balance
 - Reproducibility
 - Dropout
 - Off Scale peaks
 - Assessment/Acceptance criteria weré not all 'pass/fail' but more a comparison of the cycle numbers with each other
 - Consisted of 10x10 from 0.025ng to 0.5ng



Not so critical

- Phase 2 Mixtures intuitive assessment
 - Optimal cycle number cannot be determined by single source profiles alone
 - This phase was designed to assess the performance of mixtures with the cycle number(s) that had progressed from Phase 1
 - Mixtures to be assessed intuitively, not with STRmix due to lack of thresholds at this stage
 - Assessment criteria include:
 - Artefacts
 - Ability to determine number of contributors
 - Reproducibility
 - Mixture ratio
 - Again assessment criteria were not all 'pass/fail' but more a comparison of the cycle numbers with each other



- Phase 3 Optimising input template
 - By this phase there should only be 1 or 2 cycle numbers still being tested
 - This phase was designed to test the total DNA template input from 2ng to 0.5ng to offset the expected decrease in sensitivity resulting from the reduction in cycle number
 - Two samples to be amplified in duplicate at the following templates (in ng):
 - 2.0, 1.5, 1.4, 1.3, 1.2, 1.1, 1.0, 0.9, 0.8, 0.7, 0.6



- Assessment criteria include:
 - The peak heights need to be as large as possible without the profile becoming excess
 - The baseline needs to be as low as possible
 - The optimum input template will be the template that produces the largest peak heights with the flattest baseline
 - Templates will also be graded according to the prevalence and size of any artefacts and the ability to characterise the artefacts
- Determine optimum and maximum template
- Question: do we still consider 2 samples amped twice at each template to be enough?



- Phase 4 Interpretation parameters
 - By this phase the cycle number to be implemented will have been selected
 - This phase will determine the thresholds for analysis/interpretation

- Phase 5 Mixtures validation
 - This phase is designed to test a variety of mixtures at different templates
 - If artefacts are present, to characterise them and document how they should be considered in interpretation
 - Develop guidelines for mixture interpretation



WIT.0004.1234.0010

Assessment of Results Obtained

Two goals of the optimisation project:

- 1. Stabilise profiles obtained
- 2. Reduce the influence of artefacts
- Both of these goals will aid in the interpretation of the profiles obtained and in-turn decrease the turn around time
- As a result assessment/acceptance criteria were developed to address these aims



 Heterozygous Peak Height and Linearity vs Input Template

Acceptance Criteria – full profile must be obtained from 0.5ng, if a full profile is not obtained that cycle number may not progress to Phase 2

30 cycles 100% of alleles at 0.5ng

29 cycles 99.5% of alleles at 0.5ng

28 cycles 97.5% of alleles at 0.5ng

27 cycles 90% of alleles at 0.5ng

Based on this data 27 (and potentially 28 cycles) may not progress to Phase 2

Peak Height Ratio

Acceptance Criteria – cycles which display a peak height ratio below what is acceptable (40-50%) may not progress to Phase 2

30 cycles 65% - 85% 29 cycles 70% - 85%

28 cycles 75% - 85%

27 cycles 75% - 85%

Based on this data all cycles could progress to Phase 2

Artefacts

Assessment Criteria – ranking of samples for comparison: total number of artefacts, maximum artefact peak height, average artefact peak height, relative impact of artefacts

There was limited data as a result of the low peak heights and this would be further investigated as part of template optimisation (Phase 3)

Degradation

Acceptance Criteria - minimal degradation or decrease in slope (negative slope) will pass to next acceptance criteria, increase in slope (positive slope) may not progress further, need to identify loci which preferentially amp outside of expected degradation slope

There was preferential amplification noted but was consistent with a negative degradation slope

Inter-Locus Balance

Acceptance Criteria – need to identify loci which preferentially amp outside of expected degradation slope This was not observed at any cycle number



Reproducibility

Acceptance Criteria – ranking of samples according to standard deviation of peak heights

This data is flawed due to NATA requirements and the samples not having been on different amps – this parameter may need to be re-assessed in later testing

• Drop-Out

Acceptance Criteria – cycle numbers which display drop-out greater than 200-300RFU may not progress This was only demonstrated for one of the 30 cycle samples

Off-Scale Peaks

Acceptance Criteria – related to excess profiles but none were observed



Optimum Template vs Maximum Template

- It was expected that peak heights would reduce as the cycle number reduced.
- This affect was more pronounced than expected as even peak heights for 30 cycles were considered low.
- This introduces a new parameter ie. the input template for 30 cycles may not be optimised and the move from half volume to full volume may have needed to be accompanied by an increase in the input template.
- Data from Victorian validation indicates that optimum input template may be different for full volume and half volume reactions
- Some profiles indicate that we may be under-amping casework however we still get excess first run profiles
- Quant variation will affect the ability to compare casework and project samples
- Further investigation is required

30 cycles 0.5ng input template



Moving forward

• Original phase 1 results analysis recommended:

Alternate cycles at 0.5ng input not viable.

Two options to progress:

- 1. Accept 30 cycles at 0.5ng input as the PP21 conditions and finalise project; and commence 3500 and Globalfiler validation immediately.
- Investigate 29 cycles at increased template (0.65ng, 0.85ng and 1ng) before starting mixture studies. Postpone 3500 and Globalfiler.
- Based on the review of the data obtained to date with respect to the original assessment/acceptance criteria, it is our opinion that further investigation is supported



Moving forward

As a result we propose alternate options for moving forward:

- 1. Finalise 1B data by processing 1C data as per the project plan
 - will be a limited investigation into optimising input template
- 2. Extend scope of 1C project plan to address feedback received from project plan
 - will become similar to original phase 3 which has already been written
- 3. Return to original project plan
 - data supports continuing but does not address potential issues noted with 30 cycles
- 4. Continue with modified project plan
 - need to address new parameter of optimising 30 cycles, phase 1B data could be incorporated into phase 3 optimising input template
- 5. Discontinue project
 - not enough data has been obtained to date to support this option

It is our opinion that we should continue with a modified project plan investigating the optimum input template for 29 and 30 cycles and assess the results obtained before continuing further. This would mean completing Phase 3 before Phase 2 and adding template testing for 30 cycles





PP21 Optimisation Phase 1B

Data prepared by PA, EJC and RGM Presented by RGM



Great state. Great opportunity.

