Review of the use of STRmix by Queensland Health Forensic and Scientific Services (QH)

Requested by Commission of Inquiry

into Forensic DNA Testing in Queensland

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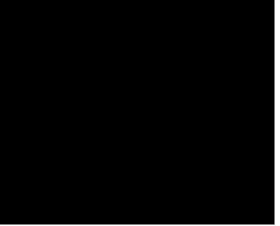

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1.0 - Purpose:

Dr Kogios and Ms Baker made a recommendation about such a review that they were not able to do as part of their overall review of the lab because of late provision of STRMix material. This is their recommendation:

Recommendation 27.

QH should facilitate an external review of the use of STRmix covering:

a. Alignment of use to in house validation and SOPs;

b. Alignment of use to STRmix recommendations.

c. Investigation of whether QHFSS' use of dropping loci in STRmix is fit for purpose;

d. Investigation of whether QHFSS' use of the STRmix diagnostic data is fit for purpose; and

e. Investigation of whether the assignment of the number of contributors is fit for purpose, both for STRmix and the implications for the wider case.

f. Investigation of the appropriate "stratification" of populations in STRMix to determine likelihood ratios.

The points in this recommendation come from the findings of Dr Kogios and Ms Baker listed in points 133 to 138 of their report (EXP.0007.0001.0060), which are:

133 The following further issues were raised in relation to the topic of DNA interpretation.

134 We have heard of instances where some staff invoke an additional contributor of DNA for mathematical modelling purposes in situations where the only indication of an additional DNA contributor is stutter above the laboratory's guideline and/or allelic imbalance.

135 It is important this claim is verified, as there are certain situations where the potential harm of such a decision far outweighs any perceived benefit to the mathematical model. An example of this is invoking an additional DNA contributor in the sperm fraction of a high vaginal swab in a sexual assault case. To an end user, this could imply an individual has had an additional sexual partner than any disclosed, causing serious harm to the individual complainant and their credibility.

99 *136 We understand there is divergent practice amongst reporting scientists*
100 *regarding double back stutter and composite stutter. It is important this claim is*
101 *verified through STRmix review, and any impact assessed.*

102 *137 There was also evidence of scientists dropping more than one loci in STRMix*
103 *and of disagreement among the reporting team as to the circumstances in which that*
104 *may be done.*

105 *138 A question was also raised about the "stratification" of populations in STRMix*
106 *to determine likelihood ratios: Instruction, 13.*

107 I have been asked by the Commission of Inquiry into Forensic DNA Testing in Queensland
108 (hereafter 'the Commission') to:
109 *a) Review the briefed material;*
110 *b) Conduct a review of the QHFSS laboratory's use of STRMix, covering at least the*
111 *following topics (Kogios and Baker report, Rec 27):*
112 *i. Alignment of use to in house validation and SOPs;*
113 *ii. Alignment of use to STRmix recommendations.*
114 *iii. Investigation of whether QHFSS' use of dropping loci in STRmix is fit for*
115 *purpose;*
116 *iv. Investigation of whether QHFSS' use of the STRmix diagnostic data is fit for*
117 *purpose; and*
118 *v. Investigation of whether the assignment of the number of contributors is fit for*
119 *purpose, both for STRmix and the implications for the wider case.*
120 *vi. Investigation of the appropriate "stratification" of populations in STRMix to*
121 *determine likelihood ratios.*
122 *c) Provide an expert opinion as to whether the QHFSS laboratory's use of STRMix is*
123 *consistent with best practice, both overall and in relation to each of paragraph (b)(i)*
124 *to (vi) above;*
125 *d) Provide an expert opinion as to what extent, if any, any deficiency in the current use*
126 *of STRMix in the laboratory could have or did have an impact on:*
127 *i. Reliably obtaining a result that could be reported to QPS and the courts;*
128 *and/or*
129 *ii. The accuracy of results reported to QPS and the courts.*
130 *e) If any deficiency in the current use of STRMix by the laboratory is identified, the steps*
131 *necessary to rectify that issue.*
132
133 I will use the terminology of Dr Kogios and Ms Baker in my review, specifically 'below
134 accepted practice', 'below recommended best practice', 'within the range of best practice',
135 and 'yet to adopt emerging best practice'. To assist me in this task the Commission have also
136 provided a number of documents (listed in Appendix I). I provide my Curriculum Vitae in
137 Appendix II.

138

139 <u>1.1 - Disclaimers</u>

140 Firstly, I declare the following points:

141 1) I am one of the technical developers of STRmix™ and have actively published
142 scientific papers, presented at conferences and conducted training workshops on
143 topics relating to the function, performance and use of STRmix. I do not personally
144 profit financially from the sales of STRmix™.

145 2) I have been involved in the training of forensic scientists in the use of STRmix™,
146 including members of the Queensland Health Forensic Science Service.

147 3) I am one of the contributing technical developers of software FaSTR™ DNA, which I
148 mention in my report. I do not personally profit financially from the sales of FaSTR™
149 DNA.

150 I do not feel that these points affect my ability to carry out the request of the Commission.

151

152 Secondly, the views expressed in this report are mine and do not reflect the position of
153 Forensic Science SA. There are sections throughout this report where I have interpreted DNA
154 profiles produced by QH to provide my own opinion. Whilst I have had 17 years of
155 experience interpreting DNA profiles, there are many other very talented people in the
156 forensic community who have had just as much, or more, experience interpreting DNA
157 profiles who may have differing opinions. Therefore, the findings in my report should be
158 considered as one person's opinion rather than the definitive truth.

159

160 **2.0 - Executive Summary:**

161 The following provides a general summary of the findings in relation to the material
162 reviewed. For more detail see the individual sections in the main body of the report:

163 2.1 - Alignment of use to in house validation and SOPs

164 In the casefile material I reviewed the use of STRmix was in alignment with the use
165 described in the QH SOPs. Within the 'Basics of DNA Profile Interpretation' SOP
166 (FSS.0001.0012.0147) there is some question around how the scientist is able to use peaks
167 below the LOR. This should be reviewed and made clearer in the SOP and to scientists.

168

169 2.2 - Alignment of use to STRmix recommendations

170 The use of STRmix as described in the 'Use of STRmix™ software SOP'
171 (FSS.0001.0001.5208) and 'Basics of DNA Profile Interpretation' SOP
172 (FSS.0001.0012.0147) generally align with what would be considered best practice in the
173 forensic community.

174 There are some passages in the 'Basics of DNA Profile Interpretation' SOP that if applied as
175 written would lead to a systematic bias towards overestimating the number of contributors to
176 a DNA profile. A systematic bias in assigning the number of contributors to a DNA profile is
177 below recommended best practice.

178 While the use of stutter thresholds, allele balance and allele reproducibility can all be factors
179 used in the assignment of a number of contributors (and their use to do so falls within current
180 best practice) I recommend the passages in the 'Basics of DNA Profile Interpretation' SOP
181 that could lead to overestimation be revised and the revisions discussed amongst the scientists
182 in QH.

183

184 2.3 - Investigation of whether QHFSS' use of dropping loci in STRmix is fit for purpose

185 The dropping of loci was raised in the statement of Emma Caunt (WIT.0004.1224.0001).
186 There are some reasons why loci need to be removed for STRmix analysis. The reason
187 highlighted by Ms Caunt is that stutter peaks are affected by pull-up. One option to consider
188 is whether the STRmix analysis can be run just removing the affected peak and not the entire
189 locus. Whether this is applicable will need to be determined on a profile-by-profile basis
190 using the STRmix deconvolution results as a guide. STRmix analysis can proceed when any
191 number of loci are removed, however the more loci that are removed the less information
192 there is available for STRmix to base its deconvolution on and the results will be less
193 reflective of the profile as a whole. The bigger issue is that if there are multiple loci that need
194 to be removed from analysis then it indicates the profile, as a whole, is likely not of a
195 standard to analyse in STRmix. There is no hard requirement for a maximum number of loci
196 that can be ignored, however (as is common in many laboratories) a maximum can be set in a
197 conservative way to ensure interpretations always remain at the highest level of rigour.

198 There is little to no guidance on this topic in the SOPs I reviewed, but I note that Emma
199 Caunt did try to rectify this with the development of a workflow diagram (an example of this
200 diagram in an email chain is seen in WIT.0004.1228.0001.pdf). This is specific to the issue of
201 dropping loci that are locus pull-up affected (which is one reason that loci might be dropped)
202 and seems to be a reasonable workflow. Some type of guidance is recommended as it will
203 minimise drift in interpretation practice over time.

204 I did not see any issues of inappropriate locus dropping in the casefiles I reviewed.

205

206 2.4 - Investigation of whether QHFSS' use of the STRmix diagnostic data is fit for purpose

207 The description of the diagnostics produced by STRmix within the QH SOPs is accurate and
208 falls within the range of current best practice. I have been told that the information about
209 when and how a review occurs is in Page 15 of *QIS 17117v21 – Procedure for case*

210 *management*. The 'Use of STRmix Software' would benefit from mentioning reviews and
211 pointing to the appropriate section of *QIS 17117v21*.

212

213 2.5 - Investigation of whether the assignment of the number of contributors is fit for purpose,
214 both for STRmix and the implications for the wider case.

215 There are passages within the 'Basics of DNA Profile Interpretation' SOP
216 (FSS.0001.0012.0147) that if applied as written would lead to a bias towards overestimating
217 the number of contributors to a DNA profile and should be reviewed. In most instances, the
218 difference these decisions will make on a prominent component of a DNA profile is
219 negligible. The main effect is to the minor component of a DNA profile. The negative
220 consequence to underestimation is to incorrectly exclude the known donors of DNA. The
221 negative consequence to overestimation is to incorrectly fail to exclude (and sometime
222 include with low levels of support) non donors of DNA. There is nothing wrong with the use
223 of sub-threshold peaks and peak balance (as outlined in the QH 'Basics of DNA Profile
224 Interpretation' SOP) to determine the number of contributors. Care should be taken though
225 that over time the application of these measures doesn't drift e.g., peak balances that used to
226 be accepted without increasing NoC now regularly lead to an increase in NoC. While it may
227 be reasonable to increase the NoC in some situations of ambiguity, a systematic bias for
228 increasing NoC should not evolve. I did see some indication of an overestimation of NoC
229 (which I detail below). I didn't see definitive evidence of a drift in interpretation practices in
230 my review, however this type of occurrence would only be noticeable in a larger review that
231 spanned a timeframe.

232 I found that very little indication of an additional contributor is needed for that additional
233 contributor to be invoked in the QH casefiles. These indications may be an imbalance (e.g., a
234 high stutter peak or imbalanced alleles), sub-threshold indications, or inconsistently amplified
235 peaks between replicates. Each of these factors can be legitimate reasons to increase the NoC,
236 the difficulty lies in determining when to accept minor occurrences of these factors without
237 increasing the NoC. In my experience the interpretations in QH increase the NoC more often
238 than I have seen in other laboratories. I found a number of instances in which I personally
239 would have assigned a lower NoC than was chosen by the scientist in the case. I couch this
240 comment by reiterating that mine is just one opinion and that I am not as familiar with the
241 performance of PowerPlex21 profiles produced at QH as those working at QH should be.
242 With this in mind, there is a risk that overestimations of NoC are occurring systemically in
243 casework at QH, which would be below recommended best practice. In order to investigate
244 whether this risk is occurring, a larger number of profiles would need to be examined, and to
245 address this I recommend a casefile review, which I expand on below.

246 There has been mention of the impact that a NoC stated in a report can have on the way the
247 results are interpreted by stakeholders (irrespective of the LRs). An example is an intimate
248 swab from a rape victim being interpreted. I found such instances in some cases I reviewed,
249 and it has possibly occurred in other prior cases I have not reviewed. A practice of tending to

250 lean towards overestimation of NoC may not be considered conservative in the sense that
251 there can be detrimental, wider-case implications if the DNA from this type of sample is
252 incorrectly interpreted as containing DNA from more than two people. As I do not have
253 information about the cases I reviewed outside the information in the casefiles, I cannot
254 identify whether this has occurred. I also cannot say whether this has occurred in other cases
255 that I have not reviewed. Much of the overestimation of NoC comes from applying stutter
256 thresholds. I suggest that a reassessment of the stutter thresholds, and the strictness by which
257 they are applied, is warranted.

258 I also recommend an external review of swabs from SAIKs in previous sexual assault cases
259 to determine which have been reported as coming from three or more people (I refer to these
260 as the 'applicable cases'). For those instances where the NoC has been assigned as three or
261 more, a review of the reasoning behind the choice to interpret the profile as coming from the
262 higher number of people should be undertaken, and how often that evidence for the extra
263 contributor is slight (i.e., one or two high stutter peaks, or an imbalance, or simply a low
264 number of minor peaks). For those profiles where, upon review, it is determined that the
265 profile should have been reported as originating from a lower number of contributors the
266 profiles should be reanalysed and reported in addendum DNA reports. Ideally this review
267 would be conducted by more than one person (to guard against individual interpretation
268 preferences) and be external to QH (such as a forensic provider in other state(s)). The review
269 should span applicable cases for the previous one-year period as this will provide a random
270 sample of cases in sufficient number to identify whether there exists a bias towards
271 overestimating the NoC to these DNA profiles.

272 In addition, a compilation of applicable cases that are as yet unresolved through the legal
273 system should be made and for those that do not fall into the previous year they should be
274 included in the review.

275 After the review, it may be found that there is no systemic over assignment of NoC. In this
276 case the action then turns to the best way to report the DNA results for these SAIK swabs so
277 that the NoC is given within some context that explains the significance of the finding. In the
278 instances of a very minor contributor to a DNA profile being present on an intimate swab,
279 there have been some suggestions by Mr Parry (WIT.0043.0001.0001 at paragraphs 40 or 41)
280 on ways that this type of scenario could be better communicated. I am not commenting on
281 suitability of the specific wording used by Mr Parry, however I believe that this type of
282 clearer communication of the significance of results may be the best option.

283 Alternatively, after the review it may be found that there is a systemic over assignment of
284 NoC. In this situation any profile in any case could be affected. It is not reasonable to review
285 all previous work and so I recommend that in this eventuality the findings of the profile
286 review be communicated to stakeholders with the offer that if they feel their case may have
287 been affected that they can bring it to the attention of QH for reassessment.

In order to monitor the performance of NoC assignment at QH it may be useful to periodically (for example every few years) generate a set of low-level mixtures and carry out a blind assignment of NoC on these profiles to monitor laboratory trends (but not as an indication of individual scientist performance).

There are several software solutions that can assist in assigning NoC that QH could investigate, which I detail later.

I also note the work carried out in document "*Forensic DNA Analysis. Single Source High Stutter Guidelines Assessment, by Angela Adamson, Cassandra James, Emma Caunt. July 2021*". In this work the authors carry out a clever and impressive investigation into the level of statistical support that one or two high stutter peaks would give to the presence of an additional contributor. They find that in all instances the presence of one or two high stutters did not support an increased NoC. Their recommendations are that single source profiles that have one, or multiple, instances of high stutter can still be interpreted as single source (and do not an increase to two contributors for analysis). The general effect they are finding is that if there is little evidence of an additional contributor then there will be little support for that additional contributor. Their recommendations also state that their findings only apply to single source profiles with high stutters (when considering if they should be interpreted as coming from two people), however this same thinking applies to any complexity of mixture, and I would recommend they apply it in that way.

<u>2.6 - Investigation of the appropriate "stratification" of populations in STRMix to determine likelihood ratios.</u>

The method of reporting a population stratified likelihood ratio (LR) carried out by QH is appropriate and in accordance with best practice (or emerging best practice). The SOPs I reviewed correctly explain the use of population stratification and the casefiles I reviewed all applied population stratification as described in the SOP.

<u>2.7 - Whether the QHFSS laboratory's use of STRMix is consistent with best practice, any deficiency in the current use of STRMix in the laboratory that could affect reliability or accuracy of reported results, and the steps required to rectify that</u>

The QH use of STRmix is within the range of current best practice and the current use of STRmix is expected to lead to reliable and accurate results.

There are a number of features of software that could be employed, or some process changes that could be made that would alleviate some of the interpretation issues that seem to be being experienced in the laboratory. I am not recommending that any of these need to be implemented or enacted for QH in order to produce reliable and accurate results. From the review I have carried out I believe that the results being reported are reliable and accurate.

325 The suggestions below are made purely as options for the laboratory to consider, and which
326 would take them to the level of emerging best practice:

- 327–332 Combined LRs in STRmix – When assessing whether multiple contributors, who all individually are supported as DNA donors, are still supported as DNA donors together current practice at QH is to carry out a manual comparison. STRmix can be used for assessing joint contribution using the LR calculation feature. This would provide an objective and consistent approach to assessing joint contribution (and would be in line with emerging best practice as recommended by Buckleton et al [1])

- 333–341 Using mixture proportion priors for sub-threshold contributors – When using sub-threshold peaks for determining the number of contributors, there is a disconnect between the data that has been used in manual interpretation and the data being provided to STRmix. In some instances, STRmix may then assign more DNA to these minor DNA donors than is intuitively expected (based on the human knowledge of peaks below the limit of reporting). A feature in STRmix that can alleviate this issue is the informed mixture proportion priors [2]. This feature is not always needed but if validated and explained in SOPs it would provide an additional tool to scientists to aid them in difficult DNA profile interpretation.

- 342–354 Mix-to-mix analysis in STRmix – This is raised in response to points expressed in Mr Parry's statement (WIT.0043.0001.0001) paragraphs 31 to 33. The concerns raised were that the existence of common unknown donors in multiple profiles within a case are not reported in a statement of witness. There is a wide variety of ways in which unknown profiles are reported and so I believe the practices of QH would fall within the range of current best practice, but perhaps at the lower end of that range. Better practice would be to identify these common unknowns. Sometimes the interpreted profiles that appear to come from a common unknown may be partial and therefore not able to be confidently identified as being in common. A tool that can assist with this is the mixture-to-mixture feature in STRmix that profiles a level of support for their being common DNA donors [3]. Using his tool then provides an objective way to determine whether there is support for interpreted unknown profiles being in common.

- 355–360 Using auto-removal of peaks below the detection threshold in STRmix – This practice would be to read profiles at the LOD in Genemapper so that all peaks above the LOD are labelled. Then in STRmix the peaks below the LOR can be automatically ignored for analysis. This way the information between LOD and LOR can be easily used to exclude or determine NoC (if that is how the QH wishes to continue) but ignored in the STRmix analysis.

- 361–363 Using the range of contributors feature in STRmix – This feature of STRmix allows an analysis to occur when a single number of contributors cannot be assigned. As such it has the ability to analyse profiles that are currently deemed uninterpretable,

364 provide a resolution pathway for disagreements over numbers of contributors, and
365 avoids the need to add a contributor in situations of uncertainty.

- 366 Use additional stutter modelling in STRmix – STRmix is able to be set up with any
367 number of stutter types. A current issue in QH seems to be the way in which double
368 stutter is interpreted, and so a practice that would align with emerging best practice
369 would be to model double back stutter within STRmix. If there are concerns over the
370 way in which this could increase the complexity of DNA profiles then there are split
371 models, whereby a standard STRmix setup (as is currently used in QH) is used for
372 most analyses and there is an 'all stutters' setup that can be used only when needed
373 i.e., when there is some dispute over whether a peak in an exotic stutter position
374 should be considered stutter or allelic. Again, this assists in resolving scientific
375 differences of opinion, and provides an objective and consistent manner of treating
376 the data.

- 377 Using FaSTR™ DNA for identifying combined stutter – FaSTR™ DNA is an
378 alternative to Genemapper and has the ability to set thresholds for combined stutters
379 and flag these as stutter peaks. Stutter peaks can be toggled on or off for profile
380 assessment within FaSTR™ DNA, and so would also assist with the assignment of
381 number of contributors.

- 382 Using FaSTR™ DNA to determine the number of contributors – FaSTR™ DNA has
383 an inbuilt tool that can be trained on a laboratory's own data to assign a number of
384 contributors to a DNA profile. This feature could be explored by QH as a helping tool
385 in their own manual assignments and may achieve a more consistent assignment.

- 386 Using FaSTR™ DNA for identifying artefacts – This is a very new feature in
387 FaSTR™ DNA. A feature exists that can assign a probability to peaks in the profile
388 for being artefactual [4]. Again, this could be used as a helper tool to assist analysts in
389 reading DNA profiles and interpreting peaks.

390 **3.0 - Introduction:**

391 3.1 - What is STRmix?

392 A common task in forensic biology laboratories is generating DNA profiles from evidence
393 items. These evidence profiles can be compared to reference DNA profiles, generated from
394 persons of interest, in order to provide opinions that assist stakeholders (such as police or
395 courts) in drawing conclusions about who may have contributed DNA to an evidence item.

396 In some circumstances the evidence DNA profiles are of good quality (i.e., containing a
397 complete complement of information at all DNA regions examined) and simple (originating
398 from a single person). In these cases, the process of interpreting the evidence DNA profile is
399 straightforward and opinions on potential DNA donors can be carried out by a direct and
400 manual comparison to reference profiles. If the reference and evidence profiles contain the
401 same information (in the form of peaks, that represent different underlying sequences of
402 DNA referred to as 'alleles') at each region (referred to as a 'locus', or in plural 'loci') then
403 they are said to 'match'. In the case of matching reference and evidence profiles, there is
404 generally extremely strong support for the reference donor also being the donor of DNA in
405 the evidence sample, as opposed to the evidence sample coming from someone else. If the
406 reference and evidence profiles don't match, then the reference donor is excluded as the
407 source of DNA on the evidence item.

408 Often evidence DNA profiles are not of good quality and are not simple. Low quality DNA
409 profiles may be generated due to there being low levels of DNA on an item under
410 examination, or due to the DNA being degraded. These factors are not issues with the
411 performance of the laboratory generating the profile, they are due to the properties of the
412 exhibit being examined and the circumstances surrounding that exhibit and are a natural and
413 expected outcome in many instances. Complex profiles arise when the exhibit being
414 examined has had DNA from multiple individuals donated to its surface.

415 In the case of complex, and/or low-quality profiles a simple manual interpretation can no
416 longer reasonably be carried out. These profiles are then analysed in software programs that
417 employ statistical and biological models referred to as 'probabilistic genotyping' (PG)
418 systems. STRmix is one such PG system. The general workflow for analysing profiles in
419 STRmix and comparing reference profiles is:

420 A) Assess the profile for suitability to carry out a STRmix analysis

421 B) Assign a number of contributors to the evidence DNA profile

422 C) Assess the case circumstances to determine whether any assumptions can, or should
423 be made about DNA contribution

424 D) Analyse the evidence DNA profile using STRmix in a process called 'deconvolution'

425 E) Assess the deconvolution results to ensure that analysis has completed successfully

426 F) Using the case circumstances, set up an appropriate analysis that compares the
427 reference DNA profile(s) to the deconvoluted evidence sample to produce a strength
428 of evidence value called a likelihood ratio (LR)

429 G) Assess the LR to ensure that the analysis has completed successfully

430 The sub-sections below highlight some of the important points when stepping through A to G
431 in the list above as they are relevant to the points in the request by the Commission.

432

433 3.2 - The use of STRmix and the importance of diagnostics

434 In general terms, when STRmix is provided a complex evidence DNA profile, it considers all
435 possible combinations of reference DNA profiles that could explain the evidence profile.
436 Each combination of reference profiles is weighed according to how well it explains the
437 evidence profile. STRmix is able to provide these 'weights' as it has biological models that
438 describe different aspects of DNA profile behaviour and statistical models that apply the
439 biological models to the data. The biological models are calibrated to the performance of the
440 laboratory during the validation of STRmix for use within a laboratory. In order to generate
441 the weights STRmix considers (along with the combination of references) different
442 combinations of DNA amounts for each contributor, level of degradation of each contributor,
443 how proficiently the different loci have amplified during PCR, and a range of other factors. In
444 statistical terms these factors are called parameters.

445 The first step of profile interpretation is for the scientists to determine whether the profile is
446 suitable for interpretation and analysis. This point is covered in more depth in section 3.4. For
447 the remainder of section 3.2 assume the determination has been made that the profile is
448 suitable for interpretation and analysis.

449 At the conclusion of deconvolution, the output of STRmix is:

450 • A list of reference combinations and their weights,

451 • An indication of the values of parameters for the biological models that best explains
452 the DNA profile, and

453 • A summary of diagnostics describing the performance of the statistical models

454 A scientist who is trained in the interpretation of DNA profiles should be able to use their
455 knowledge of DNA profile behaviour to examine a DNA profile and form an opinion on
456 which combinations of references may have given rise to it, and (in for some profiles) the
457 specific reference that is a major or minor DNA donor. While such a manual interpretation

458 would not be able to assign a numerical weight, there will be an intuitive ranking of the
459 reference combinations. These intuitive rankings should align with the weights produced by
460 STRmix, and a large part of the review of a deconvolution is checking this fact.

461 In the same vein, a scientist trained in the interpretation of DNA profiles can form an
462 intuition about the relative abundance of each DNA contributor in the DNA profile, and this
463 should align with the mixture proportions proposed by STRmix. The weights and biological
464 description of the DNA profile are the biological (or primary) diagnostics produced by the
465 software.

466 If there is a disconnect between what the analyst intuitively expects from a DNA profile, and
467 the results of a STRmix deconvolution, then a deeper investigation into the profile and the
468 STRmix analysis is required. This deeper investigation may reveal an issue with the
469 deconvolution, or it may reveal an error in the way the profile was originally set up for
470 deconvolution (such as an incorrect choice in the number of contributors), or it may give rise
471 to a new understanding of the DNA profile. The disconnect may be resolved by carrying out
472 an additional (or changed) deconvolution, carrying out additional laboratory work, or
473 updating one's opinion on the DNA profile. If the disconnect cannot be resolved, then the
474 analysis may not be reliable and should not be relied on.

475 In additional to applying their biological knowledge and training in DNA profile
476 interpretation, the scientist reviewing a STRmix deconvolution also needs to consider the
477 performance of the statistical models. STRmix provides summary statistics with each
478 deconvolution that gives information specifically on the statistical models. In order to review
479 these statistical diagnostics, the scientist will need to have undergone STRmix training. As
480 with the biological diagnostics, if the statistical diagnostics do not reflect the scientist's
481 expectations about the DNA profile analysis, then further investigation, and/or further work,
482 and/or a decision not to report the result may be appropriate.

483

484 3.3 - Assigning a number of contributors to a DNA profile

485 The interpretation of a DNA profile using STRmix™ starts with the assignment of the
486 number of contributors, NoC, to the profile. To avoid bias this is done in the absence of
487 profiling information from any persons of interest (POI) in a case. However, in circumstances
488 where an individual's DNA is expected to be present (e.g., when considering DNA results
489 produced from an intimate swab in a sexual assault case), knowledge of their DNA profile
490 could help to better inform the NoC. Further, case and sample circumstances may mean it is
491 appropriate that a person should be assumed to be a contributor of DNA to a sample during
492 deconvolution. This is done as the provision of the additional information assists the analysis
493 of the remaining contributors.

494 When considering crime scene samples, the "true" number of contributors is always unknown
495 and unknowable. It therefore falls to the analyst to utilise their knowledge, experience, and

496 expertise to provide their best estimate of the NoC. In my experience, analysts most
497 commonly use the Maximum Allele Count method in conjunction with peak height
498 information. The maximum allele count method considers that we inherit one half of our
499 DNA from each parent. The consequence of this Mendelian inheritance model is that at any
500 one region under examination in a DNA profile we may see two alleles (if the allele we have
501 inherited from our mother is different to the allele inherited from our father), or one allele (if
502 we have inherited the same allele from both parents). Using this theory means that if there are
503 three or four alleles at one region the DNA profile must come from at least two contributors,
504 if there are five or six alleles the DNA profile must originate from at least three contributors
505 and so on. Therefore, by finding the locus with the maximum allele count the minimum
506 number of contributors to the DNA profile can be determined. This minimum number may be
507 increased based on the relative abundance of the alleles at that locus. There are two
508 complicating factors to the above system of assigning a NoC.

509 The first occurs when alleles in a DNA profile are imbalanced, which can complicate the
510 assignment of a NoC. In an ideal world, when an individual donated DNA to an exhibit, that
511 DNA would be sampled and generate a profile where the alleles from that individual were all
512 in perfectly equal abundance (represented on the profile by the height of peaks, measure in
513 relative fluorescent units, or rfu). However, there are many stages along the DNA profiling
514 process where micro-variations in sampling, DNA extraction, PCR, and electrophoresis as
515 well as the effects of sampling variation will mean that alleles in a DNA profile from a single
516 individual are not balanced. This phenomenon is referred to as peak height variability (or
517 sometimes 'heterozygote imbalance') and is an example of a stochastic effect acting on a
518 DNA profile. Stochastic effects occur in all DNA profile of all intensity (and therefore of any
519 starting DNA amount). The greater the starting amount of DNA, the less that stochastic
520 effects will affect the relative balance of alleles from an individual, and conversely as the
521 starting DNA amount is reduced the greater the stochastic effects will be on the relative allele
522 balance. When DNA amounts become very low the stochastic effects can lead to the
523 complete absence of some peaks in a DNA profile. The increase in the relative effects of
524 stochastic variation as starting DNA amount decreases is a smooth, and continuous incline.
525 There is no threshold at which stochastic effects will dramatically increase or decrease in
526 aberration to the expected smooth, continuous trend. Prior to the use of probabilistic
527 genotyping systems such as STRmix it was common to set a conservative, arbitrary allele
528 intensity threshold below which interpretations were not conducted. The use of STRmix
529 negates the need for such a threshold as the model for peak height variability takes into
530 account its relationship with starting DNA amount for any profile. It follows from the above
531 that there is also no hard threshold at which two alleles of different intensity cannot be from
532 the DNA donation of a single individual. Instead, as the allele intensities diverge to greater
533 degrees the lower the probability that they will have originated from a single individual.
534 Again, probabilistic genotyping systems such as STRmix consider the DNA profile behaviour
535 in this probabilistic manner, rather than in a threshold (or rule-based) manner. From this, the
536 complication when assigning a number of contributors arises when the balance of peaks is
537 such that it is either from a single individual with an unusually high level of peak imbalance,

538 or the alleles originate two individuals donating DNA at different levels. In such a borderline
539 case is where differences in scientist DNA profile interpretations commonly occur.

540 The second common complicating factor arises as a result of a by-product of DNA profiling
541 know as stutter. Stutter is an inevitable DNA replication error that occurs during PCR. During
542 the copying of DNA fragments the DNA strands can 'slip' and the resulting copied fragment
543 can be shorter, or longer than its originally intended template. These 'stutters' occur at known
544 positions and known expected heights and so can (and are) modelled in most PG systems
545 such as STRmix. The complicating factor when assigning a NoC to a DNA profile occurs
546 when there are potentially multiple contributors to a DNA profile who have donated DNA in
547 unequal amounts. In this situation it may not be clear whether some peaks in a DNA profile
548 have originated due to stutter, or whether they are small peaks originating from a minor
549 contributor of DNA. It may also be the case that not all stutter types are included in the
550 STRmix modelling for that laboratory. Typically, when this is the case those stutter types are
551 automatically screened for during the DNA profile reading stage. However, if a peak exceeds
552 the automatic stutter screening threshold and is then present on the DNA profile it can cause
553 analysts to assign an additional contributor to explain.

554 When assigning a number of contributors, it is important to consider the consequences of an
555 incorrect assignment. Studies have shown that the statistical weighting for a known major
556 donor of DNA to a sample will not be significantly (or at all) affected by an overestimation of
557 the NoC. When there are no major DNA donors, or when considering a known minor DNA
558 donor, the effect of overestimating the NoC is that the strength of evidence supporting their
559 DNA donation will mildly decrease. However, an effect of overestimating the NoC to a DNA
560 profile is that there will be an increase in the number of false inclusions of non-DNA-donors
561 i.e., by comparison to the reference DNA profiles of individuals known not to donate DNA to
562 a sample, an overestimation in the NoC will more often incorrectly support a DNA donation
563 by those individuals. These inclusions will tend to provide mild levels of support for the non-
564 donor's inclusions. It is for this reason that usual practise when interpreting a DNA profile is
565 to assign the minimum NoC that can reasonably explain the evidence DNA profile.

566 If the NoC is underestimated, then the main effect is that individuals who are known to
567 donate DNA will be incorrectly excluded from the DNA profile i.e., the analysis will provide
568 support for no DNA donation. It is generally thought that there is no side to which
569 systematically biasing the assigned NoC is favourable.

570 Studies have shown that the ability to currently assign a NoC to a DNA profile decreases as
571 the complexity of the DNA profile increases (specifically the known number of contributors)
572 [5]. However, the misassignments tend to err on the side of underestimation. Additionally,
573 the effect of underestimating NoC tends to decrease as the complexity of the profile
574 increases. There are various actions that can be taken is assist in assigning a NoC to a profile:

575 - Additional laboratory work. It is common that to assist the scientist in assigning NoC
576 in complex profiles that an additional PCR amplification of the DNA sample is

577 carried out. Then, when the additional DNA profile is obtained an assignment of the
578 NoC occurs using the information from both profiles together. In some forensic
579 service providers this phenomenon leads to a workflow where certain DNA sample
580 types (those most likely to lead to complex mixtures) are automatically amplified
581 twice. In some circumstances the additional amplification can be carried out with
582 additional template DNA that further assists interpretation as minor DNA donors are
583 more prominently amplified. Note that additional amplification does use additional
584 DNA extract and so may not always be the favoured method for assisting in the
585 assignment of NoC.

586 • Using case context. In some circumstances the context of the case can suggest that
587 certain individuals are expected to have donated DNA. These may be individuals
588 from who the sample was taken (i.e., an intimate swab from a sexual assault victim),
589 or from an item that someone is known to have worn or touched and therefore whose
590 presence of DNA is not being contested. Use these case circumstances allows the
591 reference of those individuals to be used to assist with assigning a NoC. Note that this
592 use of reference DNA profiles does not extend to POIs who the presence of DNA is in
593 question or being contested (the scientist should be blinded to these references until
594 after the STRmix deconvolution of the evidence profile).

595 • Using sub-threshold information. When DNA profiles are read (using software such
596 as Genemapper or FaSTR DNA) there is usually a level at which peaks are not
597 labelled (assigned a peak designation by the software). This level of called the
598 analytical threshold, AT (or sometimes the 'baseline', or 'detection threshold', or
599 'limit of reporting'). Often this analytical threshold is assigned at a conservatively
600 high level, so that some peaks are still distinguishable from instrument noise below
601 this level. Whilst not labelling peaks below this analytical threshold the DNA profile
602 will still show the fluorescent signal below the threshold. In some circumstances it
603 may be appropriate to use this sub-threshold information when assigning the NoC.
604 There needs to be caution when using this information however, because while it has
605 been used by the scientist to assign a NoC, the information is not provided to STRmix
606 (as it is unlabelled) and so the NoC assignment and the STRmix analysis are based on
607 different subsets of information. In these cases, the use of a feature in STRmix called
608 'informed Mx priors' can be used to alert STRmix to the fact that a very low-level
609 contributor is present [2].

610 • STRmix (since V2.5) has the ability to model any number of stutter types as required
611 by the user. If the laboratory is finding that a stutter type is commonly occurring in
612 their profiles, or exceeding the pre-screening thresholds using during profile reading,
613 then one option is to no longer pre-screen it, and include this stutter type within
614 STRmix modelling. Doing this alleviates the issue of a peak appearing in an un-
615 modelled stutter type position and having to be accounted for by the scientist (and
616 STRmix) using an additional contributor. The disadvantage of including additional
617 stutter types in STRmix modelling is that they can further complicate the

618 interpretation of DNA profiles by the scientist (as there are additional peaks to take
619 into account when assigning a NoC). However, there are workflows that can
620 accommodate this issue. One is to use a profile reading tool that can toggle stutter
621 peaks on or off of the observed DNA profile. Another option is to have a dual
622 STRmix use system whereby a standard method accounts for the most common
623 stutter, and then a second workflow is invoked only as required which includes
624 additional stutters being retained during reading and modelled in STRmix. The second
625 workflow is then only used when ambiguity about an 'exotic' stutter type is causing
626 issues when assigning the NoC.

627 • Using a range of contributors. Sometimes, despite utilising the tools already
628 mentioned, a scientist may still not be confident in assigning a NoC to a DNA profile.
629 From STRmix V2.6 a feature is available that allows the user to specify a range of
630 contributors i.e., if the scientist was unable to choose between assigning two or three
631 contributors (for example) then the 'variable number of contributors' (VarNoC)
632 feature can be used to specify 2 to 3 contributors [6, 7]. Using the VarNoC feature,
633 STRmix will analyse the profile as each NoC within the range and then weight those
634 analyses relative to each other. When a reference profile is compared to such an
635 analysis, the final result is a single strength of evidence for that individual being a
636 DNA donor being provided that takes into account all NoCs in the range. This
637 VarNoC feature is in use within Australian forensic laboratories in casework.

638 The format of reporting results where a range of contributors is considered varies
639 between laboratories. It is common in forensic laboratories in Australia that results are
640 reported in a minimal, tabulated format and so will not be directly comparable to the
641 manner in which QH reports their results. For example, in Forensic Science SA if a
642 DNA profile is analysed using the VarNoC feature of STRmix where the range is
643 from two to three people then the profile would be described as "*Mixed DNA profile –*
644 *2 to 3 contributors*".

645

646 The final point when assigning a NoC goes beyond the effects of the statistical analysis and
647 considers the case significance. There are some situations where the assignment of a NoC can
648 have implications for how the findings are viewed that go beyond the comparison to
649 reference DNA profiles in a case. For example, an intimate swab from a sexual assault victim
650 may be compared to the reference DNA profile of the defendant and result in a likelihood
651 ratio that supports their DNA donation to the sample. However, even without changing the
652 value of this LR, the significance of the evidence may be viewed differently within the
653 greater case context if the profile was reported as originating from two people, than if it were
654 reported as originating from four people. These types of issues go beyond the use of STRmix
655 and are instead more about the communication of results. The communication may be at the
656 level of better describing the DNA profiling result (i.e., explaining if there is ambiguity in the
657 NoC) or it may be at the level of explaining the significance of DNA findings in a greater

658 case context (i.e., in what level of similar samples do we expect to see background levels of
659 DNA). This latter point reaches into the domain of activity level evaluations, which typically
660 required additional training of scientists in order to apply.

661

662 3.4 - The effect of dropping loci from the calculation

663 The first step in DNA profile interpretation (prior to analysis in STRmix, and prior even to
664 assigning a NoC) is to determine whether the profile is suitable for interpretation and
665 analysis. STRmix uses a number of biological models that describe various DNA profile
666 behaviours. If a DNA profile possess a behaviour (or feature) that is not modelling within
667 STRmix then it follows that it may not be appropriate to use STRmix to analyse that profile.
668 Under certain circumstances a solution to this issue it to ignore (or drop) loci from the
669 information provided to STRmix. Common DNA profile behaviours (or features) that are not
670 modelled in STRmix, and lead to loci being ignored are:

671 • Unresolved peaks – These are when two peaks in an electropherogram are so close in
672 size that the capillary electrophoresis instrument is unable to resolve them. Typically,
673 when this occurs, one of the peaks will be labelled and the other will not be labelled
674 and will appear as a shoulder on the first. There is no model within STRmix that can
675 account for unresolved peaks and so alleles that are missing due to being unresolved
676 have to be accounted for within STRmix using other models (in this case the dropout
677 model). In extreme cases this can deform the analysis and lead to incorrect results.
678 The solution is to ignore the locus with the unresolved peak prior to STRmix analysis.
679 When a locus has been ignored, and a reference sample is compared, then a manual
680 comparison to reference profile must be carried out to ensure that the ignored locus is
681 not exclusionary.

682 • Trisomy – This occurs when a genetic mutation has occurred in an individual leading
683 to chromosome duplication. Unlike a standard DNA profile where it is expected that a
684 single person will lead to either one or two peaks at a locus, a trisomic individual will
685 lead to three peaks at a locus. Again, there is no model in STRmix for trisomy and the
686 locus will need to be ignored in order for profile analysis to proceed.

687 These two instances of when loci can be ignored are locus specific effects i.e., the underlying
688 reason for the locus being ignored only affects the one locus. In this situation there is nothing
689 wrong with dropping more than one locus per se but doing so will lead to less information
690 being provided to STRmix to carry out deconvolution. As less information is provided to a
691 deconvolution the lower the discrimination power that the deconvolution will provide. Also,
692 care needs to be taken if multiple loci are being dropped to ensure it does not have other
693 adverse effects on the deconvolutions. For example, if the loci that are dropped are also the
694 only loci that contain a particular type of information (such as being the only loci where a
695 minor DNA donor peak is identified) then this can affect the ability of STRmix to model the
696 DNA profile.

697 It is also possible to drop loci from an analysis for issues such as pull-up affected peaks. Pull-
698 up occurs when one peak is so intense that during capillary electrophoresis the detection of
699 that peak's dye 'bleeds' into the detection of other dyes. The result is peaks occurring in the
700 profile that do not represent DNA in the DNA extract. Unlike the previous situations of locus
701 dropping, the pull-up example has an underlying reason for dropping the locus is a profile-
702 wide effect (i.e., too much DNA being amplified). Again, while there is nothing wrong with
703 dropping loci in and of itself, if multiple loci need to be dropped for a profile-wide issue such
704 as too much DNA (manifesting in the profile as pull-ups) then it may indicate there has been
705 an issue with the generation of the profile, and that it may not be suitable for analysis.

706 STRmix has the ability to model data from mildly saturated profiles, but the greater the level
707 of saturation the more the models in STRmix will be pushed to, and eventually exceed, the
708 limits of reliable use. Therefore, while there is no rule to say that multiple loci cannot be
709 dropped for analysis (even for pull-up), the better solution is to carry out laboratory work to
710 fix the biological issue first rather than trying to deal with it using statistics. For saturated
711 profiles exhibiting pull-up a common solution is to carry out a new PCR with less input
712 DNA. This solution does use additional DNA extract, and that may not be available (or it
713 may be desired that the remaining DNA extract is retained). Another solution, which does not
714 use additional DNA extract, is to carry out the capillary electrophoresis again using a diluted
715 PCR product. As this is a change in process however, a validation of the new process would
716 be required before it could be implemented.