Project Plan

- 50 samples
 - 28 cycle testing
 - 5 samples each run once at the following templates:
 - 1.5ng, 1.2ng, 1.0ng, 0.8ng, 0.7ng
 - 29 cycle testing
 - 5 samples each run once at the following templates:
 - 1.2ng, 1ng, 0.8ng, 0.7ng, 0.6ng
- Aim to provide additional information for PP21 Optimisation Project Phase 1 to aid in the decision making for progression to Phase 2.



Limitations

- Templates selected are not exhaustive
 - Aim was to extrapolate data to guide further decision making but the samples processed do not cover the full range that might reasonably be considered given previous results
- Samples are single source
 - Previous testing (original PP21 validation) has indicated that mixtures may be more informative in identifying potential interpretation issues but the scope of this additional testing is limited to single source samples



Potential Outliers

- 1 sample potentially degraded as it consistently displayed a degradation curve steeper than every other sample (Sample 3)
- 1 sample potentially affected by quant issue or prepared incorrectly as it consistently displayed peaks heights stronger than every other sample (Sample 4)
- Given the small number of samples used the presence of these potential outliers in the data has a significant affect on the results, however excluding these results reduces the amount of data available to assess. As a result data will be presented with all samples included and also with sample 3 and 4 excluded.



Results – 28 and 29 cycles – Average Peak Heights





Results – 28 and 29 cycles – Average Peak Heights





Results – Average Peak Heights

- At 28 cycles there is minimal decrease in peak heights as template decreases, except between 1.2ng and 1.0ng where there is a significant drop.
- Peaks heights are low given the amount of template used - due the limited amount of DNA present in the majority of casework samples this suggests that further investigation into 28 cycles may not be worthwhile.
- At 29 cycles there is a consistent decrease in peak heights as template decreases.
- The lack of excess profiles may indicate that the maximum template could be significantly higher than 1.2ng.



Results – 28 and 29 cycles – Degradation Slope





Results – 28 and 29 cycles – Degradation Slope





Results – 28, 29 and 30 cycles – Degradation Slope



Results – 28, 29 and 30 cycles – Degradation Slope



Results – Degradation Slope

- At 28 and 29 cycles negative degradation slope displayed as expected and shows an increase in template results in a steeper line (preferential amplification at higher templates) – STRmix will have no issue with this, the only difficulty that may arise is with the determination of number of contributors.
- With the extra data it appears that 29 cycles will follow a similar degradation curve to 30 cycles although it does appear that it might not be as steep.
- Optimisation project investigating optimum and potential maximum template – given that this preferential amplification issue is present at 30 cycles further investigation may be needed to consider preferential amplification as another measure of maximum template not just the presence of excess profiles.



Results – 28 and 29 cycles – Average Peak Height Ratio





Results – 28 and 29 cycles – Average Peak Height Ratio





Results – Average Peak Height Ratio

- No issues noted with this data.
- At both 28 and 29 cycles average peak height ratio falls within acceptable ranges.
- At 28 cycles there is an increase in balance between 1.5ng and 1.2ng (minimal).
- At 29 cycles there is an increase in balance between 1.2ng and 1.0ng (minimal).
- Could be a very early indication of where optimum values may fall.



Results – 28 Cycles – Average Peak Heights (Per Sample)





Results – 29 Cycles – Average Peak Heights (Per Sample)





Results – Average Peak Heights (Per Sample)

- This data highlights the outliers
- Sample 3 with the steep degradation slope has on average lower peak heights.
- Sample 4 with the potential quant/preparation issue has on average higher peak heights.

Potential Casework Implications

- STRmix has been developed to model negative degradation and does so well.
- It cannot model positive degradation and this is something that we see at 30 cycles where one locus is over-amped and the result is that a major contributor can be excluded even though we can see that they match.
- Assessment of these results has also included assessment of whether there are individual loci behaving outside of the expected degradation line (check of EPGs) and did not identify any issues.
- The other impacting factor is preferential amplification which is a known issue with PP21 at 30 cycles.


Further Testing

- A review of the samples selected for testing may be required:
 - Samples from Phase 1B have been identified as potentially degraded/over-amped influencing the data produced.
 - A quick review of the data that was used for Phase 1A shows that some of these results may also be affected.
 - These potential issues are not easily identified unless a review of the EPGs is also conducted.
 - The samples used should be assessed and some potentially changed to ensure the most reliable results are obtained, especially with respect to the creation of mixtures.
- Data from testing indicates that 28 cycles may not be fit for purpose and further investigation, if it is to proceed, may only need to test 29 vs 30 cycles.



HealthSupport Queensland

EC-10

PowerPlex21 Observations

October 2014



Great state. Great opportunity.





Preferential Amplification

- Pref amp is often observed in FTA processing but is not often seen in casework
- STRmix is unable to model these profiles
- The template should be reduced to improve the profile





Degradation

- Degradation is most likely a function of the sample quality rather than the kit
- Reworking often does not resolve this issue
- STRmix can model these profiles, however it may not be possible to confidently determine the number of contributors

20(LPH)

138

13

184

11

14

52

15

128

16.2

81

Profile Variability

- Reproducibility is often a concern to case managers
- Quant variation is possibly one of the largest contributors to this

 Variation in the actual quant process including pipetting error considering only using 2µL
 AB noted variation of up to 30% stating pipetting error as one of the sources
 We have observed variation of up to and exceeding (on occasion) 30%
- CE variation
 - Between machines
 - o Between runs
 - Between capillaries
- The correlation between peak height and template is the basis behind STRmix
- STRmix only uses this to determine the relative proportion of template between the contributors to the mixture
- Provided the ratios are consistent between runs then the variation in peak height should not be an issue



- Testing of 30 cycles above the input template of 0.5ng has shown that profiles do not become excess until approximately 0.8ng
- It is possible to increase template as a reworking strategy
- Be mindful of the quant variability and that your quant may not be accurate
- Use peak heights in conjunction with the quant value to determine whether amping up is a viable option
- At about 3000 rfu the baseline can become messy pull-up, more prominent +1 and -2 rpt stutter
- If you are going to amp up, suggest trying 0.65ng as a first attempt

Drop-in



- The PP21 Optimisation project has involved amping hundreds of profiles
- Rob and I have examined each of these profiles to the baseline
- No drop-in was observed

Database Search Function

- When STRmix V2.0.1 was implemented we started calculating LRs for each dataset and quoting the most conservative
- When the database search function is used the Caucasian dataset is selected
- This is the LR we quote in the EXH line if the database function has been used
- When a statement is requested, all LR ≠ 0 are recalculated in STRmix to allow HPD and Fst to be incorporated
- At this stage all 3 datasets are used to enable the most conservative to be quoted
- This may result in a significant change in the LR to that originally quoted in the EXH
- >100 billion can be anything from 10¹² upwards
- A LR of 10¹² in the database can become 30 billion when calculated as a 'normal' LR
- If this occurs, the EXH should be changed as per JAH email on 24 Oct



Emma Caunt

From:
Sent:
To:
Cc:
Subject:

ά.