717

718 <u>3.5 - likelihood ratios and population stratification in STRmix</u>

719 Once an evidence profile has been deconvoluted then STRmix can be used to compare
720 reference DNA profiles to it. This can either be a comparison to a single reference sample
721 from a POI, or it can be to a large list of reference samples, held within a database. I will
722 focus on the comparison to a single reference DNA profile as this is the most relevant to the
723 review. When comparing a reference profile to an evidence profile there are typically two
724 competing propositions (or hypotheses) considered. In general terms, one proposition
725 considers the POI as a donor of DNA to the sample, and the other proposition considers that
726 the POI is not a contributor of DNA to the sample. The ratio of the probability of obtaining
727 the DNA profiles given each of these two propositions is the likelihood ratio (LR). If the LR
728 is greater than one, then the evidence supports the first proposition compared to the second
729 (i.e., an inclusion of that person's DNA in the sample). If the LR is less than one, then the
730 evidence supports the second proposition compared to the first (i.e., an exclusion of that
731 person's DNA from the sample). If the LR is exactly one, then the evidence is neutral with
732 respect to a potential donation of DNA by the person to the sample.

733 The exact formulation of proposition (more specifically than the general form described
734 above) relies on the framework of circumstances of the case, and the details of the sample
735 that were taken. There are numerous published guidance notes on proposition formulation [1,
736 8-11].

737 The size of the LR will depend on how well the evidence profile is explained by the
738 contribution of an individual (i.e., whether their reference aligns with the references in the
739 deconvolution that possess a high weight) and also the rarity of the alleles present in the DNA
740 profiles within the population. When considering the probability of the evidence given the
741 exclusionary proposition an important point to consider is, '*if not the POI, who is the*
742 *alternative DNA donor?*'. Typically, the pool of alternative donors will include someone else
743 within the local geographical population, although there are circumstances where other
744 information in the case will suggest a specific subset (such as a particular ethnic group).
745 Additionally, the alternation DNA donor from the population may be assumed to be unrelated
746 to the POI, although STRmix is also able to consider specific relatives as alternate DNA
747 donors [12], or simply the presence of relatives of the POI in the population [13]. If the
748 alternate donor is someone in the local geographical population, then it is likely that more
749 than one ethnic group will need to be considered (i.e., someone else at random from the
750 population could be someone in any of these ethnic groups). This is important as each ethnic
751 group can have different levels of allele rarity (or frequency) and so will affect the size of the
752 LR.

753 In this situation there are several practices carried out by forensic service providers:

754 1) Using the ethnic group of the POI – this is carried out using the expectation that the
755 reference profile of the POI is going to be most commonly seen again in their own
756 ethnic group. Therefore, while not reflective of the belief that the alternate DNA is
757 someone at random in the local geographical population, it is expected to be
758 conservative (i.e., minimises the size of the LR).

759 2) Choosing the smallest LR out of several ethnic groups – this workflow calculates the
760 LR considering that the alternate DNA donor has originated from one of several
761 different ethnic groups (typically the most abundant three or four groups that make up
762 the local region). From these multiple LR calculations the smallest is chosen to report.
763 Again, the aim of this method is to be conservative with the reported LR, rather than
764 to reflect a belief that the alternative donor is from any specific ethnic group. This
765 method can be carried out in conjunction with the first i.e., calculate in several,
766 standard ethnic groups and also the ethnic group of the POI (if that is not within the
767 standard set) and report the smallest LR.

768 3) Stratify across ethnic groups – as above this method calculates the LR considering
769 that the alternate DNA donor has originated from one of several different ethnic
770 groups (typically the most abundant three or four groups that make up the local
771 region). The relative abundance of each ethnic group within the population is taken
772 into account and the final reported LR is a stratification of all individual-group LRs.

773 The first method listed above is typically no longer used as the other methods are considered
774 more robust ways to approach the problem. Therefore option 1 would be considered below
775 recommended best practice. Option 2 is in common use by forensic service providers around
776 the world (particularly in the US) and considered within the range of best practice. Both
777 option 1 and option 2 carry out approximations that are designed to be conservative in the
778 value being reported, whereas option 3 does not make those approximations and seeks to

779 provide the LR that best represents the strength of the evidence. However, there are
780 complexities with using the stratification technique as the ethnic composition of the local
781 geographical population must be known and so not all forensic service providers use this
782 method. Option 3 would be considered being the emergent best practice.

783

784 3.6 - guidance for STRmix use

785 There are numerous sources of published material that can assist in the current and emerging
786 best practice use of STRmix. These include paper that explain the underlying theory, provide
787 examples of limit testing, examples of validations, advice on implementation or use of
788 STRmix, or reports on performance to assist laboratories in their own implementation
789 strategies. In Tables 1 and 2 I update two tables from Gill et al [14] that provide references to
790 these materials.

791

Algorithms, scientific principles and methods	Version introduced	Reference
Allele and stutter peak height variability as separate constants within the MCMC	V2.0	[15]
Peak height variability as random variables within the MCMC	V2.3	[16]
Model for calibrating laboratory peak height variability	V2.0	[16]
Application of a Gaussian random walk to the MCMC process	V2.3	[17]
Modelling of back stutter by regressing stutter ratio against allelic designation	V2.0	[18-21]
Modelling of back stutter by regressing stutter ratio against LUS	V2.3	[18, 19, 21, 22]
Modelling of forward stutter	V2.4	[23]
Modelling of allelic drop-in using a simple exponential or uniform distribution	V2.0	[15]
Modelling of allelic drop-in using a Gamma distribution	V2.3	[24]
Modelling of degradation and dropout	V2.0	[25]
Modelling of the uncertainties in the allele frequencies using the HPD	V2.0	[26]
Modelling of the uncertainties in the MCMC	V2.3	[13, 26, 27]

Database searching of mixed DNA profiles	V2.0	[28]
Familial searching of mixed DNA profiles	V2.3	[12]
Relatives as alternate contributors under the defence proposition	V2.3	[12]
Modelling expected stutter peak heights in saturated data	V2.3	[23]
Taking into account the 'factor of two' in *LR* calculations	V2.3	[29]
Model for incorporating prior beliefs in mixture proportions	V2.3	[2]
Combining DNA profiles produced under different conditions into a single analysis	V2.5	[30]
Assigning a range for the number of contributors to a DNA profile	V2.6	[6]
Mixture-to-mixture comparison to identify common DNA donors	V2.7	[31]
A top-down DNA search approach	V2.8	[32]
The diagnostic outputs of *STRmix™*	V2.3	[33]
The use of artefact probabilities within STRmix deconvolution	V2.10	[34]

792 **Table 1.** publications of conceptual components of STRmix™ modelling.

793

Focus of validation	Reference
Ability of *STRmix™* to deconvolute profiles and assign *LR*s that comport to manual interpretation and human expectation	[15]
Ability of *STRmix™* to discriminate between donors and non-donors in database searches	[28]
Behaviour of *STRmix™* to assign *LR*s when dealing with multiple replicates, different number of contributors, and assumed contributors	[35]
Sensitivity of *LR* produced by *STRmix™* to different factors of uncertainty such as theta, relatedness of alternate DNA source and length of MCMC analysis	[13]
Tests to be performed when validating probabilistic genotyping, using *STRmix™* as an example	[36]

Ability of individuals from different laboratories to standardise evaluations when using *STRmix*™	[37, 38]
Ability of *STRmix*™ to reliably use peak height information in very low intensity profiles	[2, 39, 40]
Ability of *STRmix*™ to discriminate between donors and non-donors in large-scale Hd true tests, or using importance sampling	[28, 41-45]
Sensitivity of *STRmix*™ model parameters to laboratory factors	[16, 46]
Ability of *STRmix*™ to utilise information from profiles produced under different laboratory conditions within a single analysis	[30]
Effect of mixture complexity, allele sharing and contributor proportions on the ability *STRmix*™ to distinguish contributors from non-contributors	[5]
The ability of *STRmix*™ to identify common DNA donors in mixed samples	[3, 47]
The sensitivity of *LR*s produced in *STRmix*™ to the choice of the number of contributors	[48-50]
Ability to use *STRmix*™ to resolve major components of mixtures	[50]
Testing the assumption of additivity of peak heights in *STRmix*™ models	[3, 51]
Performance of the degradation model within *STRmix*™	[52]
The effect of relatedness of contributors to the *STRmix*™ analysis	[53, 54]
Testing the calibration of *LR*s produced in *STRmix*™	[55]
Validation overviews of *STRmix*™	[17, 56]
Comparison of *STRmix*™ to other probabilistic genotyping software	[36, 57-59]
Validation of the use of artefact probabilities within STRmix deconvolution	[34]

794 **Table 2.** Publications of validation of STRmix™ models.

795

796 In addition to the published material there are STRmix user's and operation manual for each
797 version of the software released. Within these manuals are information about how to use the
798 features of STRmix, how they have been validated and extensive information on the
799 underlying theory. In combination the user's manual and operation manual for STRmix V2.9
800 provide 269 pages of information (with numerous references to other additional sources of
801 information).

802 Regular training courses are provided either as short courses associated with scientific
803 conferences, or as specific STRmix courses held by the STRmix group. Information on
804 upcoming courses is presented on the STRmix website, or a site visit with training can be
805 organised.

806 Within the STRmix community there are forums by which users of STRmix can interact
807 either with developers or each other. Within Australia and New Zealand the biology statistics
808 project working group is a network of individuals from laboratories that can interact on
809 statistical matters, including those relating to STRmix. On the STRmix support website there
810 are user forums where users can post questions, or requests. There is a groups.io STRmix
811 Users Group forum that contains 765 members (as of 06/11/2022) from all around the world.
812 This group is organised completely independently from the STRmix development team.
813 Members of this group actively post questions and material specifically relating to STRmix.
814 There is a yearly STRmix User Group workshop/conference where people can present
815 validation work or their own experiences with using or implementing STRmix. Again, this is
816 a user-driven group and not controlled by the STRmix development team. The 2022 meeting
817 had over 1000 registrants.

818 All of these sources of information and interaction provide a mechanism to standardise the
819 use of STRmix on a global setting and in line with best practise. They also provide a means
820 for providing laboratories with the most up-to-date information about STRmix use.

821

822 3.7 - laboratory implementation of STRmix

823 STRmix possesses many functions and features and not all forensic service providers
824 implement all functions, or do not fully utilise all features of the functions they implement.
825 Due to this there is a broad range of STRmix use and implementation that can be considered
826 within the range of best practice. Often decisions on whether to use functions (or how fully to
827 use those functions) is based on the workflows associated with profile interpretation and
828 reporting within the laboratory, the level of training of individuals using STRmix, the level of
829 in-house validation work carried out, the philosophical preferences of the users, the level of
830 resourcing for the laboratory, and the availability of information.

831 Some examples of implementation strategies that would be considered within the range of
832 best practice that I have observed are:

833 • STRmix is not used to analyse complete, single sourced DNA profiles. Instead, a
834 default LR value (known to always be exceeded for complete single source matching
835 profiles) is reported. This is often implements as a workflow efficiency.

836 • STRmix is not used to analyse profiles above a level of complexity, as defined by a
837 NoC. For example, a laboratory may deconvolute profiles that are deemed to originate
838 from up to three people, but not from four, or more. This is often implemented as a
839 means to triage the amount of profile interpretation and deconvolution carried out.
840 The most complex profiles are chosen to be triaged out due to the fact that they are
841 the most complex to interpret and hence take the greatest amount of analyst time, take
842 the longest time to deconvolute in STRmix and take the longest time for a second
843 analyst to review.

844 • STRmix is used to model some types of stutter, but not all that are known to
845 commonly occur in a DNA profile. The stutter types not modelled in STRmix are

846 screened out during the NA profile reading stage. This is often implemented as the
847 addition of numerous stutters on a DNA profile can complicate interpretation for a
848 scientists when they assign a NoC. However, this issue is addressed in some profile
849 reading software that has the ability to toggle the presence of stutter peaks off (for
850 human assignment of a NoC) or on (for analysis in STRmix).

851 • The variable number of contributors (VarNoC) feature in STRmix is not used. This is
852 often the case as the VarNoC feature is complex and requires the additional training
853 of staff before it can safely be used.

854 • STRmix is not used to analyse weak or complex profiles. Again, this is a mechanism
855 of triaging the amount of interpretation and analysis work carried out. In a case
856 context it may be chosen not to analyse a profile if there are other more informative
857 profiles in the case that have already been analysed and reported. In a no suspect
858 workflow it may be decided not to analyse a profile in STRmix if a manual pre-
859 assessment of the DNA profile deems there is little chance of obtaining a profile that
860 would be suitable for upload to a searchable database.

861 • STRmix may be used to deconvolute a profile, but will only be used to compare
862 references to a major component of the profile and the minor component of the profile
863 is not compared to any reference profiles. This is sometimes chosen as a result of the
864 laboratory's response to in-house validations.

865 • STRmix is not utilised to compare mixed DNA profiles to a searchable DNA
866 database. This is often chosen either due to the fact that the laboratory has not
867 validated the functionality, the laboratory does not have the resourcing to include this
868 service, or the laboratory does not have access to a searchable DNA database in a
869 format required to use this feature.

870 Some of these variants of implementation strategy may be considered below the emerging
871 best practice. Some others may technically be below emergent best practice but only by
872 comparison to a world of unlimited resourcing. For example, a common task undertaken by
873 forensic service providers to limit the amount of work accepted is to triage exhibits before
874 they are submitted to the laboratory. Ideally (if resourcing where not an issue) all exhibits
875 would be accepted and tested. The choice to triage in not really then an instance of falling
876 below best practice, or even below emerging best practice. So too can the choice of a
877 laboratory not to analyse low level profiles, or complex profiles in STRmix for reasons of
878 resourcing (in this instance the time of scientists rather than the cost of laboratory reagents)
879 be viewed.

880

881 **4.0 - Standard operating procedures SOPs**

882 4.1 - Basics of DNA profile interpretation (FSS.0001.0012.0147)

883 This SOP describes the process that an analyst undertakes to interpret a DNA profile. This
884 SOP captures all of the relevant aspects of DNA profile interpretation:

885 • Number of peaks
886 • Peak balance
887 • Stutter
888 • Sub-threshold peaks
889 • Consistence of mixture proportions
890 • Conditioning on known DNA donors
891

892 As such I believe that the SOP reflects a process that would be considered within the range of
893 best practice. There are a few points I have noted below for the laboratory to consider (some
894 of these are very minor grammatical points that I noted while reading).

895 **8.4.2:** the word symmetry is used, but imbalance is the proper term. Also, in the first dot
896 point both peak height and peak area are mentioned but I think both should be peak height

897 **8.11:** a DNA profile may be considered unsuitable if there is an inability to assess the NoC to
898 the DNA profile. Since version 2.7 of STRmix a feature has been available that can assess
899 profiles over a range of contributors. In other words, if an analyst assessing a DNA profile
900 cannot determined whether it is more likely to come from two or three contributors then the
901 variable number of contributors (VarNoC) feature of STRmix can be used to analyse the
902 profile as a 2 – 3 contributor profile. I have not seen any mention of the use of VarNoC in the
903 STRmix validations or SOPs, and so I suspect it has not been investigated or validated by
904 QH. If they did validate the VarNoC feature it may alleviate some current issues, one of
905 which would be that profiles could still be analysed when a single NoC could not be
906 assigned.

907 a DNA profile may be considered unsuitable if there are too many contributors such that any
908 reference sample comparison to the DNA profile would be meaningless. The current limit for
909 complexity at QH is four-person mixtures. I don't imagine that the description above is the
910 main driver for not analysing complex profiles until they became very complex (10 or more
911 people perhaps). There are publications showing the ability to meaningfully deconvolute five-
912 person mixtures with STRmix [44], and also examples of certain types of database searching
913 analysis being carried out meaningfully on profiles of up to nine contributors in STRmix
914 [60]. Perhaps a better description would talk about limitations in computing power, analyst
915 ability to interpret complex profiles, the limits of current validation, or the limits of available
916 resourcing as reasons.

917 **9:** This section refers to the use of sub-threshold peaks in DNA profile interpretation. There
918 appears to be three categories of peaks that exist, which are to be used differently:

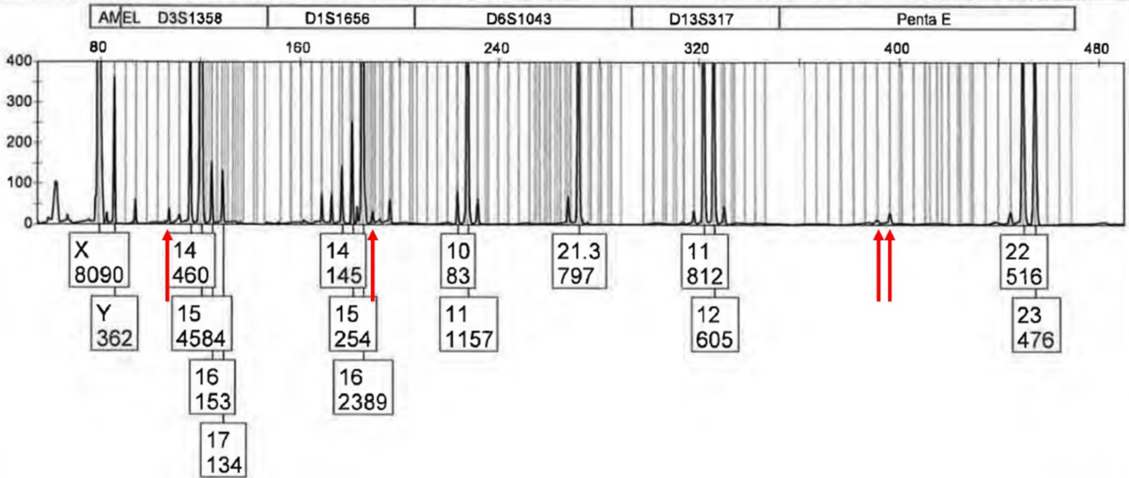
919 1) Peaks above the limit of reporting (LOR) which are used in determining the number
920 of contributors, carrying out manual checks for exclusion, and analysis within
921 STRmix

922 2) Peaks between the limit of detection (LOD) and LOR, referred to as 'sub-threshold'
923 peaks which are used in determining the number of contributors, carrying out manual
924 checks for exclusion, but not used during analysis within STRmix

925 3) Peaks below the LOD, which are not used in any capacity

926 The EPGs have peaks labelled that fall above the LOR, but not below this (i.e., peaks that fall
927 below LOD or between LOD and LOR are unlabelled). The fact that sub-threshold peaks are
928 not labelled on EPGs could cause problems, as it would not be clear to scientists, as they
929 view the EPG, which peaks fall either side of the LOD. For example, the LOD for
930 PowerPlex21 run on a 3500xl is 30rfu, a peak may be present in an EPG at 25rfu (and
931 therefore not used in interpretation) or at 35rfu (and therefore used in interpreting NoC or for
932 exclusion). The image in Figure 1 is an example from one of the casefiles I was provided.
933 The height of the peaks is indicated as the lower number within each box when the peak is
934 labelled, but is determined by comparison to the y-axis value for peaks that are not labelled.
935 In Figure 1 I have highlighted four peaks (using red arrows) which would be difficult to
936 determine if they fell above or below an LOD of 30rfu.

937

938 

939 *Figure 1: example of profile showing 4 peaks (indicated with red arrows) which may fall*
940 *below LOD*

941 I have been informed (correspondence 8.1) that:

942 "*If a peak is clearly above the LOD then the scientist will only use the zoom to assess the*
943 *peak. If the scientist thinks the peak could be close to the LOD then they will check the peak*
944 *height in GeneMapper*."

945 This alleviates the issue of any peaks being used for interpretation when they are below LOD.
946 However, it does cause additional work for scientists having to gauge which peaks are close

947 enough to the LOD to warrant further scrutiny in Genemapper, and then loading the sample
948 into Genemapper to check.

949 It is also not clear what the allelic designation of sub-threshold peaks is, which complicates
950 their use in an exclusionary capacity. I have been informed (correspondence 8.1) that:

951 "*For profiles generated using the 3500xl, peaks between the LOD and LOR are only used for*
952 *the assessment of the number of contributors, they are not used for exclusionary purposes.*
953 *For profiles generated using the 3130xl, peaks between the LOD and LOR are used for the*
954 *assessment of the number of contributors and for exclusionary purposes. If the peak is clear*
955 *on the zoomed epg then the scientist will use the printed bins to determine the designation. If*
956 *there is any ambiguity then the scientist will assess the peak in GeneMapper.*"

957 If this is the case, then the SOP 'Basics of DNA interpretation' (FSS.0001.0012.0147) section
958 9 and 16.1.6 need to be updated as they currently read:

9 Sub-threshold peaks

Sub-threshold peaks are defined as peaks that fall below the limit of reporting (LOR) and above the limit of detection (LOD).