Emma Caunt Wednesday, 8 June 2022 3:07 PM Cathie Allen Cassandra James; Justin Howes RE: Intel report_

Hi Cathie

DPP have requested a timeframe for release of the statement for this case. Can you please advise when I will be able to release the intel report and therefore the statement?

Thanks

Emma

rom: Cathie Allen		
Sent: Monday, 6 June 2022 9:51 AM		
To: Emma Caunt	>	
Cc: Cassandra James <	; Justin Howes <	>
Subject: RE: Intel report_I		0

Hi Emma

Thanks for the additional information. Please hold off on reporting the amendment until further advised.

Cheers Cathie

IC-		

Cathie Allen BSc, MSc (Forensic Science) (She/Her*) 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

Prevention Division, Queensland Health



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*If you're wondering about the use of pronouns She/Her on this signature block, I encourage you to read some resources available here





Hi Cathie

·. .

I have consulted with the original case manager and reviewer to determine the possible cause for the change in result. Both the case manager and reviewer stated that they can see my point of view with respect to calling the profile 'complex' but both indicate that they are also happy with their original interpretation. This kind of profile will always have a degree of subjectivity in the interpretation.

The outcome is that the cause of the result amendment is due to a difference of opinion. Would you like me to add this to the intelligence report?

Thanks

Emma

From: Cathie Allen <		
Sent: Friday, 27 May 2022 4:42 PM		
To: Emma Caunt <		
Cc: Cassandra James <	>; Justin Howes ·	>
Subject: RE: Intel report		

Hi Emma

Thanks for the advice regarding result amendment. Could you please advise why the amendment was necessary, ie more ref samples delivered, unintended human error, case consistency etc.

Can you please add the reason for amendment to the Intel Letter?

Cheers Cathie



Cathie Allen BSc, MSc (Forensic Science) (She/Her*) 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 Prevention Division, Queensland Health



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*If you're wondering about the use of pronouns She/Her on this signature block, I encourage you to read some resources available here



rom: Emma Caunt <	
Sent: Wednesday, 25 May 2022 12:59 PM	
'o: Cathie Allen and an ann an an an ann an an ann an ann an ann an	
C c: Cassandra James <	
Subject: Intel report_	

Hi Cathie

I am writing a statement for **provide statement** and have a result that needs to be incorrected. The profile was originally reported as a 4 person mixture but looking at the mixture in detail, including the ratios across the profile and the contribution of the reference samples in the case, I think that the profile indicates 5 people and is therefore complex.

I have attached the draft intel report for your review. Could you please let me know if you are happy for this report to be issued?

Thanks

5.mma



Forensic and Scientific Services

INTELLIGENCE REPORT

To: Senior Sergeant Stephan FOXOVER DNA Management Section Forensic Services Group Operations Support Command Queensland Police Service Client Reference :



Re: Update of DNA profiling result for sample

(owing a reassessment of this result at statement preparation stage, the reported interpretation of this DNA profile requires updating and correction in the Forensic Register.

The DNA profiling result obtained from this sample was initially reported as follows:

Mixed DNA profile Mix – supports non contribution Mix – support for contribution >100 billion Mix – support for contribution 1 million to 100 billion Mix – inconclusive

The updated DNA profiling result obtained from this sample will now be reported as follows:

Complex mixed profile unsuitable for interp or comparison

The pdf document in this email is the report issued by Forensic and Scientific Services. Any other attachments or information provided is not considered to be the issued report.

s information has been peer-reviewed in accordance with standard laboratory Quality Assurance protocols.

Peer Reviewed by Cassandra James, Scientist Forensic DNA Analysis 25 May 2022 Emma Caunt, Scientist Forensic DNA Analysis 25 May 2022 Phone



NATA Accredited . Laboratory 41 Accredited for compliance with ISC/IEC 17025 -Testing PO Box 594 Archerfield QLD 4108 AUSTRALIA Phone Fax Email

Email	string	2

Emma Caunt

From:	Allison Lloyd
Sent:	Monday, 30 May 2022 11:35 AM
To:	Emma Caunt; Tegan Dwyer
Subject:	RE: Intel report_

Hey,

I'm ok if you want to complex it, I can see it both ways, and having to make the assumption that both refs are present.

AL

l

From: Emma Caunt		
Sent: Monday, 30 May 2022 9:51 AM		
_ To: Tegan Dwyer <	: Allison Lloyd <	
ubject: RE: Intel report_		

Hi Tegan

Thank you for this. I'm not sure about the intel report. I guess the reason would be difference in opinion so I'll add that to the report and see if Cathie is happy with that.

Allison – do you have anything to add?	
Thanks	
Emma	
From: Tegan Dwyer < Sent: Monday, 30 May 2022 9:40 AM	8
To: Emma Caunt; Allison Lloyd	

Hi Emma,

I think this one is borderline for me, I can see why you would call it complex, especially at D8 with the 7 allele being high stutter but that would be based on assuming the two reference samples are there.

I haven't done a great deal of incorrects/Intel reports - how will your intel report differ if it's a difference of opinion vs now we all agree? Is it not unintentional human error either way?



*** ***

Forensic & Scientific Services, Prevention Division, Queensland Health

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a 39 Kessels Road, Coopers Plains, QLD 4108

e www.health.gld.gov.au/healthsupport/businesses/forensic-and-scientific-services

Queensland Health acknowledges the Traditional Owners of the land,	and pays respect to Elders past, present and future.
From: Emma Caunt <	
Sent: Monday, 30 May 2022 8:12 AM	
To: Tegan Dwyer <	>; Allison Lloyd
Subject: FW: Intel report	

Hi Tegan and Allison

Please see below email string. As the case manager and reviewer would you be able to have a look at this sample and let me know what you think the reason for the incorrect is. For example, are you still happy with your original interp and therefore we have a difference of opinion or maybe you agree that the profile is complex. This will help me to inform Cathie of the reason for the incorrecting of the result.

I don't think that the reason is receipt of another reference sample or case consistency as suggested as possible reasons by Cathie.

Thank you

Emma

From: Cathie Allen	
Sent: Friday, 27 May 2022 4:42 PM	-
To: Emma Caunt <	
Cc: Cassandra James	>; Justin Howes <
Subject: RE: Intel report_	

Hi Emma

hanks for the advice regarding result amendment. Could you please advise why the amendment was necessary, ie more ref samples delivered, unintended human error, case consistency etc.

Can you please add the reason for amendment to the Intel Letter?

Cheers
Cathie
Cathie Allen BSc, MSc (Forensic Science) (She/Her*)
Managing Scientist
Social Chair, Organising Committee for 25th International Symposium of the
Australian and New Zealand Forensic Science Society (ANZESS), Brisbane, 11 – 15 Sept 2022
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· · · · · ·



From: Emma Caunt
Sent: Wednesday, 25 May 2022 12:59 PM
To: Cathie Allen
Cc: Cassandra James <
Subject: Intel report_

Hi Cathie

I am writing a statement for **provide the statement** and have a result that needs to be incorrected. The profile was originally reported as a 4 person mixture but looking at the mixture in detail, including the ratios across the profile nd the contribution of the reference samples in the case, I think that the profile indicates 5 people and is therefore complex.

I have attached the draft intel report for your review. Could you please let me know if you are happy for this report to be issued?

Thanks

Emma

email string

Emma Caunt

From: Sent: To: Subject:

ter

. S

Emma Caunt Monday, 13 June 2022 2:10 PM Sharon Johnstone RE: Intel report_

Hi Sharon

Yes, Cassie has reviewed the sample and agreed that it is complex. I also got her to review the intel report before sending it to Cathie.

Thanks

Emma

From: Sharon Johnstone	
ent: Monday, 13 June 2022 2:03 PM	
To: Emma Caunt <	
Subject: RE: Intel report	

Hi Emma, Has this casefile been given to Cassie for review yet?

If not I think what would be best is to see what her interp for this sample is independently before we decide how this sample should be reported.

I had more of a look at this sample for case context and there is 2 x knives in the case. For this particular knife the only other profiles are of the deceased or complex. This result may have more implications if changed than you first indicated.

Can you please have Cassie do her review and see what comes of it.

Thanks, haron



Sharon Johnstone Senior Scientist – Forensic Reporting and Intelligence Team

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

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	CLEAN HANDS SAVE LIVES Wash your hands regularly to stop the spread of germs.
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	Hi Sharon
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	From: Cathie Allen < Sent: Monday, 6 June 20 <u>22 9:51 AM</u> To: Emma Caunt < Cc: Cassandra James <; Justin Howes · Subject: RE: Intel report
	Hi Emma
	Thanks for the additional information. Please hold off on reporting the amendment until further advised.
	Cheers Cathie

2



Cathie Allen BSc, MSc (Forensic Science) (She/Her*)

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

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Hi Cathie

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The outcome is that the cause of the result amendment is due to a difference of opinion. Would you like me to add this to the intelligence report?

Thanks

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Cheers

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At D18 the allelic peaks are 12,14,15,16,17,19. Both of the refs are 12,16 leaving 14,15,17,19. However the 14 is too high to pair exclusively with the 15,17,19 indicating that there is another portion of 14. This would make the profile 5p.

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 Cc: Cassandra James

 Subject: RE: Intel report

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Thanks

Emma

C



Emma Caunt

From: Sent: To: Subject:

See 4.

Emma Caunt Thursday, 16 June 2022 10:26 AM Sharon Johnstone RE: Intel report_

Hi Sharon

Do you have any updates on this yet?

Thanks

Emma

l

From: Sharon Johnstone	
Sent: Monday, 13 June 2022 2:03 PM	
`o: Emma Caunt ·	
subject: RE: Intel report	

Hi Emma,

Has this casefile been given to Cassie for review yet?

If not I think what would be best is to see what her interp for this sample is independently before we decide how this sample should be reported.

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Sharon Johnstone Senior Scientist – Forensic Reporting and Intelligence Team

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

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Cathie Allen BSc, MSc (Forensic Science) (She/Her*)

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Cathie



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I have attached the draft intel report for your review. Could you please let me know if you are happy for this report to be issued?

Thanks

Emma



Emma Caunt

From:
Sent:
To:
Subject:

Emma Caunt Monday, 20 June 2022 2:44 PM Tegan Dwyer; Sharon Johnstone RE: Intel report_

Thank you Tegan, that helps.

From: Tegan Dwyer <		
Sent: Monday, 20 June 2022 2:43 PM		
To: Emma Caunt <	Sharon Johnstone •	>
Subject: RE: Intel report_		

Hi Emma,

I think the error is that I have missed a contributor, looking at it now I can see evidence of 5p at D8, and potentially t D18 as well, which I didn't see at the time.

Hope that helps,

Tegan

From: Emma Caunt <	
Sent: Monday, 20 June 2022 2:23 PM	
To: Sharon Johnstone	78
Cc: Tegan Dwyer	
Subject: RE: Intel report_	

Hi Sharon and Tegan

I am happy to state "unintentional human error" in the report if I know what the error is. Can you please let me know what error has occurred for the result to need to be changed?

hanks

Emma

From: Sharon Johnstone	
Sent: Monday, 20 June 2022 2:20 PM	
To: Emma Caunt <	
Cc: Tegan Dwyer <	
Subject: RE: Intel report_	

Hi Emma,

I have just had a conversation with Tegan and she said that she is happy for the intel report to state "unintentional human error" as the reason in the change in result. I have CCd her for transparency.

Cathie as managing scientist has asked for the intel report to include the reason for this change in result. The report will still need to go to her for approval prior to being sent.

Regards,

4 ja: • ja:

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.



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R Contraction	

From: Emma Caunt ·	
Sent: Monday, 20 June 2022 2:07 PM	
To: Sharon Johnstone <	
Subject: RE: Intel report	

Hi Sharon

' think that Tegan's email is asking whether a difference of opinion is the same as unintentional human error. I don't
 Delieve that she is saying that human error has occurred.

I don't understand where human error has played a part in this change in interpretation. I also don't understand the reluctance to state that is it possible for two scientists to interpret a profile differently, especially since because we have had lots of discussions about subjectivity.

I am still reluctant to state, in writing, that a human error has occurred in this instance.

Can I please send the report without stating a reason for the change?