Sub-threshold peaks can be used to inform the number of contributors and to exclude the donor of a reference sample, however they do not form part of the statistical interpretation of the profile and are not included in the LR.

959

960 And

16.1.6 Sub-threshold peaks

Forensic DNA Analysis has validated a limit of reporting for the purpose of confidently distinguishing true allelic peaks from background noise [4]. This means that only those peaks above the limit of reporting can be used in the statistical analysis of DNA profiles. It is noted however that there is a chance that peaks below this level could be from DNA and the closer these peaks are to the limit of reporting, the more likely they are to be from DNA. Where there is a low level contribution to the profile it is expected that these sub-threshold peaks could interfere with the interpretation of the allelic peaks above the limit of reporting and therefore should be considered in the determination of the number of contributors. The results of the testing have demonstrated that it is appropriate to use sub-threshold peaks during the interpretation of a DNA profile. Following on from this, if these sub-threshold peaks are used in the determination of the number of contributors it is expected that they would also be used for exclusionary purposes.

Note: Sub-threshold peaks should only be considered if they are distinct from baseline, above the LOD, below the LOR and not potential stutter peaks.

961

962 There are examples in other forensic laboratories where sub-threshold peaks are used in an
963 exclusionary capacity, and so this practice is not outside the range of current best practice. A
964 potential workflow that could assist streamline the process of DNA profile interpretation
965 would be to read the DNA profiles in Genemapper at the LOD. This would mean that all
966 peaks that can be used in manual interpretation (whether or not they are used for exclusionary
967 purposes) and determination of NoC are present on the EPG. The benefit of doing this is that

968 the scientist doesn't need to judge whether the peaks are close enough to LOD to open them
969 in Genemapper, nor do they need to rely on bins to determine allelic designation. Therefore
970 any additional time spent during read the DNA profiles at the LOD is outweighed by time
971 saving downstream during interpretation. In STRmix if an analysis is set up with a profile
972 that has peaks below the LOR (a setting called the 'detection threshold' in STRmix) then a
973 warning will be shown, and the user will have the option of proceeding with analysis either
974 retaining or removing those peaks from the analysis. In the case of QH the use would select
975 to remove them from analysis so that the deconvolution could proceed as it currently does,
976 using only peaks above the LOR, but with the benefit that the scientist has information about
977 all peaks above the LOD. There is also an administrative benefit as if all peaks above the
978 LOD are labelled then there is no need to include a zoom of each EPG. Instead,
979 interpretations can be carried out completely on the information in the full scaled EPG.

980

981 **10.1.1:** "…*and if their genotype combinations are the best supported throughout the mixture*
982 *taking into account the peak heights, then the numerator can be assumed to be one*". If fact
983 this occurs if their combination of genotypes is the <u>only</u> one possible (as opposed to the best
984 out of multiple, in which case the numerator is not one, but rather the weight associated with
985 that combination).

986 **10.3 scenario 3** (two unknowns). Just a note that there are situations where you may choose
987 to have a prosecution proposition with multiple knowns who are all considered unknown in
988 the defence proposition, and this doesn't necessarily require the DNA to be deposited at the
989 same time. For example, imagine a scenario where a POI is accused of stabbing person A on
990 Monday and person B on Friday with the same knife. A swab from the knife blade (taken
991 from the POI when they were arrested) comes back as a mix of person A and person B. The
992 prosecution proposition is that the DNA comes from person A and person B. Defence don't
993 concede the presence of either person on the knife blade and so their proposition is two
994 unknowns.

995

996 **Increasing NoC:** There are a few sections within the SOP appendix that all tend to suggest
997 increasing the NoC. Section 16.1.8 suggests that if

998 "*profiles have different alleles (either above or below threshold) in the low level contribution,*
999 *however it appears that overall there is only one low level contributor*"

1000 Then:

1001 "*In this instance, there is no certainty that there is only one contributor to the low level*
1002 *contribution and a contributor should be added*".

1003 I believe that this practise, if strictly applied, would lead to a systematic overestimation of
1004 NoC in some cases. When there is a low-level contributor to a DNA profile, that sits around
1005 the LOR then it would be expected to see different alleles from that contributor on multiple
1006 PCR amplifications. Following the guidance as described would very regularly lead to such
1007 profiles having an additional contributor added. It also somewhat clashed with section 16.1.5
1008 which states if there are four or fewer alleles in the minor then rework is not necessary. It is
1009 likely in these situations that rework would lead to different alleles.

1010 This same idea is again reinforced in the '4P Mixture Discussion Paper (v15)', which states
1011 under 'points to consider':

1012 "*For 2P mixtures, assuming n+1 contributors is preferable unless confidence is high that this*
1013 *is not warranted. This is not necessary with 3P or 4P mixtures*"

1014 It appears that this general preference of increasing the NoC in the case of two-person
1015 mixtures is based on some work carried out within the laboratory "*Moran R and Caunt E*
1016 *(2014) Assessment of the Number of Contributors for Mixed PowerPlex® 21 DNA Profiles*
1017 *within Forensic DNA Analysis*". I received this document on 16/11/2022 and while this is the
1018 source of the recommended interpretation approach it points to another document (E. Caunt,
1019 R. Morgan, J. Howes, C. Allen, *Development of guidelines for the determination of number*
1020 *of contributors to a PowerPlex®21 profile*. 2015) that details the study carried out to develop
1021 these recommendations. I have not seen this document and so can only speculate that within
1022 the study there were a number of instances where three-person mixtures presented profiles
1023 appearing as two-person mixtures, leading to the current recommendations. If this is the case
1024 then I provide the following advice, which may already be realised, but is worth checking.
1025 The experiment I just described would determine the probability of three-person mixtures
1026 presenting as two-person mixtures. This is not the same as the probability of profiles that
1027 present as two-person mixtures being three person mixtures. The difference between the
1028 statements is the order of the evidence and the conditional statement (and the latter requiring
1029 case context). For example, the probability of an apparent low-level two-person mixture
1030 actually being a three-person mixture is much lower for an intimate swab in a sexual assault
1031 than for a public object (like a door handle). This is what may be driving some
1032 overestimation of NoC in DNA profiles from sexual assault cases.

1033 I note however, that there is some further guidance to this idea of reproducibility of a minor
1034 component in section 16.1.9 part 8. I saw a number of instances where I personally would
1035 have interpreted the DNA profile as originating from less contributors than was assigned by
1036 the reporting scientist. However, it is also worth noting that there were instances in the
1037 casework I reviewed where reamplifications did not lead to reproducible peaks in a minor
1038 component, and this was not used as a reason to increase the NoC beyond what was required
1039 to explain the peaks seen. Therefore, I do not know if this passage in the SOP is being
1040 followed strictly as it is written, or instead is being supplanted by a more suitable to
1041 interpretation style. I note that I was only able to review 13 casefiles. A larger review of cases
1042 could reveal the extent to which any bias in assigning NoC is occurring. One of my

1043 recommendations (number 8) is a review of DNA profiles from SAIK swabs and these could
1044 be used to investigate the concern listed immediately above.

1045 Again, a similar sentiment on reproducibility and the NoC is seen in section 16.1.9 section
1046 8a. In section 16.1.9 section 8d the suggestion is that if there is a deviation from the expected
1047 ski-slope trend of peak heights in a DNA profile then the NoC should be increased by one.
1048 However significant deviation from this trend commonly, due to locus amplification
1049 efficiency differences, and do not necessarily suggest an additional contributor to the DNA
1050 profile. Again, taken literally this practice may lead to systematic over assignments of NoC. I
1051 did not notice any applications of this guidance being used to increase the assigned NoC,
1052 however I was only able to review 13 casefiles and so have a limited view. As before, the
1053 review suggested in recommendation 8 could serve as a means to also review whether there
1054 were any instances of guidance 16.1.9 section 8d being applied, and whether it is done so
1055 appropriately.

1056

1057 **16.1.10**: This section states:

1058 "*Although the LRs are calculated separately for each reference sample in the case, the*
1059 *manual comparison should include a check of all reference samples together, particularly for*
1060 *strong profiles with low mixture ratios*"

1061 I agree with this sentiment, but just make the note that there is no need to do this manually.
1062 An easy implementation is to set up a LR calculation in STRmix considering both people in
1063 the prosecution proposition and no-one in the defence proposition. You can then set a LR
1064 threshold to determine whether there is evidence that they can both be contributors. This
1065 avoids length and subjective manual interpretations, which can be particularly complicated
1066 when there is not good resolution between components in the DNA profile.

1067 There is also a paragraph that talks about how to report a result where it is found that some
1068 people cannot both have contributed. An option that is not considered in this paragraph is to
1069 report the two individual LRs (one for each person) but then provide a caveat that states they
1070 cannot both be contributors to the DNA profile.

1071 The final paragraph talks about reworks and whether there is an intuitive correctness to the
1072 comparison of the reference to the evidence. I just note here again that the VarNoC feature in
1073 STRmix can be useful in these situations.

1074

1075 **16.4**: second and third paragraphs talk about multiple 'runs' but I think this is meant to be
1076 referring to 'amplifications'.

1077

1078 **16.5:** This section refers to stutters. In the first paragraph it states:

1079 "*The -1 repeat stutter thresholds are used during case management for the determination of*
1080 *number of contributors, whilst -2 repeat and +1 repeat stutter thresholds are used at plate*
1081 *reading to determine which peaks should be removed before the profile is suitable for*
1082 *interpretation.*"

1083 However, I note that the +1 repeat stutter type is included in STRmix for 3500 data and so
1084 presumably not removed from the profile at the reading stage.

1085 Also in the following paragraph is states:

1086 "*As STRmix™ cannot model -2 repeat and +1 repeat stutter peaks…*"

1087 However, STRmix has been able to model any number of stutters (and at any position relative
1088 to their parent peak) since version 2.5. This may also affect the wording of the paragraph just
1089 below the figure, as STRmix can (and will) model the possibility of combined stutters adding
1090 to each other's expected peak height to create a total expected height.

1091

1092 **16.6:** This section provides guidance on manual exclusions and provides a comprehensive
1093 table on when a reference should be manually excluded. This is ok, and there are a number of
1094 laboratories that carry out manual exclusions. I just make the note that the interpretation of
1095 the profile and the use of this table and both quite time-consuming tasks and will give rise to
1096 difference of opinion between scientists. An easy solution is not to carry out manual
1097 exclusions, and instead calculate LRs against every reference in the case. This would ensure
1098 consistency of opinion and would also take no additional time to carry out (as references are
1099 already compared in batches anyway).

1100

1101 <u>4.2 - Use of STRmix™ software (FSS.0001.0001.5208)</u>

1102 This SOP goes through the use of the STRmix™ software within the QH workflow. In
1103 general, all aspects of this SOP are within the range of current best practice. I have a few
1104 suggestions for addition:

1105 1. In the diagnostics section it would be good to talk about the use of the weights as the
1106 primary diagnostic for interpreting the DNA profile

1107 2. It may be useful to have a component in here about review, or a mention of review
1108 with a pointer to the document where the review process is described (which I have
1109 been informed in correspondence 8.1 is Page 15 of *QIS 17117v21 – Procedure for*
1110 *case management*).

1111 3. It may be useful to include a section on the use of informed mixture proportion priors.
1112 This is a feature that can be particularly important when sub-threshold peaks are used
1113 to determine NoC, as this decision is made on information that will not be provided to
1114 STRmix. An explanation of the use of sub-threshold peak information and mixture
1115 proportion priors is give in Taylor et al [2].

1116

1117 It is stated in this SOP that STRmix is validated for the analysis of DNA profiles with 1 – 4
1118 contributors. It was common for the complexity of STRmix analyses to be capped at four due
1119 to:
1120 • Complexity of interpretation increasing as the number of contributors increased
1121 • Limitations in computing power, coupled with the efficiency of STRmix coding
1122

1123 With regards to this latter point, STRmix coding has become more efficient over the previous
1124 three versions, so that much less RAM is required to carry out complex DNA profile
1125 analyses. For five-person mixtures STRmix V2.7 typically required 100 – 300GB of RAM.
1126 For the same mixtures STRmix V2.8 typically required 50 – 100GB of RAM. For the latest
1127 version of STRmix, V2.9, STRmix typically requires 10 and 20GB of RAM and can be
1128 restricted to work with under 8GB of RAM. This brings five-person mixture analysis into the
1129 realm of being able to be run on standard desktop PCs. As such, laboratories are now
1130 validating STRmix for use on five-person mixtures more regularly.

1131 With regards to the first point, the interpretation of complex five-person mixtures can be
1132 aided by the use of helper tools for assignment of NoC, such as exists in FaSTR™ DNA. A
1133 known limitation of assigning a NoC is that as the NoC increases, the ability for analysts to
1134 assign the known NoC decreases. In a study by Bright et al [5] the known NoC to artificially
1135 constructed mixed DNA profiles was assigned for 3, 4, 5 and 6 person mixtures 98%, 76%,
1136 36% and 0% respectively.

1137 It is therefore still up to individual laboratories as to whether they choose to validate and
1138 analyse five-person mixtures in casework. There are laboratories that do analyse five person
1139 mixtures. Another common variant in use is that five-person mixture are interpreted when at
1140 least one person’s DNA can be assumed to have contributed (which can reduce the
1141 complexity of the interpretation). The use of STRmix for 1 – 4 people is still within the
1142 current range of best practice.

1143

1144 4.3 - Procedure for Profile Data Analysis using the Forensic Register (FSS.0001.0002.0272)

1145 This SOP is about the use of the Forensic Register (FR) for recording results and progressing
1146 work. I am not familiar with the FR and have no comments on this SOP.

1147

1148 4.4 - Procedure for Resolving DNA Profile Interpretation Differences of Opinion
1149 (WIT.0017.0013.0001)

1150 This SOP deals with the situation where there is a difference of opinion between scientists.
1151 Differences of opinion are expected to occur in any forensic laboratory from time to time. It
1152 comes as different people have different experiences, knowledge, understanding, or belief
1153 about DNA profiling. Over time the collective knowledge of the group is expected to grow
1154 with exposure to each other's ideas and differences. It is expected that aspects which were
1155 common ground for differences of opinion are resolved through a collective increase in
1156 knowledge and experience. When this occurs, differences of opinion turn to more nuanced
1157 aspects of DNA profile interpretation, until they took are collectively understood, and so on.
1158 The SOP produced by QH to resolve differences is quite large and the process quite
1159 formalised compared to others I have seen. The process outlined, whilst formal, seems fine
1160 and I believe would fall into the range of current best practice.

1161 I have only one note for QH to consider. The final stage of the disagreement resolution
1162 process, if a resolution cannot be found, is for the case to be reassigned (presumably to a
1163 scientist who agrees with the opinion of the remaining scientist). Another option, and
1164 arguably one that better reflects the fact that there is a divergence of opinion over the DNA
1165 profile is to report the fact that there is a divergence of opinion and provide both opinions in
1166 the report (with both scientists then signing the report).

1167 An example of such a policy can be found in the Forensic Science SA 'Casefile review' SOP.
1168 This SOP outlines the process for disagreements between scientists first being to take the
1169 matter to an appropriate senior staff member (such as the line manager), who determines
1170 whether there is validity to both dissenting opinions. If this is the case, then the reporting
1171 scientist must acknowledge the presence of the divergent views in their report. The report,
1172 with both views, can then be signed off by the line manager. Alternatively, two reports can be
1173 issued, referring to each other and each providing one of the dissenting views (signed by the
1174 scientist holding that view). All reports and records of the dissenting views are captured in
1175 the laboratory information management system.

1176 I was not involved in the creation of this policy, nor the writing of this SOP and so I cannot
1177 comment on the level of stakeholder engagement that was involved in the generation of this
1178 procedure. I am also unaware of the process for dispute resolution in other forensic
1179 laboratories.

1180

1181 **5.0 - casefiles**

1182 5.1 - [redacted] P1: 6.1

1183 The use of STRmix within this case adheres to SOPs.

1184 I notice in this case there was an instance of a DNA profile that appeared to have an anomaly,
1185 which rendered it unsuitable for reporting. An attempt was made to carry out STRmix, and
1186 the diagnostics within the STRmix analysis indicate an issue. The STRmix results were
1187 correctly not used to carry out LR calculations. Of interest that while the result was deemed
1188 not suitable for reporting in a court report it was manually compared to reference DNA
1189 profiles and the information provided in an intelligence report. I agree with the interpretation
1190 that was carried out, but I do not recall seeing any provisions in SOPs for the ability to use
1191 differing interpretation styles and results in an intelligence capacity compared to a court
1192 reporting capacity. It may be that I missed this information, or that it is in an SOP I have not
1193 been provided, or that it is not in an SOP. If the latter is the case, then there may need to be
1194 an addition to an SOP that described this process and the bounds under which it can occur.

1195

1196 5.2 - [redacted] P1: 6.2

1197 I believe the use of STRmix within this case adheres to SOPs. This case has an example of a
1198 locus that was dropped due to 'known vWa 13 issue'. I am aware of this issue and if it is
1199 occurring in the case then dropping the locus is an appropriate action. There were also a
1200 number of instances where peaks in the EPG were removed due to being 'N-2 rpt'. As N-2
1201 repeat stutters are not modelled within STRmix they should indeed be removed from input
1202 files. Presumably there are filters in Genemapper that automatically remove these peaks and
1203 those left on the profile (and deemed to be N-2 repeats) must be instances of that stutter peak
1204 type that has exceeded the threshold. If this is occurring often then it may be worth QH
1205 reassessing their N-2 repeat stutter threshold in Genemapper to ensure it is not too low. A
1206 small caution here is that there are instances where the removal of N-2 repeat peaks can cause
1207 issues, mainly when there is an allelic component to the peak in the N-1 stutter position. This
1208 issue comes when STRmix trials an allele in the N-1 stutter position, and then looks for its N-
1209 1 repeat (which would fall into the N-2 repeat position), can't find it and has to invoke
1210 dropout. The consequence is that the allele designation to the N-1 repeat position is less than
1211 it should be. This is only a rarely occurring issue and diagnosable from the STRmix output,
1212 but worth bearing in mind when considering a raising of the N-2 stutter filter in Genemapper.

1213 Alternatively N-2 repeat stutters could be added to the STRmix model and then left on EPGs,
1214 which does not have the same issue.

1215 Also, if scientists are able to remove peaks in N-2 positions that have fallen above the current
1216 Genemapper filter I did not see in the SOPs any guidance about the range over which this can
1217 occur. It may be that there is no defined upper limit to a scientist's ability to remove peaks,
1218 and the limit is based on experience of them and their reviewer. If so, that is fine, but should
1219 just be noted in an SOP.

1220

1221 In some instances, it was not clear to me why a NoC was chosen. For example, sample
1222 ████ was interpreted as coming from three people and I am not sure what information
1223 has been used by the scientist to go beyond two. They may be interpreting a third person due
1224 to peaks being in stutter positions but deemed too high (such as a peak in a N-2 repeat
1225 position at D16, or a peak in an N-1 and N+1 repeat positions at D18). I noted a couple of
1226 instances across the casefiles where notes were made in FR in the sample notes section that
1227 highlighted the reasons for the NoC assignment. For example, in case ████(case
1228 6.8), sample ████, a note was given:

Sample Notes

KML 14.07.2020 - Deemed 2p due to high stutter @vWA and sub threshold peaks.

1229

1230 And in case ████(case 6.10) sample ████:

Sample Notes

████ 08/02/2022 Batch notes checked, results OK to use. 3P based on peaks over -rpt stutter threshold at D2 & FGA and over +1rpt stutter threshold at D16 & D12

1231

1232 This type of note is very useful and can serve not only as an indication to others reviewing
1233 the work the reason behind an interpretation decision, but can also act as a record to remind
1234 the reporting scientist down the track as to their reasoning (for example if they were being
1235 questioned in court). Many forensic service providers have proformas, or areas within their
1236 information management systems where such reasoning can be specified and I would
1237 encourage their use.

1238

1239 Finally, there are some instances where it is not clear to me why additional PCRs are
1240 requested, or not requested. For example, some profiles (such as ████) have 3 PCRs,
1241 whereas others that seem similar (such as ████) have only one PCR and could have
1242 been amplified with additional DNA to aid interpretation. I do not think there is any reason to
1243 doubt the reliability or accuracy of reported results, I just make this note as a comment that
1244 some decisions are not transparent. Perhaps a note in the Forensic Register, or written on the
1245 EPG itself when further work is requested would benefit reviewers and make transparent the
1246 decision making process. This is only a very minor point.

1247

1248 5.3 - ████: P1: 6.3

1249 The use of STRmix within this case adheres to SOPs.

1250 This case possessed two instances where intimate samples had their NoC increased due to
1251 what appears to be a single high stutter. I agree that these stutters would be unusually high
1252 but are still considered as being non-allelic by STRmix for some portion of the time. I would

1253 be cautious increasing the NoC based on a single observation of a single high stutter peak but
1254 can understand the scientist's reason for doing so in these profiles. This may be an example
1255 of where qualifying statements in the report about the putative nature of the third contributor
1256 (as given by Mr Parry in paragraphs 34 to 42 of his statement, WIT.0043.0001.0001) might
1257 be a better description of the profile.

1258 Also, this case is an example of one where there is a common unknown male profile observed
1259 across several samples. Whilst not essential, it is common practice for forensic laboratories to
1260 report the presence of unknowns, or multiple occurrences of the unknowns in a court report.
1261 Again, this is something brought up by Mr Parry in his witness statement (paragraphs 31 to
1262 33 of WIT.0043.0001.0001).

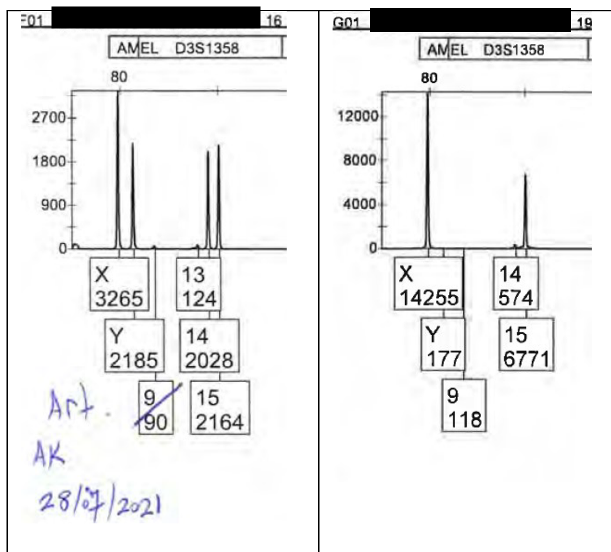
1263

1264 5.4 - ██████: P1: 6.4

1265 The use of STRmix within this case adheres to SOPs.

1266 There is a small inconsistency about the way that the 9 allele at D3S1358 is handled between
1267 scientists. Sometimes it is removed as an artefact (as in case ██████ in sample
1268 ██████) and sometimes it is left labelled (as in sample ██████ in case
1269 ██████). Figure 2 below shows these two examples.

1270



1271 *Figure 2: 9 allele being treated as artefactual in case ██████ in sample ██████*
1272 *(left) and allelic in sample ██████ in case ██████ (right)*

1273

1274 The area in which the 9 allele is observed in these profiles is a known artefact zone. Below I
1275 paste a portion of table 4 from the Promega PowerPlex® 21 System for Use on the Applied
1276 Biosystems® Genetic Analyzers technical manual:

Table 4. DNA-Dependent Artifacts Observed in Amplification Reactions with Human Genomic DNA.

Dye Label or Locus	Artifact Size
Fluorescein	~88–112 bases[1]

1277

1278 [1]For artifacts in this size range, rfu approximately 1.5% or less of the main peaks may be observed in the D3S1358 locus.

1279

1280 The issue of the 9 not being removed in most instances is only minor as STRmix has drop-in
1281 models that can account for additional peaks. Also (as is the case for the sample shown in Fig
1282 2, right) the mixture was assigned as coming from three people and as the 9 allele is the only
1283 minor peak neither contributor is forced to possess a 9 and so individuals will not be falsely
1284 excluded on this basis. The main issue that the retention of the 9 allele could cause is if it was
1285 used as the decision point for assigning a NoC. Nevertheless, as a known artefact it should be
1286 removed, and perhaps a reminder to staff of the known Powerplex21 artefacts would assist.

1287

1288 For sample ████████ I note the Gelman Rubin convergence diagnostic is 1.42. In the Use
1289 of STRmix software SOP (FSS.0001.0001.5208) it states:

1290 "*Deconvolutions with a Gelman-Rubin convergence diagnostic (GR) value of >1.2 (after*
1291 *additional iterations) should only be accepted for reporting after close scrutiny where the*
1292 *deconvolution is intuitively correct, and all other Diagnostic tools have given satisfactory*
1293 *results. Considerations should first be given to re-working and / or repeat STRmix™*
1294 *analysis.*"

1295 I cannot see where any consideration of additional reworking or repeat STRmix analysis has
1296 been recorded. I would have expected to see a second STRmix analysis carried out with
1297 increased iterations. I have seen instances of this occurring in other cases (such as case
1298 ██████████ sample ████████), which are noted in the sample notes (as shown below)
1299 and evident in the STRmix analysis files.

1300

Sample Notes
STRmix run with double accepts as unable to resolve with default accepts. EJC 10.03.2022

1301

1302 I suspect the cause of the high GR value is the overamplification of locus D8S1179. The
1303 other diagnostics for the deconvolution appear fine and so I do not believe there is any risk of
1304 an unreliable result having been reported.

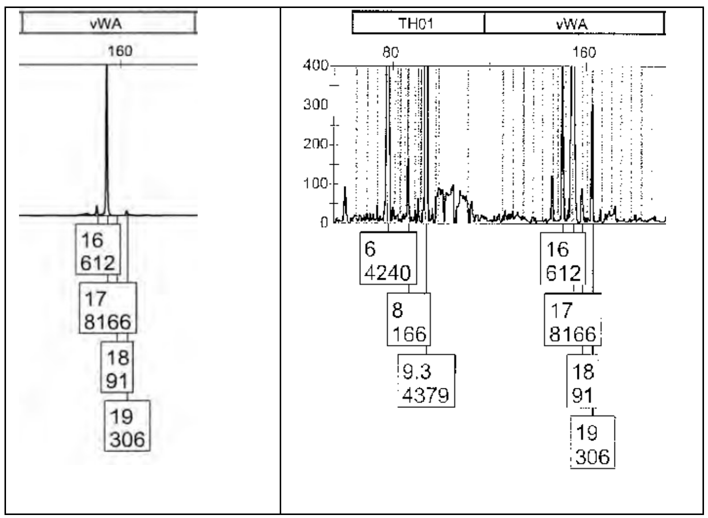
1305

1306 5.5 - [redacted]: P1: 6.5

1307 The use of STRmix within this case adheres to SOPs.

1308 This case has an example of where the inclusion of a N-2 repeat model would be beneficial.
1309 In sample [redacted] on PCR2 the vWa locus (shown in Figure 3 at full scale on the left
1310 and zoomed on the right) has a 15 peak that has been screened out (presumably at the
1311 Genemapper stage) as N-2 repeat stutter. The quality of the zoom is not great, but you can see
1312 the 15 peak sits just above 100rfu, and hence above the LOR for this profile (80rfu).

1313



1314 *Figure 3: Sample [redacted], PCR2, vWa at full scale on the left and zoomed on the right*

1315

1316 The person being compared to this sample is [15,19] at this region and in the LR supports
1317 exclusion at this locus (LR = 0.0416) whereas all other are inclusionary. The exclusionary LR
1318 at this locus comes from the fact that the missing 15 must be accounted for by dropout, and
1319 hence incurs a penalty. This is where even a dual-STRmix-kit system would be beneficial i.e.,
1320 if QH maintained their standard STRmix kit and also had a STRmix kit set up that included
1321 additional stutter types (such as N-2 repeats) then in this instance the profile could be re-read
1322 in Genemapper retaining all stutter peaks and then analysed in STRmix with the expanded
1323 kit. STRmix could then weight the possibility of this peak being N-2 repeat stutter vs allelic
1324 and calculate the LR accordingly.

1325 There are two points to make here: a) the manner in which QH has carried out the analysis
1326 and interpretation of this profile (and all profiles in this case) is within the bounds of SOPs,
1327 and is within the range of best practice (there are many other laboratories that treat data in
1328 this same way), and b) the overall LR (for the whole profile) is strongly inclusionary and the
1329 result at this locus, if changes by several orders of magnitude in either direction would not
1330 change the reported result.

1331

1332 5.6 - [redacted]: P1: 6.6

1333 The use of STRmix within this case adheres to SOPs. I have no further comments on this
1334 case.

1335

1336 5.7 - [redacted]: P2: (Sexual Offences): 6.8

1337 The use of STRmix adheres to SOPs within this case. There are instances of NoC being set
1338 based purely on the presence of non-reproducible sub-threshold peaks. The STRmix analyses
1339 are adequately reflecting the presence of very low-level contributors in these instances and so
1340 will be providing appropriate strengths of evidence.

1341 I noticed that in this case the scenario (as given on the QPS submission of articles for forensic
1342 examination form) was:

Offence Details – Brief summary
On the 25.01.2020 the victim was asleep in her camp room at [redacted] camp. The victim awoke at 0400 to the sound of an alarm and the named person ([redacted]) has been present in the room and inserted his finger/s into the victims vagina. The victim has said stop twice to the named person. The named person has then decamped in an unknown direction by unknown means.

1343

1344 Despite the allegation of digital assault, the vaginal swabs appear to have been screened for
1345 semen. This is ok, but perhaps unnecessary given the scenario. However, it there may be
1346 reasons for doing this (e.g., MOU with QPS on how to deal with SAIK samples, uncertainty
1347 around the circumstances of this particular case, etc). Once screened for sperm and semen
1348 and found to be negative for both the swabs were still processed with a differential extraction
1349 (designed to separate sperm from non-sperm cells). Based on the case circumstances and the
1350 screen results, this type of extraction could have been avoided and a standard DNA extraction
1351 performed. If it is standard to carry out differential extractions on all SAIK samples then this
1352 is something that could be review, but I note this is out of the scope of what I am reviewing.

1353 Finally, in the report only the results of the sperm fractions of the swabs were reports, with
1354 the epithelial fractions reported as:

Endocervical swab (1) – epithelial fraction

This fraction was not processed at this time due to reasons detailed in the Appendix.

1355

1356 The appendix states:

The current practice within Forensic DNA Analysis is for epithelial fractions from internal female sexual assault investigation kit (SAIK) samples to be stored following a differential lysis extraction process. This is because when these fractions are profiled, they are generally found to be a single contributor match to the person from whom the sample was taken. Given the nature of these samples, this finding is not unexpected. These epithelial fractions are stored indefinitely, and can be sent for DNA profiling at a future date if required.

1357

1358 However, in this case the alleged assault was digital, and so if the POIs DNA was going to be
1359 in one of the fractions, then it would be the epithelial fraction. I would therefore suggest the
1360 epithelial fraction as the most important one in this instance (noting that I believe a
1361 differential extraction didn't need to be carried out in the first place). Again, it may be that
1362 the standard reporting of differential samples is to report only the sperm fraction, however if
1363 this is the case perhaps more case context should be taken into account. A final note for this
1364 point is that the Quantifiler trio kit possesses an indicator of the level of male DNA in the
1365 sample, and it is common for laboratories to use this as a guide on whether to proceed sexual
1366 assault samples through to analysis.

1367

1368 I also note that the high vagina swab (1) is reported as:

This DNA profile also indicates the presence of possible low-level DNA which is below the Forensic DNA Analysis reportable threshold. The possible additional DNA is unsuitable for comparison purposes and in my opinion does not interfere with the interpretation described above.

1369

1370 However, I cannot see where these possible indications are in the profile, or the zooms of the
1371 profile baseline.

1372

1373 5.8 - [redacted]: P2: (Sexual Offences): 6.9

1374 The use of STRmix within this case adheres to SOPs. I note that there are differences in the
1375 way that sexual assault swabs are reported. For example, in case 6.9 the results are reported
1376 as:

Endocervical swab

This swab was submitted for DNA analysis to undergo a differential lysis extraction procedure.

Spermatozoa were not microscopically observed in the spermatozoa fraction and a sample from the swab tested negative for seminal fluid. In the absence of any semen being detected, the spermatozoa fraction was not processed further.

1377

1378 Whereas in case 6.8

Endocervical swab (1)

Semen was not detected in a sample prepared from the endocervical swab (1). The swab was submitted for DNA Analysis to undergo a differential lysis extraction process.

1379

1380 I realise from the wording of the report in case 6.9 that the differential extraction comes
1381 before the examination for sperm, and so some of my comments questioning the use of
1382 differential extraction when no sperm were detected do not apply. This point on wording is
1383 only very minor and I do not believe there either misrepresent the findings in any way. But it
1384 does show that even for someone who fluent with forensic techniques, subtle differences in
1385 wording can lead to different understandings.

1386 There also seems to be some difference as to whether epithelial fractions are processed and
1387 reported, or not between cases. Again, I am not concerned that there is any misrepresentation
1388 of results, this is just a small point.

1389 I also note that there is a general difference in the level of reworking carried out by different
1390 scientists. The scientist with conduct of this case tends to carry out two or three
1391 amplifications for mixed samples, whereas (for example) the author of case 6.10 only tends to
1392 carry out a single amplification. This difference in the level of reworking is of minor
1393 consequence. While forensic laboratories strive for all their scientists to reach a consistent
1394 standard of evidence processing, differences such as the one highlighted are ubiquitous.
1395 There is a balance when producing SOPs, on one hand they cannot be so prescriptive that
1396 there is no room for expertise or human judgement. On the other hand, they cannot be so
1397 lenient that they provide no guidance for how a case should be handled. In the grey zone
1398 between these extremes exist differences between analysts which are healthy and lead
1399 (through case discussions and review) to different ways of looking at cases. Ultimately the
1400 combination of these individual experiences and perspectives between scientists are a benefit
1401 to the entire laboratory, and it is just a matter of ensuring that diversity of opinion all lies
1402 within sensible scientific bounds. An activity undertaken by some forensic laboratories is to
1403 have regular 'DNA profile interpretation' meetings. In these meetings scientists bring DNA
1404 profiles they found challenging to interpret so that they can be discussed amongst the group
1405 and a range of opinions heard. Often these discussions lead to greater understanding and
1406 consistency within the group not just on DNA profile interpretation, but also on thought
1407 processes behind when reworking is carried out.

1408

1409 I note that in a 2015 document "*Assessment of the Number of Contributors for Mixed*
1410 *PowerPlex® 21 DNA Profiles within Forensic DNA Analysis*" by Robert Morgan, Emma
1411 Caunt (FSS.0001.0079.2173), the following passage gives guidance on reworking.

7. Reworking
The aim of the rework should be to confirm how a profile is behaving, assess the reproducibility of a component(s) for which the number of contributors is unclear or to potentially provide additional information in the form of additional peaks. Where more extract can be included in the amplification without overloading or increasing baseline noise, this should be done. For mixed DNA profiles the input can be increased above 0.5ng where it is suitable to do so based on the peak heights and the complexity of the profile [3]. In other cases where an increase in template is not possible a repeat amplification is sufficient. Where reworks have been performed, the minimum number of contributors may need to be reassessed based on the reproducibility of peaks or additional information that may have been obtained. It is recommended that, where a contribution in the stochastic range is thought to be single source, two reworks are performed so that final assessment can be made with a total of three amplifications. Depending on whether the input template is being increased or kept the same[4], these reworks may be ordered at the same time. It is also recommended that no more than three amplifications are performed for the determination of the number of contributors, unless there is an issue with one or more of these runs, since more amplifications may increase the complexity of the interpretation.

1412

1413 However, I do not recall seeing this a part of an SOP and so should be considered only as
1414 guidance rather than a laboratory procedure.

1415

1416 5.9 - [redacted] P2: (Sexual Offences): 6.10

1417 The use of STRmix within this case adheres to SOPs. This case has an example of unresolve
1418 peaks. Specifically, D12S391 in sample [redacted] an 18.3 peak for which a 17.3 stutter is
1419 expected, but absent, from the DNA profile. There is an 18 peak present which is obscuring
1420 the 17.3 peak. The absence of this peak is noted in STRmix as an 'evidence issue':

EVIDENCE PEAK ISSUES

[redacted]

LOCUS	PEAK	ISSUE	DECISION
Missing Stutter Peaks			
D12S391	17.3	Allele 18.3 is missing Back Stutter at position 17.3 (expected height of 206 RFU)	-

1421

1422 This occurrence is a regular but infrequent occurrence in DNA profiling. The absence of an
1423 expected stutter like this can be dealt with by either:

1424 • Continuing with the analyses despite the missing peak (which is the course of action
1425 taken for this sample by the scientist). This is acceptable, because STRmix possesses
1426 models for peak dropout that can explain the absence. Whilst not designed to model
1427 unresolved peaks, the models are robust enough that experience has shown they can
1428 account for some instances of unresolved peaks. When an analysis is progressed,
1429 despite their being unresolved peaks, there will be a tendency for the peak height
1430 variability to be elevated for that stutter type, which is exactly what has occurred in
1431 this case. Particular care with the scrutiny of resulting diagnostics is required in these
1432 instances to ensure the remainder of the deconvolution is not affected by this issue.

1433 • If on review of the STRmix analysis described in the previous point it is decided that
1434 the remainder of the deconvolution has been adversely affected then the

1435 deconvolution should be run again, ignoring the locus with the peak resolution issue.
1436 This locus would then need to be compared to references manually to ensure the
1437 information it possessed (now unseen by STRmix) is not exclusionary.

1438 I believe in this case the deconvolution of the sample with the unresolved peak has provided
1439 reasonable results, and there is no issue of unreliable opinions having been provided. A
1440 bigger issue can occur when the unresolved peak is potentially allelic (rather than being
1441 stutter) as this can significantly affect the LR when having to be accounted for as dropout.

1442 The final point I noted in this case is that there can sometimes be an apparent different
1443 treatment of the same type of data in different profiles. For example, on the issue of high
1444 stutters (and whether they lead to the addition of a contributor) in sample [redacted] a
1445 sample note states:

Sample Notes

1446 [redacted] 06/01/2022 - batch note checked, high stutter@D3[18] D16[12],

1447 Thereby accepting the presence of the high stutters without increasing the NoC, whereas on
1448 other samples, such as [redacted], the note states:

Sample Notes

1449 [redacted] 08/02/2022 Batch notes checked, results OK to use. D12 (19) peak added into profile (was labelled incorrectly as a spike in GMIDX) 3P interp based on peaks at D3, D1 & D12 over stutter thresholds.

1450 Thereby not accepting the presence of high stutters and increasing the NoC. I do not believe
1451 that there is any issue with the interpretation of either of these samples, and I believe the
1452 results produced and the opinions reported are reliable and accurate. I would have also
1453 accepted the results and had the same opinion if in the case of sample [redacted] the peaks at
1454 D3, D1 and D12 had been accepted as high stutters and the profile analysed as a two-person
1455 mixture. Or if this result were produced in a laboratory that had validated the VarNoC
1456 functionality in STRmix I would also accept a range from 2 to 3 contributors. All
1457 interpretations would fall within the range of best practice, and these types of differences in
1458 the application of expert discretion do occur (in all forensic laboratories). Sometimes there
1459 are other extrinsic features of the profile that are taken into account (which may have been
1460 the case here). I mainly point this out as it directly relates to the Commission instructions
1461 with regards to the assignment of number of contributors and the treatment of stutter peaks. It
1462 may be worth QH reviewing some casefiles and finding examples of where peaks have been
1463 accepted as high stutter compared to when they have invoked an increase in NoC. There may
1464 be some defining features that distinguish these two groups, or it may be an opportunity to
1465 simply have a discussion amongst reporting scientists, with examples, to reach a group
1466 understanding (or highlight areas in the group where differences of opinion currently exist,
1467 which could dictate some further research to assist everyone). In the recommendation section
1468 I recommend a review of cases with SAIK swabs and the resulting DNA profile
1469 interpretations. Carrying out the review of SAIK swab DNA profiles could also be used to
1470 capture the data I have suggested immediately above. These types of activities should be a
1471 regular occurrence within a forensic laboratory.

1472

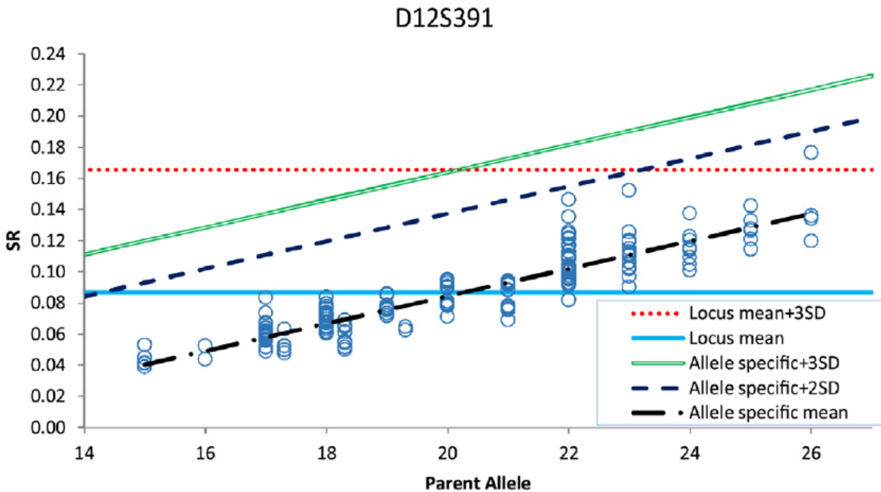
1473 5.10 - [redacted]: P2: (Sexual Offences): 6.11

1474 The use of STRmix within this case adheres to SOPs. Sample [redacted] is an intimate
1475 swab from a victim of alleged sexual assault. It has been designated as a three-person
1476 mixture; however, I cannot see what information in the profile requires more than two
1477 contributors. As I do not see any sub-threshold peaks in the baseline zoom of the profile
1478 (although this is difficult to see clearly) I suspect the scientist must have been basing their
1479 opinion on stutter peak heights. Given that stutter peak heights appear to be a common cause
1480 for increasing the number of contributors I wonder whether a reassessment of the stutter
1481 thresholds is required.