Thanks

Emma

From: Sharon Johnstone <s< th=""><th></th></s<>	
Sent: Monday, 20 June 2022 1:54 PM	
To: Emma Caunt <	
Subject: RE: Intel report	

>

Hi Emma,

Thanks for providing the email string attached. It is noted that it was suggested by Tegan the reason for the change is unintentional human error. I think given that has been suggested by the original case manager, it is reasonable for that to be the reason on the intel report.

If the intel report is addressed I can't see why the statement can't be sent out by your leave period.

Regards, Sharon

e



Sharon Johnstone Senior Scientist – Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream

revention Division, Queensland Health

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From: Emma Caunt	ľ
Sent: Monday, 20 June 2022 12:58 PM	
To: Sharon Johnstone <	
Subject: RE: Intel report	

Hi Sharon

Please see attached email string. The basis of the difference of opinion is whether you can use the fact the both refs appear to be present but both of them being present changes the number of contributors.

Thanks

Emma

From: Sharon Johnstone < Sent: Monday, 20 June 2022 12:37 PM

WIT.0004.1237.0023

To: Emma Caunt <<u>I</u> Subject: RE: Intel report_

HI Emma,

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Given the conversations you had with the original reporter and reviewer, what is the basis of the difference of opinion?

Regards,

Sharon



Sharon Johnstone Senior Scientist – Forensic Reporting and Intelligence Team

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

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rom: Emma Caunt <	Þ
Sent: Monday, 20 June 2022 9:42 AM	
To: Sharon Johnstone <	
Subject: RE: Intel report_	

Hi Sharon

The original case manager is happy with their original interpretation therefore the reason is not human error. Since the original interpretation was performed with all reference samples available it is also not a change due to reference samples being received.

I do not want to write something in a report that I do not believe to be true. If a reason needs to be included in the report then the reason is a difference of opinion, otherwise it would be best to leave the reason out of the report.

Thanks

Emma

From: Sharon Johnstone < Sent: Monday, 20 June 2022 9:37 AM To: Emma Caunt Subject: RE: Intel report

Hi Emma,

So usually the intel reports state either human error or change in interp due to reference samples. But as I understand it the reference samples were taken into consideration when the result was reported. Which one do you believe to be the most suitable explanation?

Regards, Sharon

Sharon Johnstone

Senior Scientist – Forensic Reporting and Intelligence Team

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

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From: Emma Caunt <	
Sent: Friday, 17 June 2022 12:06 PM	
To: Sharon Johnstone <	
Subject: FW: Intel report_	

Hi Sharon

My understanding from our conversation yesterday is that the change in interpretation is due to human error as the 7 peak at D8 is above stutter threshold making the profile 5p without consideration of the reference profiles.

I have had another look at the profile and some of my assessments in my email below were incorrect.

At D18 the 15 peak is below stutter threshold, therefore if both reference samples have contributed then the profile would be 4p at this locus.

At D8 the 11 and 13 peaks are below stutter threshold. Both of the reference samples are 12,14 leaving alleles 7, 8, 10 and 15. Given the height of the 7 peak in relation to the heights of the 8,10 and 15 peaks, it is my opinion that the 7 cannot pair with any of them. This means that without consideration of the reference samples the profile appears to be 4p, but if both refs are present then the profile is 5p.

This takes us back to my original assessment that the change in result is due to a difference of opinion rather than human error.

Can you please reply to this email to let me know how you would like me to proceed given that there is a committal hearing on Wednesday next week and DPP has asked again for a timeframe.

Thanks

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Emma

From: Emma Caunt
Sent: Monday, 13 June 2022 11:47 AM
To: Sharon Johnstone
Subject: FW: Intel report

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I've had another look at this in detail.

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Cheers Cathie

Cathie Allen BSc, MSc (Forensic Science) (She/Her*) Managing Scientist

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From: Emma Caunt <
Sent: Wednesday, 25 May 2022 12:59 PM
To: Cathie Allen <
Cc: Cassandra James <
Subject: Intel report_

Hi Cathie

I am writing a statement for **control of** and have a result that needs to be incorrected. The profile was originally reported as a 4 person mixture but looking at the mixture in detail, including the ratios across the profile and the contribution of the reference samples in the case, I think that the profile indicates 5 people and is therefore complex.

I have attached the draft intel report for your review. Could you please let me know if you are happy for this report to be issued?

Thanks

Emma
· Vec · see

email string (6)

Emma Caunt

From: Sent: To: Cc: Subject:

Cathie Allen Tuesday, 21 June 2022 10:26 AM Emma Caunt Cassandra James; Justin Howes; Sharon Johnstone RE: Intel report_

Hi Emma

We will request that the Intel Report is issued by Tegan. I've asked Sharon to discuss this with Tegan.

Cheers Cathie

Cathie Allen BSc, MSc (Forensic Science) (She/Her*) 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

Prevention Division, Queensland Health

a 39 Kessels Road, Coopers Plains, QLD 4108 e www.health.gld.gov.au/fss

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From: Emma Caunt	
Sent: Tuesday, 21 June 2022 10:21 AM	
To: Cathie Allen	
Cc: Cassandra James <	; Justin Howes <
Sharon Johnstone <	•
Subject: RE: Intel report	

Hi Cathie

My understanding is that intel reports have been provided to the courts in the past. In fact I believe that Insp Neville has told one of our reporters that they are included in the court briefs. Additionally, when casefiles are requested by

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the courts they may also contain intel reports. On this basis I consider them to be court documents and therefore should be treated as such.

My preference would be to not include the reason for the change in result in the report as this seems to be a contentious issue.

However, if you are directing me to use the standardised wording then I will do so.

Thanks

Emma



li Emma

Thanks for the information regarding the additional line from a quality perspective. I see the value in the organisation including this in reports and will ensure that all other Intel reports I'm made aware of include it.

The Intel Report is a summary provided to the QPS to communicate the reasoning for an amendment or correction of reported results and what the changes are. The report is provided to the QPS, and in the context of its provision is not hearsay, given it's not being provided to the Court.

The standardised wording of 'Following a reassessment of this result at statement preparation stage, the reported interpretation of this DNA profile requires updating and correction in the Forensic Register, due to an unintentional human error.' states facts – a reassessment had been undertaken, the reported interpretation requires correction, and this was due to unintentional human error – each of those things have occurred.

What is your proposal to move forward regarding this?

Cheers athie



Cathie Allen BSc, MSc (Forensic Science) (She/Her*) 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

Prevention Division, Queensland Health

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Hi Cathie

Unfortunately I don't feel that I am able to use the standardised wording for this intel report. Since the error is not mine, I am using the information provided by somebody else that the difference in the interpretation is in fact due o a human error. For me to state that the reason for the change is human error is therefore hearsay and I think it is important for me to acknowledge this in my report.