1482 Sample [redacted] is also an intimate swab from the victim. It has also been assigned as a
1483 three-person mixture. I believe in this instance the reason for the assignment is a high N+1
1484 repeat stutter (in position 28) at locus D21S11. While I agree that this peak would be an
1485 unusually high stutter, I would be hesitant to increase the NoC based on this alone and would
1486 have at least carried out an additional amplification to gain clarity on the nature of the peak.

1487 As it stands the sperm fraction from both intimate swabs were reported as three person
1488 mixtures. While the STRmix analyses have provided an appropriate strength of evidence for
1489 the comparison to the suspect's reference sample, these samples fall into the category
1490 highlighted in paragraph 135 of the report from Dr Kogios and Ms Baker.

1491 In this case there are several instances of a high stutter at D18 being commented in the
1492 sample notes. The victim in this case has a 22 allele at this locus, which in the upper end of
1493 the range of alleles for D18. If the current stutter thresholds being used by QH are set by the
1494 average observed stutter ratio plus three standard deviations (which is a common method in
1495 forensic validations) then published studies have shown that alleles in the upper range for the
1496 locus can exceed a threshold set in this way. The graph in Figure 4 is from Kalafut et al [61]
1497 showing the observed stutter ratios (blue circles) at locus D12S1391, the average stutter ratio
1498 (blue line), the average plus three standard deviations (red line). Note how the observed
1499 stutter ratios at the higher allele exceed the red line (i.e., the threshold).



1500
1501 *Figure 4: Graph from Kalafut et al [61] showing observed stutter ratios (blue circles) at*
1502 *locus D12S1391, the average stutter ratio (blue line), the average plus three standard*
1503 *deviations (red line).*

1504

1505 While an even higher threshold could be set to account for the largest alleles, this can become
1506 overly conservative for the smaller alleles, and so generally this is not done. On 16/11/2022 I
1507 was provided a spreadsheet used by QH to assist in determining the stutter thresholds to
1508 apply during DNA profile evaluation. On this sheet there are locus specific stutter thresholds,
1509 but also an indication of the observed stutter ratio of large alleles (which often exceed the
1510 stutter ratio for the locus). This a useful spreadsheet to have access to for interpretations and
1511 can be used to address the point I raised above regarding large alleles. It would be worth
1512 checking that scientists at QH are aware of this spreadsheet and the behaviour of using stutter
1513 thresholds for large alleles and that they take this into account when carrying out assignments
1514 of NoC.

1515 Alternatively, the use of a reading software that can highlight stutters (such as FaSTR) would
1516 assist.

1517

1518

1519 5.11 - ██████████: P2: (Sexual Offences): 6.12

1520 The use of STRmix within this case adheres to SOPs.

1521 I am not sure what pushed the interpretation of sample ███████ from a two-person to a
1522 three-person mixture. I could not see any indications of sub-threshold peaks that would cause
1523 this (although again my ability to carry out interpretations on the zooms is limited) and so I
1524 assume it may be due to the scientist's interpretation of stutters. I note that at D12S391 the

1525 victim in this matter possesses an allele (allele 27) in the upper range of alleles for that locus.
1526 The apparently high stutter at this locus is quite possibly a standard stutter, with the height
1527 just a product of the fact that it is such a large allele. On 16/11/2022 I was provided a
1528 spreadsheet used by QH to determine stutter thresholds. The stutter ratio for locus D12S391
1529 in the spreadsheet is 18% and the observed stutter ratio in the profile is 17.3% and so does
1530 not exceed this threshold. I note that the allele 27 is beyond the largest allele in the stutter
1531 threshold spreadsheet and so its stutter ratio should be interpreted with caution even if a
1532 stutter ratio was observed at a level higher than the 'largest allele threshold' column. The
1533 resulting LRs for this sample by comparison to the victim's and suspect's references would
1534 not be affected by whether the sample analysed as either a two-person or three-person profile
1535 as the main two contributors are both distinguishable from any potential trace component.

1536 Again, with sample [redacted] I am not sure what pushed the interpretation of sample
1537 from a two-person to a three-person mixture. I could not see any indications of sub-threshold
1538 peaks or high stutters. In this case the LR produced for the comparison to the suspect's
1539 reference is likely to be lower than if the profile was analysed as a two-person mixture. Even
1540 as a three-person mixture the support given to the suspect being a donor of DNA is extremely
1541 strong and so I would still consider the reported opinions as reliable.

1542

1543 5.12 - [redacted] P2: (Sexual Offences): 6.13

1544 The use of STRmix within this case adheres to SOPs.

1545 In this case there were definite indications of three contributors to the sperm fractions of the
1546 high ([redacted]) and low ([redacted]) vaginal swabs. Given the last previous consensual
1547 sexual partner the victim had was one day prior to the alleged assault it is quite possible that
1548 the unknown component in these samples was this previous partner. This is an instance where
1549 it may have been useful to the court for the scientist to report the fact that there was an
1550 interpretable component from an unknown individual that was common to both samples. I
1551 realise that this does not seem to be currently possible within the SOPs of QH (and based on
1552 the comments of Mr Parry on this topic).

1553

1554 5.13 - [redacted]: P2: (Sexual Offences): 6.14 (NOT REVIEWED)

1555

1556 5.14 - [redacted]: P2: (Sexual Offences): 6.15 (NOT REVIEWED)

1557

1558 5.15 - [redacted]: P2: (Sexual Offences): 6.16 (NOT REVIEWED)

1559

1560 5.16 - [redacted]: P2: (Sexual Offences): 6.17 (NOT REVIEWED)

1561

1562 5.17 - [redacted]: P2 (Murder): 6.19 (NOT REVIEWED)

1563

1564 5.18 - [redacted]: P2 (Murder): 6.20

1565 The use of STRmix within this case adheres to SOPs. I did not receive any STRmix analysis
1566 reports for this case but given what I saw in the file I don't really need to see them (I don't
1567 expect to find anything that directly relates to the points raised in the Commission brief). I
1568 have no further comments on this casefile.

1569

1570 5.19 - [redacted]: P2 (Murder): 6.21

1571 The use of STRmix within this case adheres to SOPs. There is an opportunity for QH to yield
1572 more information from the profiles they analyse by leveraging the assumed contributor
1573 feature of STRmix. This may already be occurring and just not printed out for casefiles and
1574 so I haven't seen it. An example is sample [redacted] in this case which has mixture
1575 proportions of approximately 0.62 : 0.21 : 0.13 : 0.05. Main contributor to the profile has 10
1576 alleles that can be interpreted with probability greater than 0.99. This is not sufficient for an
1577 upload and search of NCIDD. One of the references ([redacted]) in the case has extremely
1578 strong support for being a contributor of DNA and aligns with the 0.21 contributor. It would
1579 be possible to carry out a second deconvolution of sample [redacted] assuming the
1580 presence of [redacted], which would markedly improve the power in the analysis to resolve
1581 the major contributor's profile. The result would likely yield the profile of the major DNA
1582 donor at a level that was now uploadable to NCIDD. The result of this second deconvolution
1583 wouldn't be reported in a court report as it was for intelligence purposes.

1584

1585 I have also found that there are differences in the way that different scientists deal with peaks
1586 in N-2 repeat stutter positions. Figure 5 shows four examples of peaks in this position from
1587 four different cases (the first of which comes from case 6.21), two of which the scientist has
1588 removed and two of which the scientist has retained. In the case of the fourth example
1589 ([redacted] I believe this is one of the only (or maybe the only) indication used to
1590 indicate the profile came from two contributors rather than from a single contributor.

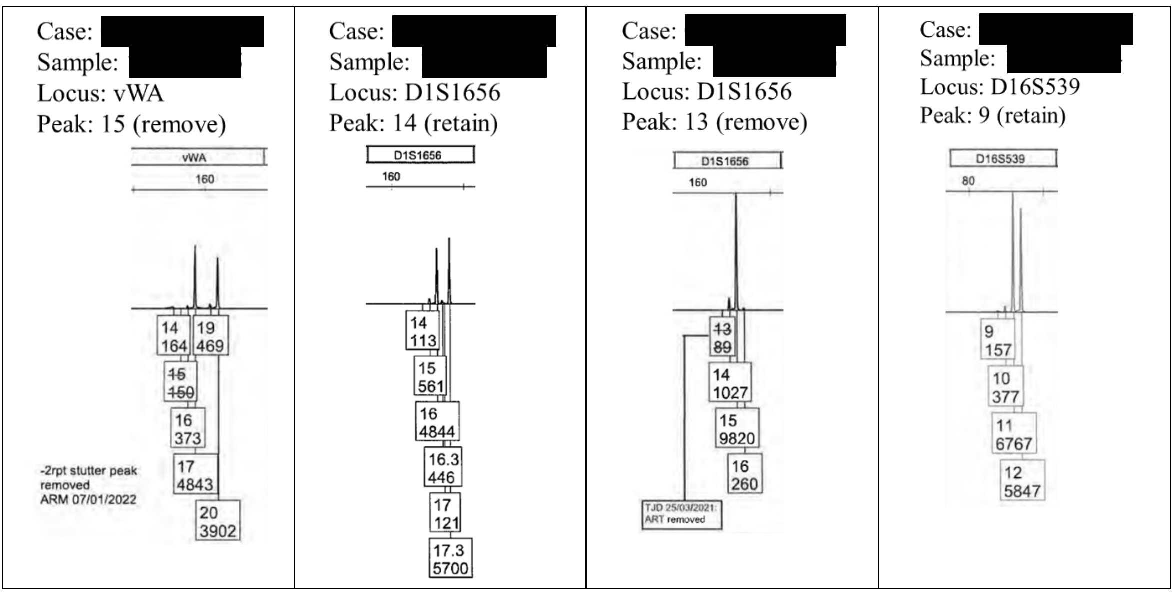


Figure 5: Four examples of peaks in N-2 repeat stutter positions showing the different way in which the scientist dealt with them

The issue highlighted in Figure 5 is not major, because the models in STRmix can deal with missing peaks via a dropout model and can handle additional peaks via a drop-in model. There are, however, two instances where opposing choices can have a larger effect. The first is when the removal of the peak causes modelling issues that drives STRmix away from assigning an allele component to the N-1 position (as previously discussed for case 6.2). The second is when the N-2 peak in contention is the decision point for the assignment of a NoC. Despite the above point that the choice of whether to retain or remove the peak will usually have a minor effect on outcome, the ideal situation in a forensic laboratory is to have a consistent approach across all scientists for dealing with these types of peaks. There are a number of avenues to improve the consistency of approach, which (in order of preference; highest to lowest) are:

- Model the N-2 stutter type in STRmix in all profiles so that those peaks are always left on profiles and always deal with probabilistically
- Model the N-2 stutter types in STRmix in a secondary kit setup that is used only when there are N-2 peaks in a DNA profile that are in contention
- Retain all existing practices but devise a series of rules that dictate when a peak in an N-2 position should be removed or retained
- Retain all existing practices and guidelines, gather case examples of N-2 peaks being removed or retained and conduct group interpretation discussions with a view to reaching a group understanding on when to remove or retain N-2 peaks
- Raise the N-2 stutter threshold in Genemapper so that they are screened out at a higher level and do not allow scientists to further override peaks that exceed the

1616 threshold (noting that this will increase the instances of peak removal causing
1617 modelling issues)
1618

1619 For sample [redacted] there is a sample note:

1620

Sample Notes
[redacted] 07/01/2022 CSTRAMP20211213-01 batch notes checked, no results affected, noted that D8 did not resolve as expected from detected, expected genotype weighted at ~95%, decon accepted as no effect on reported LR

1621 I agree with the comment that the reported LR for the comparison to reference [redacted]
1622 [redacted] will not have been affected (and so there is not an issue on an unreliable
1623 result having been reported), but the minor contributor LRs could have been affected. If there
1624 is an issue where the STRmix output has not met intuitive expectation, then this should not be
1625 accepted. In this case I also agree that the genotype of the major DNA donor at D8 should
1626 have been resolved 100% of the time (as opposed to the noted 95%). Potential solutions are
1627 to run the deconvolution for a larger number of iterations (giving it more time to find the
1628 appropriate sample space) or to use mixture proportion priors to enforce the obvious major :
1629 minor relationship between donors.

1630

1631 My final note for this case is that one of the samples [redacted] was interpreted as a single
1632 source profile, with no indication in the sample note on FR of a mixtures. This sample was
1633 reported as:

[redacted] - C - DRY RED ELONGATED DROP 0.2CM X 0.2CM STAIN [SWBL] On inside surface of front entry door frame
The DNA profile obtained from this sample matches the DNA profile of [redacted]. Based on statistical analysis, it is estimated that the DNA profile obtained is greater than 100 billion times more likely to have occurred if [redacted] had contributed DNA rather than if she had not.

This DNA profile also indicates the presence of possible low level DNA which is below the Forensic DNA Analysis reportable threshold. This possible additional DNA is unsuitable for comparison purposes and in my opinion does not interfere with the interpretation described above.

1634

1635 It is fine to have made this interpretation. However, if the interpretation of the reporting
1636 scientist is that a low level second contributor may be present then ideally a comment in the
1637 sample note section of the FR should be made. This would then align the electronic stored
1638 record of interpretation with the reported result.

1639

1640 I also note that reporting the result in this way is an example of the type of contextual
1641 information around the assignment of a NoC that was suggested by Mr Parry in his statement
1642 (WIT.0043.0001.0001 at paragraphs 40 and 41). This shows that there is scope for this type
1643 of information to be provided within the existing reporting system of QH.

1644

1645 5.20 - [redacted]: P3: 6.23 (NOT REVIEWED)

1646

1647 5.21 - [redacted]: Intel: 6.24 (NOT REVIEWED)

1648

1649 5.22 - [redacted]: Intel: 6.25 (NOT REVIEWED)

1650

1651 5.23 - [redacted]: Intel: 6.26 (NOT REVIEWED)

1652

1653 5.24 - [redacted]: Intel: 6.27 (NOT REVIEWED)

1654

1655 5.25 - [redacted]: Intel: 6.28 (NOT REVIEWED)

1656

1657 5.26 - [redacted]: Intel: 6.29 (NOT REVIEWED)

1658

1659 5.27 - [redacted]: Intel: 6.30 (NOT REVIEWED)

1660

1661 5.28 - [redacted]: Dropped Loci: 6.32 (NOT REVIEWED)

1662

1663 5.29 - [redacted]: Dropped Loci: 6.33

1664 In this case there are instances of STRmix analyses that have been carried out where one or
1665 two have been ignored due to pull-up affected peaks. I have only reviewed sample
1666 [redacted], in which D16S539 and D12S391 were ignored, in both instances due to the
1667 potential effects that pull-up had on peaks sitting in N+1 stutter positions. The STRmix
1668 analysis in this instance appears to have performed appropriately and there is no issue of
1669 unreliable results produced from this analysis.

1670 The analysis could have occurred in a staged approach, trialling different levels of
1671 information loss. For example, the analysis of the DNA profiles could have been carried out
1672 without the removal of the pull-up affected peaks and the deconvolution scrutinised to

1673 determine whether the weights were acceptable. If they were deemed unacceptable, then the
1674 analysis could have been conducted with just those affected peaks removed (rather than the
1675 entire loci ignored, and again the deconvolution result could have been assessed for
1676 appropriateness. It is only if this last deconvolution was found to still be unacceptable that the
1677 deconvolution could have been carried out with loci ignored.

1678 This staged approach is along the lines of flow diagram suggested by Emma Caunt on how to
1679 deal with profiles with loci that potentially needed to be ignored. However, I note that it does
1680 mean the profile has to potentially be deconvoluted and reviewed multiple times.

1681

1682 **6.0 - Recommendations and considerations**

1683

1684 6.1 - Recommendations

1685 1. Clarify whether peaks between the LOD and LOR can be used for exclusionary purposes
1686 and potentially amend the 'Basics of DNA Profile Interpretation' SOP. Then clarify with
1687 scientists what the correct procedure is for using sub-threshold peaks. Consideration 1
1688 provides some alternative workflows that QH could investigate.

1689 2. Add some material into an SOP that provides guidance on when it is appropriate to ignore
1690 a locus, and whether (and under what conditions) multiple loci can be ignored, and any
1691 steps or checks that need to be carried out to ensure robust opinions are still being
1692 provided. As part of the instructions there should be advice on how loci to be potentially
1693 dropped can first be investigated without dropping the locus, and/or dropping just one
1694 allele before dropping the locus. Guidance should also include information on when it is
1695 deemed that a profile is too affected by saturation or pull-up to be analysed in STRmix.
1696 Developing this guidance could be based on an analysis of profiles that have already been
1697 produced by QH at different levels of intensity.

1698 3. Review the areas of the 'Basics of DNA Profile Interpretation' SOP highlighted in section
1699 4 that suggest a practice that could lead to systematic overestimation of number of
1700 contributors. I recommend an approach that is conservative, but not systematically biased
1701 to over or underestimating the number of contributors.

1702 4. Review the process of SAIK swab processing to ensure that the extraction method reflects
1703 the circumstances of the case (i.e., not proceeding to differential lysis if there is no
1704 indication of sperm in the case scenario), and that the results reported (i.e., sperm or
1705 epithelial or both) are the most appropriate.

1706 5. Review the current stutter thresholds to ensure they are achieving appropriate coverage.
1707 This could be achieved by retrospective data analysis of references samples that have

1708 been generated at QH under the same PCR conditions as evidence profiles. This review
1709 should include an exploration of the limits of applying thresholds, for example their
1710 performance on large alleles. Scientists in QH should then be made aware of the
1711 performance of the current threshold, and the limitations of these thresholds (from
1712 inhouse data analysis and as reported in literature).

1713 6. Work towards standardising the treatment of peaks in N-2 stutter positions. Some options
1714 to improve the consistency of approach (in order of preference; highest to lowest) are:
1715 • Model the N-2 stutter type in STRmix in all profiles so that those peaks are always
1716 left on profiles and always deal with probabilistically
1717 • Model the N-2 stutter types in STRmix in a secondary kit setup that is used only when
1718 there are N-2 peaks in a DNA profile that are in contention
1719 • Retain all existing practices but devise a series of rules that dictate when a peak in an
1720 N-2 position should be removed or retained
1721 • Retain all existing practices and guidelines, gather case examples of N-2 peaks being
1722 removed or retained and conduct group interpretation discussions with a view to
1723 reaching a group understanding on when to remove or retain N-2 peaks
1724 • Raise the N-2 stutter threshold in Genemapper so that they are screened out at a
1725 higher level and do not allow scientists to further override peaks that exceed the
1726 threshold (noting that this will increase the instances of peak removal causing
1727 modelling issues)

1728 7. Ensure that the reported interpretation stored in FR aligns with the reported interpretation
1729 of the results (see comments for case 6.21). This can be achieved through sample notes.
1730 This practice should be noted in an SOP (possibly Procedure for Profile Data Analysis
1731 using the Forensic Register, FSS.0001.0002.0272).

1732 8. An external review be conducted of swabs from SAIKs in previous sexual assault cases to
1733 determine which have been reported as coming from three or more people (I refer to these
1734 as the 'applicable cases'). For those instances where the NoC has been assigned as three
1735 or more, a review of the reasoning behind the choice to interpret the profile as coming
1736 from the higher number of people should be undertaken, and how often that evidence for
1737 the extra contributor is slight (i.e., one or two high stutter peaks, or an imbalance, or
1738 simply a low number of minor peaks). For those profiles where, upon review, it is
1739 determined that the profile should have been reported as originating from a lower number
1740 of contributors the profiles should be reanalysed and reported in addendum DNA reports.
1741 Ideally this review would be conducted by more than one person (to guard against
1742 individual interpretation preferences) and be external to QH (such as a forensic provider
1743 in other state(s)). The review should span applicable cases for the previous one-year
1744 period as this will provide a random sample of cases in sufficient number to identify
1745 whether there exists a bias towards overestimating the NoC to these DNA profiles.

1746

1747 In addition, a compilation of applicable cases that are as yet unresolved through the legal
1748 system should be made and for those that do not fall into the previous year they should be
1749 included in the review.

1750 After the review, it may be found that there is no systemic over assignment of NoC. In this
1751 case the action then turns to the best way to report the DNA results for these SAIK swabs
1752 so that the NoC is given within some context that explains the significance of the finding.
1753 In the instances of a very minor contributor to a DNA profile being present on an intimate
1754 swab, there have been some suggestions by Mr Parry on ways that this type of scenario
1755 could be better communicated.

1756 Alternatively, after the review it may be found that there is a systemic over assignment of
1757 NoC. In this situation any profile in any case could be affected. It is not reasonable to
1758 review all previous work and so I recommend that in this eventuality the findings of the
1759 profile review be communicated to stakeholders with the offer that if they feel their case
1760 may have been affected that they can bring it to the attention of QH for reassessment.

1761

1762 6.2 - Considerations

1763

1764 1. Consider adopting a procedure whereby DNA profiles are read to the LOD but analysed
1765 in STRmix at the LOR (using the inbuilt feature of STRmix to ignore peaks below this
1766 level). This should assist in achieving some workflow efficiency within DNA
1767 interpretation. Alternatively, consider having only one threshold, above which all
1768 information is used in evaluation and STRmix, and below which information is not used.

1769 2. Consider using the Y-chromosome quantification and autosomal quantification value
1770 from Quantifiler Trio as a decision point on whether to carry out STR analysis on SAIK
1771 swabs.

1772 3. Consider using STRmix to carry out joint LRs i.e., where multiple people have
1773 individually given inclusionary LRs, calculate an LR where they are all included in the
1774 prosecution proposition. The value of this joint LR is not reported, but rather used as a
1775 check that the individuals can be contributors together.

1776 4. Consider using the mixture-to-mixture feature of STRmix to assist in determining when
1777 unknown that have been interpreted from multiple profiles in a case could be the same
1778 individual.

1779 5. Consider using STRmix to carry out LR calculations against all references in a case,
1780 rather than relying on manual intuitive exclusions. There is negligible additional time
1781 required to carry out these calculations and they will achieve a greater level of consistent

1782 interpretation. Note that in my review of the casefiles I didn't see any instances of a
1783 manual intuitive exclusion and so it may be that whilst technically possible according to
1784 the SOP, they are not commonly used.

1785 6. Consider the validation and use of the variable number of contributors feature in STRmix.
1786 This is a tool that allows analysis of a profile when a single number of contributors cannot
1787 be assigned. This will assist in being able to analyse currently uninterpretable profiles, it
1788 will assist in resolving differences in scientific opinions, and it could assist in issues
1789 surrounding the change of interpretation for a sample result that has already been added to
1790 the Forensic Register. I note that in the document "*Forensic DNA Analysis. Single Source*
1791 *High Stutter Guidelines Assessment, by Angela Adamson, Cassandra James, Emma*
1792 *Caunt. July 2021*" this feature was used, and so there is some familiarity with it within the
1793 members of QH.

1794 7. Consider the validation and use of FaSTR DNA, which has the ability to highlight stutters
1795 on a DNA profile (including taking into account combined stutters), has a tool that
1796 assigns a number of contributors, and a tool that assists with identifying artefacts. All of
1797 these features would assist scientists in interpreting DNA profiles and achieve greater
1798 consistency of interpretation.

1799 8. Consider expanding the models in STRmix to include additional stutter types. This will
1800 assist in DNA profile interpretation as peaks in these positions do not need to be screened
1801 out in Genemapper or accounted for as allelic during interpretation or deconvolution.

1802 9. Consider reporting both interpretations in the instance of an unresolvable difference of
1803 opinions. Rather than re-allocating cases where a disagreement cannot be resolved it may
1804 be better to report the fact that there is a disagreement in the way the profile is interpreted,
1805 report both and have both scientists sign the statement. This is more reflective of how the
1806 courts should consider the evidence rather than the report giving the impression that
1807 everyone agrees on how the profile should be interpreted.

1808 10. Encourage the use of the sample notes section of FR to indicate why a NoC has been
1809 chosen if it is not immediately obvious from the profile (i.e., if it relies on a peak
1810 imbalance, sub-threshold peaks, or high stutters).

1811 11. Consider reviewing a number of casefiles to find examples of where peaks have been
1812 accepted as high stutter and when they have invoked an increase in NoC. Identify whether
1813 there are defining features that distinguish these two groups. Discuss the findings
1814 amongst reporting scientists, with the examples, to reach a group understanding, or
1815 highlight areas in the group where differences of opinion currently exist (which could
1816 dictate some further research). Note that this could be done in conjunction with
1817 recommendation 8 and discussed as per consideration 14.

1818 12. Consider developing an intelligence process (if it does not already exist) whereby
1819 references in cases can be assumed to have contributed to evidence samples purely as a
1820 means to gain better resolution in the genotypes of other contributors so that they may be
1821 uploaded and searched against NCIDD.

1822 13. Consider developing court report wording that reflects the presence of a very minor and
1823 putative contribution to a DNA profile. This should alleviate some issues with a number
1824 of contributors being detrimental to a case within the wider context.

1825 14. Consider holding regular DNA profile interpretation meetings. In these meetings
1826 scientists bring DNA profiles they found challenging to interpret so that they can be
1827 discussed amongst the group and a range of opinions heard. Often these discussions lead
1828 to greater understanding and consistency within the group on DNA profile interpretation,
1829 and the thought processes behind when reworking is carried out.

1830

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2014

2015

2016 **Appendix I: material provided by the Commission**

2017 Review of STRMix – updated 14 November 2022

2018

No.	Document description	Date	Doc ID
Instructions			
1.1	Terms of Reference	10.06.2022	COI.9999.0025.0001
1.2	Instructions to expert	7.11.2022	
Standard Operating Procedures			
2.1	35007v4 - Use of STRmix Software	06.09.2021	FSS.0001.0001.5208
2.2	33773v3 - Procedure for Profile Data Analysis using the Forensic Register	10.03.2022	FSS.0001.0002.0272
2.3	36061v1 – Procedure for Resolving DNA Profile Interpretation Differences of Opinion	10.09.2021	WIT.0017.0013.0001
2.4	17168v14 – Basics of DNA profile interpretation	13.07.2020	FSS.0001.0012.0147
2.5	33188v4 – Introduction to DNA profile interpretation	10.06.2020	FSS.0001.0012.0986
Equipment manuals / recommendations / guidance			
3.1	STRmix 2.8 User's Manual	20.10.2020	FSS.0001.0001.2697
3.2	STRmix v2.8.0 Release and Testing Report	29.09.2020	
3.3	STRmix 2.8 Test Report	29.09.2020	
3.4	STRmix 2.8 Implementation and Validation Guide	20.10.2020	
3.5	STRmix 2.8 Operation Manual	29.09.2020	
3.6	STRmix 2.8 Installation Manual	29.09.2020	
3.7	STRmix guideline document: 'The highest posterior density for the Monte Carlo effect in STRmix	July 2020	
3.8	Advice from STRmix to Emma Caunt regarding changing from 9700 to Proflex thermocyclers	21.04.2021	
3.9	QHFSS internal document: 4P Mixture Discussion Paper	01.10.2020	
3.10	QHFSS internal document: Single Source High Stutter Guidelines Assessment	July 2021	
3.11	Assessment of the Number of Contributors for Mixed PowerPlex® 21 DNA Profiles within Forensic DNA Analysis	16.11.2022	FSS.0001.0079.2173
Validation documentation			

4.1	Project 231 Implementation Plan – Verification of STRmix v2.8	Undated	FSS.0001.0023.8133
4.2	Proposal 231 – Verification of STRmix v2.8.0 Report Attachments: Excel workbook Excel workbook Excel workbook	March 2021	FSS.0001.0023.8154 FSS.0001.0023.8170 FSS.0001.0023.8171 FSS.0001.0023.8172
4.3	Email correspondence regarding feedback on Verification of STRmix v2.8.0 Report	25.10.2021	WIT.0006.0040.0001
Witness statements			
5.1	Statement of Rhys Parry: Number of contributors: [34] – [42]	28.09.2022	WIT.0043.0001.0001_R WIT.0043.0002.0001_R to WIT.0043.0004.0001_R
5.2	Statement of Emma Caunt: Stutter threshold, combined stutter, removing loci: [3] – [31] STRMix validation: [32] – [39] Mixture searching: [131] – [133]	06.10.2022	WIT.0004.1224.0001 WIT.0004.1225.0001 to WIT.0004.1244.0001
5.3	Statement of Kylie Rika: Verification of Proflex for PP21: [38] – [47] Advice from STRMix: KR-03-1	06.10.2022	WIT.0006.0145.0001 WIT.0006.0146.0001 to WIT.0006.0164.0001
5.4	Statement of Justin Howes: Interpretation of DNA profiles: [137] – [155]	06.10.2022	WIT.0016.0188.0001
Example casefiles			
Priority 1			
6.1	Full casefile and STRMix reports for ██████ (P1)	Various	FSS.0001.0081.7410 + Reports (awaiting Orb upload)
6.2	Full casefile and STRMix reports for ██████ (P1)	Various	FSS.0001.0081.7479
6.3	Full casefile and STRMix reports for ██████ (P1)	Various	FSS.0001.0081.7623
6.4	Full casefile and STRMix reports for ██████ (P1)	Various	FSS.0001.0081.7723
6.5	Full casefile and STRMix reports for ██████ (P1)	Various	FSS.0001.0081.7856
6.6	Full casefile and STRMix reports for ██████ (P1)	Various	FSS.0001.0081.7971

6.7	Bundle of P1 casefile Quant Batch Results for above casefiles 6.1 – 6.6	Various	COI.0294.0005.0001
Priority 2 (Sexual Offences)			
6.8	Full casefile (including additional testing) and STRMix reports for ███ (P2 – SAIK)	Various	FSS.0001.0081.8087 + Reports (awaiting Orb upload) + Additional testing (awaiting Orb upload)
6.9	Full casefile and STRMix reports for ███P2 – SAIK)	Various	FSS.0001.0081.8267
6.10	Full casefile and STRMix reports for ███(P2 – SAIK)	Various	FSS.0001.0081.8366
6.11	Full casefile and STRMix reports for ███(P2 – SAIK)	Various	FSS.0001.0081.8526
6.12	Full casefile and STRMix reports for ███(P2 – SAIK)	Various	FSS.0001.0081.8621
6.13	Full casefile and STRMix reports for ███ (P2 – SAIK)	Various	FSS.0001.0081.8716
6.14	Full casefile and STRMix reports for ███(P2 – SAIK)	Various	FSS.0001.0081.8820
6.15	Full casefile and STRMix reports for ███(P2 – SAIK)	Various	FSS.0001.0081.8994
6.16	Full casefile and STRMix reports for ███(P2 – SAIK)	Various	FSS.0001.0081.9077
6.17	Full casefile and STRMix reports for ███(P2 – SAIK)	Various	FSS.0001.0081.9237
6.18	Bundle of P2 - SAIK casefile Quant Batch Results for above casefiles 6.8 to 6.17	Various	COI.0294.0007.0001
Priority 2 (Murder)			
6.19	Full casefile and STRMix reports for ███(P2 – Murder)	Various	FSS.0001.0082.0001 + Reports (awaiting Orb upload)
6.20	Full casefile and STRMix reports for ███(P2 – Murder)	Various	FSS.0001.0082.0271
6.21	Full casefile and STRMix reports for ███(P2 – Murder)	Various	FSS.0001.0082.0386
6.22	Bundle of P2 - Murder casefile Quant Batch Results for above casefiles 6.19 to 6.21	Various	COI.0294.0007.0001
Priority 3			
6.23	Full casefile, STRmix reports and Quant Batch Results for ███(P3)	Various	FSS.0001.0082.0489 FSS.0001.0083.2081

			FSS.0001.0083.2103 + Reports (awaiting Orb upload)
Intel cases			
6.24	Full casefile and STRMix reports for [redacted](Intel)	Various	FSS.0001.0082.0512 + Reports (awaiting Orb upload)
6.25	Full casefile and STRMix reports for [redacted](Intel)	Various	FSS.0001.0082.0744
6.26	Full casefile and STRMix reports for [redacted](Intel)	Various	FSS.0001.0082.1015
6.27	Full casefile and STRMix reports for [redacted](Intel)	Various	FSS.0001.0082.1123
6.28	Full casefile and STRMix reports for [redacted](Intel)	Various	FSS.0001.0082.1354
6.29	Full casefile and STRMix reports for [redacted](Intel)	Various	FSS.0001.0082.1512
6.30	Full casefile and STRMix reports for [redacted](Intel)	Various	FSS.0001.0082.1592
6.31	Bundle of Intel casefile Quant Batch Results for above casefiles 6.24 to 6.30	Various	COI.0294.0003.0001
Dropped loci			
6.32	Full casefile, STRmix reports and Quant Batch results for [redacted](1 locus dropped)	Various	
6.33	Full casefile for [redacted](2 loci dropped)	Various	
Expert opinions			
7.1	K&B Report: Concerns about additional contributors, stutter, dropping loci and stratification: [133] – [139]	28.10.2022	EXP.0007.0001.0001_R
Correspondence			
8.1	Email from Emma Caunt to Commission of Inquiry regarding STRmix including attached Minor Change – PowerPlex21 (Casework Baseline on 3500xL using Data Collection version 4)	11.11.2022	

2019

2020

2021 **Appendix II: Curriculum Vitae**

2022

2023 **Education**
2024 2017-2018: Deep learning specialisation, Coursera
2025
2026 2016-2019: PhD (Discipline of statistics, College of Science and Engineering), Flinders
2027 University. Thesis title: Improving the statistical evaluation of forensic DNA evidence
2028
2029 2016: Machine Learning, Coursera
2030
2031 2015-2016: Certificate of Advanced Studies in "Statistics and the Evaluation of Forensic
2032 Evidence" offered through the Formation Continue UNIL-EPFL Lausanne - Suisse
2033
2034 2014: Lean Six Sigma – Yellow Belt (Advanced)
2035
2036 2008-2011 - Diploma in Biostatistics, Biostatistics Collaboration of Australia.
2037
2038 2001-2005: PhD (School of Biological Sciences), Flinders University and South Australian
2039 Museum. Thesis title: Genetics Using DNA markers for wildlife management and protection:
2040 a study of the population structure and systematics of the Australian carpet pythons utilising
2041 STRs, mitochondrial DNA sequence and allozymes.
2042
2043 2001: Honours Degree, Flinders University. Thesis title: Stable isotope ratio analysis of the
2044 human bone retrieved from St Mary's churchyard, and chemical analysis of the surrounding
2045 soil.
2046
2047 1998-2000: Undergraduate Degree, Flinders University - Forensic and Analytical Chemistry.
2048
2049 1997: Matriculation, Prince Alfred College.
2050
2051 **Research history**
2052 Statistics:
2053 Peer-reviewed publications: 123
2054 i10 index: 72
2055 h-index: 34
2056 (as per google scholar):
2057 https://scholar.google.com.au/citations?hl=en&pli=1&user=F38i2VMAAAAJ
2058 Article review : publication ratio of 1:1
2059
2060 **Peer reviewed publications:**
2061
2062 2009:
2063 1. Duncan A. Taylor, Julianne M. Henry, Simon J. Walsh. South Australian Aboriginal
2064 sub-population data for the nine AMPFlSTR® Profiler Plus™ short tandem repeat
2065 (STR) loci. Forensic Science International: Genetics, 2009 2(2):e27-e30.
2066
2067 2012:

2068 2. Taylor DA, Henry JM Haplotype data for 16 Y-Chromosome STR loci in Aboriginal
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2072 3. Taylor DA, Nagle N, Ballantyne KN, van Oorschot RA, Wilcox S, Henry J,
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2078 4. Ottens R, Taylor D, Abarno D, Linacre A. Successful direct amplification of nuclear
2079 markers from a single hair follicle. Forensic Science Medicine and Pathology. 2013;
2080 9(2):238-43
2081
2082 5. Jo-Anne Bright, Duncan Taylor, James M. Curran and John S. Buckleton.
2083 Degradation of forensic DNA profiles. Australian Journal of Forensic Sciences. 2013;
2084 45(4):445-449
2085
2086 6. Jo-Anne Bright, Duncan Taylor, James M. Curran, and John S. Buckleton .
2087 Developing allelic and stutter peak height models for a continuous method of DNA
2088 interpretation Forensic Science International: Genetics. 2013; 7(2), 296-304.
2089
2090 7. Duncan Taylor, Jo-Anne Bright and John S. Buckleton. The interpretation of single
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2092 516-528.
2093
2094 2014:
2095 8. Buckleton, J., H. Kelly, J.-A. Bright, D. Taylor, T. Tvedebrink and J. M. Curran.
2096 Utilising allelic dropout probabilities estimated by logistic regression in casework.
2097 Forensic Science International. Genetics, 2014; 9: 9-11
2098
2099 9. Tegan E. Collins, Renée Ottens, Kaye N. Ballantyne, Nano Nagle, Julianne Henry,
2100 Duncan Taylor, Michael Gardner, Alison J. Fitch, Amanda Goodman, Roland A.H.
2101 van Oorschot, R. John Mitchell and Adrian Linacre Characterisation of novel and rare
2102 Y-chromosome short tandem repeat alleles in self-declared South Australian
2103 Aboriginal database, International Journal of Legal Medicine. 2014; 128(1): 27-31.
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2105 10. Jo-Anne Bright, Duncan Taylor, James Curran, John Buckleton. Searching mixed
2106 DNA profiles directly against profile databases. Forensic Science International:
2107 Genetics. 2014; 9: 102–110
2108
2109 11. Duncan Taylor. Using continuous DNA interpretation methods to revisit likelihood
2110 ratio behaviour. Forensic Science International: Genetics. 2014; 11: 144-153
2111
2112 12. D Taylor, JA Bright, J Buckleton, J Curran. An illustration of the effect of various
2113 sources of uncertainty on DNA likelihood ratio calculations. Forensic Science
2114 International: Genetics. 2014; 11: 56-63
2115

13. J Buckleton, JA Bright, D Taylor, I Evett, T Hicks, G Jackson, JM Curran. Helping formulate propositions in forensic DNA analysis. Science & Justice 2014; 54(4): 258-261

14. Jo-Anne Bright, Cathie Allen, Shelley Fountain, Kerryn Gray, Denise Grover, Sharon Neville, Adam L Poy, Duncan Taylor, Gavin Turbett, Linzi Wilson-Wilde. Australian population data for the twenty Promega PowerPlex 21 short tandem repeat loci. Australian Journal of Forensic Sciences. 2014; 46 (4): 442-446

15. Ballantyne KN, Ralf A, Aboukhalid R, Achakzai NM, Anjos MJ, Ayub Q, Balažic J, Ballantyne J, Ballard DJ, Berger B, Bobillo C, Bouabdellah M, Burri H, Capal T, Caratti S, Cárdenas J, Cartault F, Carvalho EF, Carvalho M, Cheng B, Coble MD, Comas D, Corach D, D'Amato ME, Davison S, de Knijff P, De Ungria MC, Decorte R, Dobosz T, Dupuy BM, Elmrghni S, Gliwiński M, Gomes SC, Grol L, Haas C, Hanson E, Henke J, Henke L, Herrera-Rodríguez F, Hill CR, Holmlund G, Honda K, Immel UD, Inokuchi S, Jobling MA, Kaddura M, Kim JS, Kim SH, Kim W, King TE, Klausriegler E, Kling D, Kovačević L, Kovatsi L, Krajewski P, Kravchenko S, Larmuseau MH, Lee EY, Lessig R, Livshits LA, Marjanović D, Minarik M, Mizuno N, Moreira H, Morling N, Mukherjee M, Munier P, Nagaraju J, Neuhuber F, Nie S, Nilasitsataporn P, Nishi T, Oh HH, Olofsson J, Onofri V, Palo JU, Pamjav H, Parson W, Petlach M, Phillips C, Ploski R, Prasad SP, Primorac D, Purnomo GA, Purps J, Rangel-Villalobos H, Rębała K, Rerkamnuaychoke B, Gonzalez DR, Robino C, Roewer L, Rosa A, Sajantila A, Sala A, Salvador JM, Sanz P, Schmitt C, Sharma AK, Silva DA, Shin KJ, Sijen T, Sirker M, Siváková D, Skaro V, Solano-Matamoros C, Souto L, Stenzl V, Sudoyo H, Syndercombe-Court D, Tagliabracci A, Taylor D, Tillmar A, Tsybovsky IS, Tyler-Smith C, van der Gaag KJ, Vanek D, Völgyi A, Ward D, Willemse P, Yap EP, Yong RY, Pajnič IZ, Kayser M. Towards male individualization with rapidly mutating Y-chromosomal STRs . Human Mutation. 2014; 35(8): 1021-1032

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23. JA Bright, IW Evett, D Taylor, JM Curran, J Buckleton. A series of recommended tests when validating probabilistic DNA profile interpretation software. (2015) Forensic Science International: Genetics 14, 125-131

24. Duncan Taylor, John Buckleton, Ian Evett. Testing likelihood ratios produced from complex DNA profiles. Forensic Science International: Genetics 16 (2015) 165–171

25. Renée Blackie, Duncan Taylor, Adrian Linacre. Successful direct amplification of nuclear markers from single dog hairs using DogFiler multiplex. Electrophoresis (2015) 36(17), 2082-2085

26. JEL Templeton, D Taylor, O Handt, P Skuza, A Linacre. Direct PCR Improves the Recovery of DNA from Various Substrates. Journal of forensic sciences (2015) 60(6), 1558-1562.