Please see attached email for the reason for the additional wording 'The pdf document in this email is the report issued by Forensic and Scientific Services. Any other attachments or information provided is not considered to be the issued report.'

Thanks

Emma

From: Cathie Allen <	>
Sent: Tuesday, 21 June 2022 8:56 AM	
To: Emma Caunt < <u>I</u>	>
Cc: Cassandra James <	>; Justin Howes <
Subject: RE: Intel report	
n i i i i i i i i i i i i i i i i i i i	

Hi Emma

Thank you for the opportunity to review the Intel Report prior to its release.

Can you please ensure that the wording within the report is the standardised wording and in line with other Intel Reports that have been issued. Specifically, the following wording being used 'Following a reassessment of this result at statement preparation stage, the reported interpretation of this DNA profile requires updating and correction in the Forensic Register, due to an unintentional human error.'

Also, are you able to advise the reasoning for the inclusion in the Report of the following 'The pdf document in this email is the report issued by Forensic and Scientific Services. Any other attachments or information provided is not considered to be the issued report.' I don't think I've seen this in any other Intel Reports.

Cheers Cathie

Cathie Allen BSc, MSc (Forensic Science) (She/Her*)

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

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25TH INTERNATIONAL SYMPOSIUM	MARK YOUR DIARY	
BRISBANE SH 2022	NOSTED BY Australian and New Zealand FORENSIC SCIENCE SOCIETY	
From: Emma Caunt		
Sent: Monday, 20 June 2022 3:09 PM		
To: Cathie Allen <		
Cc: Cassandra James <	; Justin Howes <	
Subject: RE: Intel report_		
Importance: High		

Hi Cathie

• have attached the updated Intel Report for your information. I would appreciate it if could please respond with any -reedback by noon tomorrow (Tuesday) to facilitate release of the statement prior to the committal hearing on Wednesday.

Thanks

Emma

From: Cathie Allen	
Sent: Monday, 6 June 2022 9:51 AM	_
To: Emma Caunt <	
Cc: Cassandra James <	>; Justin Howes <
Subject: RE: Intel report_	

Hi Emma

Thanks for the additional information. Please hold off on reporting the amendment until further advised.

Cheers



Forensic and Scientific Services

INTELLIGENCE REPORT

To: Senior Sergeant Stephan FOXOVER DNA Management Section Forensic Services Group Operations Support Command Queensland Police Service

Client Reference :



Re: Update of DNA profiling result for sample

Ulowing a reassessment of this result at statement preparation stage, the reported interpretation of this DNA profile requires updating and correction in the Forensic Register. I have been advised that the reason for the update is unintentional human error.

The DNA profiling result obtained from this sample was initially reported as follows:

Mixed DNA profile Mix – supports non contribution 2000 billion 2000 Mix – support for contribution 1 million to 100 billion 2000 Mix – inconclusive 2000 Mix – inconclusive 2000 Mix – micenclusive 2000 Mix – Mixel 2000 Mix – Mixel 2000 Mix

The updated DNA profiling result obtained from this sample will now be reported as follows:

Complex mixed profile unsuitable for interp or comparison

The pdf document in this email is the report issued by Forensic and Scientific Services. Any other attachments or information provided is not considered to be the issued report.

This information has been peer-reviewed in accordance with standard laboratory Quality Assurance protocols.

Peer Reviewed by Cassandra James, Scientist Forensic DNA Analysis 21 June 2022 Emma Caunt, Scientist Forensic DNA Analysis 21 June 2022 Phone



Cathie

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, •



Cathie Allen BSc, MSc (Forensic Science) (She/Her*)

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

Prevention Division, Queensland Health

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From: Emma Caunt	
Sent: Tuesday, 31 May 2022 11:19 AM	
Tel Cathia Allan	
	- Luckin Haussian
Lc: Cassandra James <	>; Justin Howes <
Subject: RE: Intel report	

Hi Cathie

have consulted with the original case manager and reviewer to determine the possible cause for the change in result. Both the case manager and reviewer stated that they can see my point of view with respect to calling the profile 'complex' but both indicate that they are also happy with their original interpretation. This kind of profile will always have a degree of subjectivity in the interpretation.

The outcome is that the cause of the result amendment is due to a difference of opinion. Would you like me to add this to the intelligence report?

Thanks

Emma

From: Cathie Allen <	
Sent: Friday, 27 May 2022 4:42 PM	
To: Emma Caunt <	
Cc: Cassandra James <	; Justin Howes
Subject: RE: Intel report_	

1 (* 1) ; * ₁

Hi Emma

Thanks for the advice regarding result amendment. Could you please advise why the amendment was necessary, ie more ref samples delivered, unintended human error, case consistency etc.

Can you please add the reason for amendment to the Intel Letter?

Cheers Cathie



I have attached the draft intel report for your review. Could you please let me know if you are happy for this report to be issued?

Thanks

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C

(

Emma

email string

Cassandra James

From: Sent: To: Cc: Subject:

Cathie Allen Tuesday, 21 June 2022 11:36 AM Tegan Dwyer Cassandra James; Justin Howes; Sharon Johnstone RE: Intelligence Report

Hi Tegan

Thanks for preparing the Intel Report. I'm happy for this to progress through to peer review.

Cheers Cathie

e



Cathie Allen BSc, MSc (Forensic Science) (She/Her*) 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

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From: Tegan Dwyer <	
Sent: Tuesday, 21 June 2022 11:22 AM	
To: Cathie Allen	>
Cc: Cassandra James	
Subject: Intelligence Report	

Hi Cathie,

I believe you are aware of this sample already, please let me know if you would like any change to this Intel Report before it heads to peer review.

Thank you,

47 <u>,</u> ^

Tegan



Tegan Dwyer (she/her) Reporting Scientist – Forensic Reporting & Intelligence Team

Police Services Stream, Forensic & Scientific Services Prevention Divsion, 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.

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Forensic and Scientific Services

INTELLIGENCE REPORT

To: Senior Sergeant Stephan FOXOVER DNA Management Section Forensic Services Group Operations Support Command Queensland Police Service

Client Reference :



Re: Update of DNA profiling result for sample

owing a reassessment of this result at statement preparation stage, the reported interpretation of this DNA profile requires updating and correction in the Forensic Register, due to an unintentional human error.

The DNA profiling result obtained from this sample was initially reported as follows:

Mixed DNA profile Mix – supports non contribution Mix – support for contribution >100 billion Mix – support for contribution 1 million to 100 billion Mix – inconclusive

The updated DNA profiling result obtained from this sample will now be reported as follows:

Complex mixed profile unsuitable for interp or comparison

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is information has been peer-reviewed in accordance with standard laboratory Quality Assurance protocols.



Peer Réviewed by Cassandra James, Scientist Forensic DNA Analysis 21 June 2022





PO Box 594 Archerfield QLD 4108 AUSTRALIA Phone Fax Email

Cassandra James

From:	Cassandra James
Sent:	Tuesday, 21 June 2022 11:51 AM
То:	
Cc:	Tegan Dwyer
Subject:	
Attachments:	Intel reportdocx; Intel Report
	Sample

Good Morning,

Please find attached an Intelligence Report detailing the amendment to result **exercises** in relation to this case.

The pdf document in this email is the report issued by Forensic and Scientific Services. Any other attachments or information provided is not considered to be the issued report

'ind Regards
Cassie James



Cassandra James 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.

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WIT.0004.1238.0001

EC-12 Incorrect result 1 25/05/22. -Sent email to CIA with intel report for incorrect result (1 27/05/22 1 Received email from CJA asking for I reason for incorrect 30/05/22 Sent email to Tegan & Allison (original (mer + reviewer) to ask thom what they thought was reason for incorrect 11 30/05/22 Received email from Tegan stating reason as difference of opinion 1 -30/05/22 -Received enail from Allison stating 100 that she can see both points of view 100 110 31/05/22 110 Sent email to CJA stating reason as 1200 difference of opinion 1225 110 100 NIFS www.nifs.org.au

06/06/22 Received email from Cathie terring me to hold off on report - report reporting the incorrect 08/06/22 Emailed GJA for update as DPP had asked for a timeframe (committed Listed for 22/06/22) 13/06/22 Emailed Sharon to follow up due to timograme request 13/06/22 Sharon called me. She said she was supposed to follow up with me last weak She asked me to explain my reasoning For Conpu 1 13/06/22 1 Emailed sharon to explain my reasoning R K in more detail. Sharon replied to ask if Cassie had resieved yet www.nifs.org.au

16/06/22 Emailed Sharon for update 16/06/22 Sharon spoke to me + said that the 7208 is ~ D.1° b over threshold Shich makes the profile Sp without the refs and therefore the reason is human error l'explained That I thought that wa a weak reason + asked if ld send out the report without a reason. She Said that I had to include a reason + that reason is human error 17/06/22 Sent email to Sharon explaining that 1 still thought the reason was difference of opinion 17 06/22 Spoke to have + asked what I should do if I an directed to write something in a report that I do not agree with are advised to get the direction writing + then write in the report have been directed to www.nifs.org.au

Emma Caunt

From:Cathie AllenSent:Monday, 30 April 2018 11:24 AMTo:Emma CauntCc:Andrew RiddellSubject:Meeting

Sensitivity:

Confidential

Hi Emma

I would like to meet with you on Wednesday, 2nd of May at 3pm in Conference Room 113, to discuss a workplace matter relating to compliance with workplace record keeping practices in which you may have further information and or have been involved. Before I decide how to proceed in this matter I would like to give you an opportunity to respond.

Andrew Riddell, Manager HR and Business Relationships, will be in attendance at the meeting. You may bring a support person with you. Your support person may be a union representative, friend or family member, your support person does not participate in the interview. If you cannot have a support person attend the interview face-to-face, arrangements can be made so that they can attend by telephone. Can you please advise Andrew of your support person to ensure appropriateness.

I reiterate I am only considering this matter at this stage. No decision has been made as to what, if any, further action will be taken in relation to this matter. If, after considering your response I determine further action is required, I will contact you again to provide details of the further action.

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, legal representative or employee assistance. If you need to discuss this matter with any staff member you should make this request through myself on telephone in the first instance.

Employee assistance

Employee assistance offers a confidential counselling service which is free of charge to all employees of HSQ for up to six sessions per calendar year. Access to this service is by self-referral. If you wish to utilise this service, please contact Optum on 1800 604 640. More information on employee assistance can be found at http://gheps.health.gld.gov.au/eap.

Cheers Cathie



Cathie Allen

Managing Scientist - Police Services Stream

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



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Emma Caunt

From: Sent: To: Subject: Brian Newman < Wednesday, 2 May 2018 6:31 AM Cathie Allen Re: Proposed meeting with Emma Caunt and Kylie Rika

Cathie

Thank you for your email.

We have been retained by both Emma and Kylie to support them now, but as we discussed yesterday, I will not be available tomorrow due to a matter before the QIRC.

Both of our clients have expressed concerns in relation to this meeting and both have no other support person available and do not want to proceed with the meeting without a support person of their choosing present, such is their right.

With that said, I am available tomorrow morning.

I am aware that there was another staff member who had their meeting moved forward.

This was not an option you had discussed yesterday and had you done so, we may have been able to facilitate the meeting then.

Nevertheless, the fact remains that our clients feel threatened and they are not willing to attend without a support person of their choosing, nor should they be forced to.

Can you please confirm that you agree to move this proposed meeting to tomorrow (Thursday)?

Regards Brian Newman Workers First Pty Ltd

Sent from my iPhone X

On 1 May 2018, at 16:04, Cathie Allen <

wrote:

Hi Brian

We are available to meet tomorrow afternoon at 1630 if that assists. If you're unable to attend in person, we are able to set up a teleconference for the meeting, or include another representative from your organisation.

Cheers Cathie



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From: Brian Newman [mailto: Sent: Tuesday, 1 May 2018 12:14 PM To: Cathie Allen Subject: Re: Proposed meeting with Emma Caunt

Cathie

Thank you for the accommodation, however, as I am sure you may be aware, it would unlikely that I would get away from the QIRC prior to 1600.

On that basis, and given the significant concerns which are apprehended by our client with respect to this matter and recent workplace matters which have been witnessed and cause for serious concern of our client and her welfare, we respectfully request that this matter is postponed for another day.

For your additional consideration, we have also been contacted by other staff in the workplace to support them in what appears to at least be a similar meeting in the same workplace at a similar time and place with the same people and agenda.

Once we have taken formal instructions from those staff, we would be making the same or similar request for postponement of the proposed meeting.

Yours truly Brian Newman JP(Qual) Director of Investigations & Industrial Advocacy

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On Tue, May 1, 2018 at 11:52 AM, Cathie Allen

wrote:

Hi Brian

The purpose of the meeting is to bring clarification regarding workplace record keeping and to gain information that Emma may have regarding this. I'm unable to provide any further information until we meet to discuss it.

The meeting is scheduled for 1500 (not 1400) on Wednesday 2nd of May. We are able to schedule the meeting later in the afternoon, however the latest that the start time could be is 1600 on Wednesday.

Cheers

Cathie



Cathie Allen

Managing Scientist - Police Services Stream

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



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From: Brian Newman [mailto: Sent: Tuesday, 1 May 2018 9:33 AM To: Cathie Allen Subject: Proposed meeting with Emma Caunt

Dear Cathie

We have been requested to attend a proposed meeting with our client, Mrs Caunt on Wednesday 2 May 2018 at 1400.

Agenda and further and better particulars

The agenda for the meeting is unclear and the particulars outlined in your email are vague.

Would you please outline a clear agenda for the meeting and provide particulars which are to be discussed at the meeting.

Proposed date and time of meeting

I am committed to a Queensland Industrial Relations Commission (QIRC) conference in Brisbane city at the proposed time of the meeting and respectfully request that this meeting is moved to another time to enable my attendance as Mrs Caunt's support person.

We would appreciate your urgent reply to this request to move the meeting time and enable my attendance to support our client.

Kind regards

Brian Newman

Workers First Pty Ltd



(url) <u>www.workersfirst.com.au</u>

(a) PO Box 1152 Beenleigh Qld 4207

Sent from my iPad Pro

--

Yours truly Brian Newman Workers First Pty Ltd

Beenleigh Office 11b/20 Main Street, Beenleigh Queensland 4207

Postal address PO Box 1152 Beenleigh Queensland 4207

Office

Facsimile

Website www.workersfirst.com.au

Email

ABN 65 600 518 278

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Emma Caunt

From: Sent: To: Subject: Cathie Allen Tuesday, 1 May 2018 2:26 PM Emma Caunt Meeting

Hi Emma

I understand that your support person is unable to make the meeting time on Wednesday afternoon. Is it possible for you to ask another representative to attend the meeting with you as we'd like to resolve the matter in a timely fashion.

I would like to discuss with you further clarification on a query that was raised regarding workplace record keeping and gather some information from you.

Cheers Cathie



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Emma Caunt

From: Sent: To: Subject: Emma Caunt Tuesday, 1 May 2018 3:26 PM Brian Newman RE: Meeting

I'm terrified

From: Brian Newman [mailto:brian.newman@workersfirst.com.au]
Sent: Tuesday, 1 May 2018 3:24 PM
To: Emma Caunt
Subject: Re: Meeting

Well done

Yours truly Brian Newman JP(Qual) Director of Investigations & Industrial Advocacy

You can visit our website to JOIN NOW.

p: ______ | e: ______ | e: ______ | 24/7 text: ______ | 24/7 text: ______ | f: ______ | f: ______ | www.workersfirst.com.au | PO Box 1152 Beenleigh QLD 4207

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On Tue, May 1, 2018 at 3:08 PM, Emma Caunt <

> wrote:

Hi Cathie

I am being represented by Workers First and as such would like all correspondence regarding this matter to be forwarded to them. I have no other support person available to me and therefore will only be supported by Workers First.

Regards

Emma

From: Cathie Allen Sent: Tuesday, 1 May 2018 2:26 PM To: Emma Caunt Subject: Meeting

Hi Emma

I understand that your support person is unable to make the meeting time on Wednesday afternoon. Is it possible for you to ask another representative to attend the meeting with you as we'd like to resolve the matter in a timely fashion.

I would like to discuss with you further clarification on a query that was raised regarding workplace record keeping and gather some information from you.

Cheers

Cathie



Cathie Allen Managing Scientist – Police Services Stream Forensic & Scientific Services, Health Support Queensland, Department of Health

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Time/Date	User	Labgrp	Test			Event
1:58 26-Feb-21	smjf1	SS		Transferred from rack	to nac	¢
3:09 10-Apr-18	prf2	SS	_	Transferred from rack	to rac	<
120 80 Yat - 18	0,411	55		Transferred from rack	10 190	
1:53 03-NOV-14	pattl	22		Transferred from rack	to raci	
1:31 ZB-APT-13	part1	22	CODONT	Iransterred from rack	10 Nat	CK.
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Emma Caunt

From:
Sent:
To:
Subject:

Emma Caunt Thursday, 25 August 2022 8:02 AM Justin Howes; Sharon Johnstone RE: DBLR workshop

Hi Justin

That's great, could you please ask Cathie if the department would fund this workshop.

Thanks

Emma



Hi, no I haven't heard from Cathie of any unwillingness for the dept to fund.

Justin



Justin Howes

Team Leader - Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream, Forensic & Scientific Services 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.

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



Although attendance at the DBLR workshop would be partly for my own professional development, I would consider that it would also be of benefit to Forensic DNA Analysis given that we are currently evaluating DBLR. I understand that the department can use their discretion when funding such workshops. In this instance can you please confirm that Cathie is unwilling for the department to fund my attendance at the workshop.

Thanks

Emma



Hi Emma

Cathie advised the Policy C42 would apply here, where Attachment 1 mentions where PDA is appropriate.

There is a point in the policy that 'Evidence of professional development expenditure within the previous 12 months (to an amount equivalent to the employee's PDA) is to be provided by the employee when requesting additional departmental funding for the professional development purposes.'

I would think this is the first step in that if wanting additional departmental funding, the evidence of PDA use would be needed and then Attachment 3 is used to record and demonstrate the use of PDA.

Regards Justin



Justin Howes

Team Leader - Forensic Reporting and Intelligence Team

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



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Wash your hands regularly to stop the spread of germs.

From: Emma Caunt ₄______ Sent: Monday, 22 August 2022 1:04 PM To: Sharon Johnstone < Subject: DBLR workshop

Justin Howes

Hi Sharon and Justin

I am attending the ANZFSS conference this year and am self-funded. Would I be able to get funding to attend the DBLR workshop (\$195)? I would be happy to attend the Sunday workshop in my own time. I have completed the online DBLR training but there are still some aspects of the program that I don't understand. I think this workshop would be beneficial for me and would assist with the write up of the DBLR project report.

Thanks

Emma



Emma Caunt Scientist

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

a 39 Kessels Road, Coopers Plains, QLD 4108 e www.health.qld.gov.au/fss

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