27. Simone Gittelson, Tim Kalafut, Steven Myers, Duncan Taylor, Tacha Hicks, Franco Taroni, Ian W Evett, Jo-Anne Bright, John Buckleton. A Practical Guide for the Formulation of Propositions in the Bayesian Approach to DNA Evidence Interpretation in an Adversarial Environment. Journal of Forensic Sciences. (2015) 61(1), 186-195

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2222 35. D Taylor, D Abarno, E Rowe, L Rask-Nielsen. Observations of DNA transfer within
2223 an operational Forensic Biology Laboratory. Forensic Science International: Genetics
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2234 38. D Taylor. The evaluation of exclusionary DNA results: A discussion of issues in R v
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2237 39. D Taylor. Is technology the death of expertise? Forensic Science International:
2238 Genetics (2016) 24, e1-e3
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2240 40. D Taylor. Probabilistically determining the cellular source of DNA derived from
2241 differential extractions in sexual assault scenarios. Forensic Science International:
2242 Genetics (2016) 24, 124-135
2243
2244 41. D Taylor, T Hicks, C Champod. Using sensitivity analyses in Bayesian Networks to
2245 highlight the impact of data paucity and direct future analyses: a contribution to the
2246 debate on measuring and reporting the precision of likelihood ratios. Science &
2247 Justice (2016) 56(5), 402-410
2248
2249 42. D Taylor, D Powers. Teaching artificial intelligence to read electropherograms.
2250 Forensic Science International: Genetics (2016) 25, 10-18
2251
2252 43. R Blackie, D Taylor, A Linacre. DNA profiles from clothing fibers using direct PCR.
2253 Forensic Science, Medicine, and Pathology (2016) 12 (3), 331-335
2254
2255 44. Geoffrey Stewart Morrison, David H Kaye, David J Balding, Duncan Taylor, Philip
2256 Dawid, Colin GG Aitken, Simone Gittelson, Grzegorz Zadora, Bernard Robertson,
2257 Sheila Willis, Susan Pope, Martin Neil, Kristy A Martire, Amanda Hepler, Richard D
2258 Gill, Allan Jamieson, Jacob de Zoete, R Brent Ostrum, Amke Caliebe. A comment on
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2636 **Published books:**
2637 Forensic DNA Evidence Interpretation Second Edition. Editors John Buckleton, Jo-Anne
2638 Bright, Duncan Taylor. (2016) CRC Press. ISBN 9781482258899
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2640 Forensic Biology Evidence Evaluation: Utilizing Activity Level Propositions and Likelihood
2641 Ratios. Duncan Taylor and Bas Kokshoorn. (2022) CRC Press. ISBN 9781032225814
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2643 **Contribution to books:**
2644 Parentage analysis and other applications of human identity testing (Chapter 82). Duncan
2645 Taylor, In I. Freckelton & H. Selby (Eds), *Expert evidence*. North Ryde, Australia; Thomson
2646 Lawbook Co.
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2648 ‘Complex Mixtures’ (Chapter 19). Duncan Taylor, John Buckleton, Jo-Anne Bright, In
2649 Encyclopaedia of Forensic Science, Third Edition: Section 10023... Senior Editor Max
2650 Houck. Academic Press, Elsevier. ISBN 978-0-12-382165-2
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2652 **Grants**
2653 2019 – 2023 – $27 000 – South Australian Police - Humphries M, Roughan M, Taylor D.
2654 “Recommender systems for forensic evidence triage”
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2656 2021 – $15 000 – Australian Academy of Forensic Science Research Fellowship Award.
2657 Duncan Taylor, Adrian Linacre, Russel Brinkworth. “Using machine learning to improve
2658 PCR”

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2661 **Patents**
2662 63/037,475 - 10 June 2020 - provisional patent - Methods and systems for identifying nucleic
2663 acids
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2665 **Contributions to Forensic Science**
2666 Technical developer of software STRmix™, used for DNA evidence interpretation in
2667 Australia, New Zealand and parts of USA. STRmix™ training courses provided in:
2668 - Melbourne, Australia
2669 - Auckland, New Zealand
2670 - Manchester, England
2671 - Washington, USA
2672 - Las Vegas, USA
2673 - Belfast, Northern Ireland
2674 - Dublin, Ireland
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2676 2017 - Pioneered Activity level evaluation of DNA evidence in Australia. Member of the
2677 Australia New Zealand working group to produce the ANZPAA-NIFS "An introductory
2678 Guide to Evaluative Reporting".
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2680 2019 - Technical co-developer of common DNA donor analysis in software DBLR™.
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2682 2020 - Technical developer of Artificial Neural Network functionalist in FaSTR™, a DNA
2683 profile reading software.
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2685 2020 - Invited to be involved in the development of expert assessment and registration for
2686 activity level evaluation by Nederlands Register Grechtelijk Deskundigen (Netherlands
2687 Register for Court Experts).
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2689 2021 – Membership of Standards Australia Committees and Joint Standards
2690 Australia/Standards New Zealand Committees for work on ISO 21043
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2692 **Work presented at conferences**
2693 Presented at the Australia and New Zealand Forensic Science Society symposium in
2694 Melbourne 2008:
2695 - Y-chromosome short tandem repeat (Y-STR) diversity in South Australian Aboriginal
2696 and Caucasian populations – Duncan A. **Taylor**, Robert J. Mitchell, Roland van
2697 Oorschot, Nano Nagle, Julianne M. Henry.
2698
2699 Presented at the International Society of Forensic Genetics symposium in Buenos Aires 2009:

2700 • Knowing your DNA database: issues with determining ancestral Y haplotypes in a Y-
2701 Filer database - Duncan A. **Taylor**, Robert J. Mitchell, Roland van Oorschot, Nano
2702 Nagle, Julianne M. Henry.
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2704 Presented at the Australia and New Zealand Forensic Science Society symposium in Sydney
2705 2010:
2706 • Modelling stochastic effects from empirical data to develop interpretational tools and
2707 guidelines – Duncan Taylor, Christopher Hefford – Won the award for best biology
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2709 • Introducing rules to a staff DNA profile validation process to improve the detection
2710 rate of contamination events – Duncan Taylor, Christopher Hefford.
2711 • Resolving the extent of admixture in an Australian Aboriginal Y-STR database -
2712 Duncan A. Taylor, Robert J. Mitchell, Roland van Oorschot, Nano Nagle, Julianne M.
2713 Henry.
2714 • Novel and Rare Y-Chromosome Short Tandem Repeats At DYS456 And DYS635 In
2715 Australian Aborigines – Tegan E Collins, Michael Gardner, Julianne M Henry,
2716 Duncan A Taylor, Alison J Fitch, Amanda Goodman.
2717 • The Derivation of a Paternity Index where the Mother and Father are Biological
2718 Brother and Sister - Damian Abarno, Duncan Taylor.
2719
2720 Presented at the International Society of Forensic Genetics symposium in Vienna 2011:
2721 • Novel and rare Y-chromosome short tandem repeats in Australian Aborigines
2722
2723 Asian Forensic Sciences Network in 2011:
2724 • Population frequency study for Y-STR loci for Brunei Darussalam Malay and
2725 Chinese
2726
2727 Presented at the Australia and New Zealand Forensic Science Society symposium in Hobart
2728 2012:
2729 • STRmix: sophisticated DNA profile analysis for forensic scientists (Keynote address)
2730 • Evaluation and statistical analysis of data pertaining to the persistence of seminal
2731 components after sexual assault
2732 • A brother comes to the rescue when a mother is not enough
2733 • Quick and easy semi-automated DNA reporting using Microsoft Office
2734 • DNA profiling of soils using next generation sequencing
2735 • The impact of Aboriginal database admixture on weight of evidence calculations for
2736 uniparental and autosomal markers
2737
2738 Presented at International Society of Forensic Genetics symposium in Melbourne 2013:
2739 • How certain are we about our statistics? - D. Taylor, J. Bright, J. Buckleton, J. Curran
2740 • Going totally Bayesian: Lab experiences when moving to a continuous DNA
2741 interpretation model – D. Taylor
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2743 Presented at the Australia and New Zealand Forensic Science Society symposium in Adelaide
2744 2014:
2745 • Removing the need to specify a number of contributors for DNA interpretation - D.
2746 Taylor, J. Bright, J. Buckleton
2747 • Using continuous DNA interpretation systems to revisit likelihood ratio behaviour -
2748 D. Taylor
2749 • Contamination or coincidence: Determining the appropriate likelihood ratio threshold
2750 for contamination detection using STRmix™ - J. Henry, D. Abarno, D. Taylor
2751 • The effectiveness of STRmix™ software to detect contamination of forensic samples
2752 by laboratory personnel - J. Henry, D. Abarno, D. Taylor
2753 • Direct PCR improves the recovery of DNA from various substrates - Jennifer E.L
2754 Templeton, Renée Ottens, Oliva Handt, Duncan Taylor, Adrian Linacre
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2756 Presented at the 5th International Conference on Evidence Law and Forensic Science in
2757 Adelaide 2015:
2758 • Using Bayesian Networks to put DNA findings in a greater case context
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2760 Presented at International Society of Forensic Genetics symposium in Krakow, Poland 2015:
2761 • Using Hd true tests to inform on model performance and address adventitious
2762 matching – Duncan Taylor, John Buckleton, Ian Evett
2763 • The interpretation of y chromosome mixtures - Moretti T. R., Myers S. P., Taylor D.,
2764 Bright J. A., Buckleton J. S
2765 • Interpreting mixed DNA profiles considering a range in the assigned number of
2766 contributors - Cooper S. J., McGovern C. E., Abarno D., Bright J. A., Taylor D.,
2767 Buckleton J. S.
2768 • Vectors of DNA transfer in a laboratory environment – Taylor D., Abarno D., Rowe
2769 E., Rask-Nielsen L.
2770 • DNA profiles from fingermarks - Templeton J. E. L., Blackie R., Taylor D., Handt O.,
2771 Linacre A.
2772
2773 Presented at the Australia and New Zealand Forensic Science Society symposium in
2774 Auckland 2016:
2775 • Using sensitivity analyses on Bayesian networks to assess sampling uncertainty and
2776 direct further research – Duncan Taylor, Tacha Hicks, Christophe Champod (oral)
2777 • Direct PCR: successes and limitations. Templeton J, Blackie R, Rowe E, Taylor D,
2778 Handt O, Linacre A (poster)
2779 • Is standardisation of DNA profile interpretation achievable? Stuart Cooper. Laura
2780 Russell, Jo-Anne Bright, Catherine McGovern, Duncan Taylor, John Buckleton (oral)
2781 • Direct PCR of Hair Samples – A success story? Oliva Handt, Mel Sifis, Duncan
2782 Taylor (oral)
2783
2784 Presented at the Australia and New Zealand Forensic Science Society symposium in Perth
2785 2018:

2786 • How much DNA accumulates on untouched items in the home? - Taylor D, Moroney
2787 M, Linacre A (poster)
2788 • Evaluating mixed Y-STR profiles - Taylor D, Curran J, Buckleton J (oral)
2789 • Introducing activity level reporting to casework - Taylor D (oral)
2790 • Using artificial neural networks to read electropherograms - Taylor D, Harrison A,
2791 Kitselaar M, Powers D (oral)
2792 • SNP panel DNA profiles from touched sample. Adrian Linacre, Duncan Taylor (oral
2793 presented by Linacre)
2794 • Validation of the Qiagen Argus x-12 QS X-STR PCR kit for use in familial search
2795 candidate exclusionary work. Abarno DV, Pearce M, Rowe E, Scandrett L, Taylor
2796 DA, Linacre A (poster presented by Abarno)
2797 • Musings on the first Australian conviction resulting from a familial search - Abarno
2798 DV, Summers C, Sobieraj TC, Taylor DA (oral presented by Abarno)
2799 • DNA profiles from touched samples - Martin B, Blackie R, Kirkbride P, Taylor D,
2800 Linacre A (oral presented by Linacre)
2801 • Assessment of changes to DNA database interrogation at forensic science SA -
2802 Collins T, Dubrich J, Stankovic D, Williams T, Windram R, Taylor DA, Abarno DV
2803 (oral presented by Collins)
2804 • An introductory guide to evaluative reporting - Catoggio D, Bunford J, Taylor D,
2805 Wevers G, Ballantyne K, Morgan R (poster presented by Morgan)
2806
2807 Presented at International Society of Forensic Genetics symposium in Prague, Czezh
2808 Republic 2019:
2809 • Applying autosomal STR probabilistic genotyping models to SNP data using
2810 hierarchical Bayesian modelling – Duncan Taylor, Julianne Henry, Catherine
2811 Hopkins, James Curran (poster)
2812 • Modelling DNA transfers in complex scenarios - Duncan Taylor, Tacha Hicks-
2813 Champod, Christophe Champod (oral)
2814 • From reference to mixture to mixture to mixture and beyond - Maarten Kruijver,
2815 Duncan Taylor (oral presented by Kruijver)
2816 • Application of the GNano 31-plex ancestry prediction assay in an Australian context -
2817 Catherine Hopkins, Duncan Taylor, Kelly Hill and Julianne Henry (poster presented
2818 by Henry)
2819 • Verification of the GNano 31-plex ancestry prediction assay for forensic casework -
2820 Julianne Henry, Catherine Hopkins, Kelly Hill and Duncan Taylor (poster presented
2821 by Henry)
2822
2823 Presented at the American Academy of Forensic Sciences in USA, 2021:
2824 • A two-trace problem in probabilistic genotyping: should the evidence be combined or
2825 not - Maarten Kruijver, Duncan Taylor (oral presented by Kruijver)
2826
2827 Presented at the Australia and New Zealand Forensic Science Society symposium in Brisbane
2828 2022:

2829 • Do we need to read profiles anymore? Combining neural network profile processing
2830 and probabilistic genotyping - Duncan Taylor, John Buckleton (oral presented by
2831 Duncan Taylor)
2832 • Examples of new investigative leads generated from large-scale, inter-case crime
2833 scene profile comparisons – Duncan Taylor, Damien Abarno (oral presented by
2834 Duncan Taylor)
2835 • Covert sampling, familial searching and extradition. Solving the murder of Suzanne
2836 Poll – Anthony van der Stelt, Duncan Taylor (oral presented jointly by Anthony van
2837 der Stelt and Duncan Taylor)
2838 • AI and machine learning for DNA evidence: addressing the practical legal issues –
2839 Kerry Ann Andresen, Caitlin Williams, Linzi Wilson-Wilde, Duncan Taylor (oral
2840 presented jointly by Kerry Ann Andresen and Caitlin Williams)
2841 • Real data making big data: using Bayesian networks to build a digital twin for case
2842 exhibit submission – Louise Campbell, Melissa Humphries, Duncan Taylor (poster
2843 presented by Louise Campbell)
2844 Level of incidental DNA transfer from bedding – Denise Ward, Oliva Handt, Duncan Taylor
2845 (poster presented by Denise Ward)
2846
2847 Presented at International Society of Forensic Genetics symposium in Washington DC, USA
2848 2022:
2849 • An experimental extension to the discrete Laplace method for Y-STR haplotype
2850 frequency estimation - Maarten Kruijver, Duncan Taylor, John Buckleton (oral
2851 presented by Maarten Kruijver)
2852 • DNA transfer between exhibits, evidence bags & workspaces - Claire Mercer, Adrian
2853 Linacre, Duncan Taylor, Dr Julianne Henry (oral presented by Claire Mercer)
2854
2855 **Lecturing Duties**
2856 Lectures given in:
2857 2011 – present: Flinders University - BIOL3792 (Forensic Biology)
2858 2017 – 2019: Murdoch University - Forensic Science Professional Practise
2859
2860 **Student Supervision**
2861 Current:
2862 – Claire Mercer – PhD – trace DNA transfer during DNA exhibit transport and analysis
2863 – Louise Campbell – PhD – using recommender systems for forensic exhibit triage
2864 – Isla Madden – Honours – Predicting probative levels of touch DNA on forensic DNA
2865 tapelifts using Diamond™ Nucleic Acid Dye
2866 – Druvi Patel – Honours – Collection of DNA from Airspaces – a look at contactless DNA
2867 transfer
2868 – Caitlyn McDonald – Honours – Applying machine learning to PCR conditions to improve
2869 DNA profiling
2870
2871 2022 – Honours – Ayesha Khalid Ahmed Khan - Improving PCR efficiency by using API

2872
2873 2022 – Kerry Andresen and Cailin Williams – Adelaide Uni Law School project - The
2874 application of artificial intelligence and machine learning to DNA profiling: admissibility
2875 under the rules of evidence issues paper
2876 2022 – Bridget Alyward – 3rd year Adelaide Uni Law School project – Admissibility issues
2877 around the use of activity level evaluations in South Australian Courts
2878
2879 2021 – Lingchen Wang – Honour – adapting standard PCR thermocyclers to provide real-
2880 time feedback to a machine learning system
2881 2018 – 2021 Belinda Matulick (nee Martin) – PhD – trace DNA analysis on improvised
2882 explosive devices
2883
2884 2020 – Lucas Puliatti – Honours – Investigating the level of DNA transfer from a brief visit
2885 2020 – Sasha Carson – Honours – Investigating the potential for cross-contamination at a
2886 crime scene
2887 2020 – Cara-Mae Shipley – Honours – Validation of the HIrisPlex SNP kit
2888
2889 2019 – 2020 – Partho Protim Gosh – Masters – Using ANN to determine number of
2890 contributors
2891
2892 2019 – Phola Ramos – Honours – DNA transfer to clothing during simulated sexual assaults
2893
2894 2017 – 2019 - Suni Edson – PhD student from Armed Forces DNA Identification Laboratory
2895 (AFDIL) dealing with the processing and profiling of bone samples.
2896
2897 2018 – Joshua Sweaney – honours – application of artificial neural networks to forensic
2898 biology DNA detection and analysis
2899 2018 – Catherine Hopkins – honours – Creation of a GNano SNP database for Aboriginal
2900 Australians
2901 2018 – Kirsten Heuer – honours – Development of an in-house Y-SNP ancestry assay for the
2902 enhancement of the FSSA Aboriginal Y-STR DNA database
2903
2904 2017 – Michael Kistelaar – Work placement student from Flinders University - Using deep
2905 learning neural networks for interpretation of complex electrophoretic data
2906 2017 – Belinda Matulick – Honours – Developing a SNaPshot panel for the identification of
2907 ancestry Aboriginal Y-chromosomes
2908 2017 – Monique Moroney – Honours – Analysis of trace DNA transfer and persistence to fill
2909 activity level reporting gaps
2910 2017 – Jess Champion – Honours – DNA transfer and persistence
2911
2912 2016 – Ashleigh Harrison – Summer student from Flinders University - Using deep learning
2913 neural networks for interpretation of electrophoretic data
2914 2016 – Melissa Drogemuller – Summer student from Flinders University - Using deep
2915 learning neural networks for forensic handwriting comparison

2016 – Renée Blackie (nee Ottens) – PhD student from Flinders University - Direct PCR as a means to generate DNA profiles from trace material such as hair and fibres
2013 - 2016 – Jennifer Templeton – PhD student from Flinders University – Studies on Low Template DNA for Forensic Human Identification

2011 – Renée Ottens - Honours Student from Flinders University - Novel Y-Chromosome Short Tandem Repeat Sequences.

2009 – Tegan Collins - Honours Student from Flinders University - Novel and Rare Y-Chromosome Short Tandem Repeats at DYS456 and DYS635 in Australian Aborigines.

2008 – Ankita Chitalia - Masters Student from Flinders University - Forensic DNA profiling technology: Driving trace DNA profiling to its technical limit; particularly with post-amplification procedures.

Invited speaking events & workshop presentations
2022 – presented series of six workshops on activity level evaluation as part of an ANZPAA-NIFS training workshop

2021 – 13th Asian Forensic Science Network Annual Meeting and Symposium – Discussion panel on "Reporting probabilistic genotyping in court; lessons from the stand"

2021 – 7th Annual STRmix workshop – 'Y-STRs in STRmix (a.k.a. STRmixY)'

2021 – 3rd Annual Northeast Forensic Laboratory Probabilistic Genotyping Users Group Meeting - 'Factoring uncertainty into evaluations—The HPD interval in STRmix'

2021 - 6th annual Questioning Forensics conference hosted by the DNA Unit of the Legal Aid Society in New York City DNA Unit speaking on Bayesian Networks and activity level reporting

2019 - 2020 – Lectures given in online course 'DNA Interpretation given activity level propositions' run by Tacha Hicks from Lausanne University

2019 – Ontario Centre of Forensic Sciences workshop on evaluative reporting – "Australian practitioner perspective on evaluative reporting"

2019 – Web series: Probabilistic Genotyping of Forensic Evidentiary Typing Results – "What can 'big data' tell us about performance? Multi-lab studies, PCAST, sensitivity/specificity and ROC plots"

2019 – Australian Defence Lawyers Alliance Conference – "What do the DNA results really mean?"

2960 2018 – Australia and New Zealand Forensic Science Society symposium in Perth 2018
2961 workshop – "Activity Level Inference in Forensic Genetics"
2962
2963 2018 – Adelaide Festival of Ideas "My teacher said I'd need maths one day: Mathematical
2964 techniques you never knew were being used by Forensic Science SA to solve crime"
2965
2966 2018 – Gordon Research Conference: Forensic Analysis of Human DNA. Maine USA –
2967 "Probabilistic genotyping software"
2968
2969 2017 – Open State. Future Forensics: Crime scene to courtroom discussion panel. The Dome.
2970 Victoria Square Adelaide.
2971
2972 2017 - Griffith University Innocence Project and the Griffith Law Criminal Justice
2973 Symposium: Lifting the Veil on DNA Evidence: What Do the Statistics Really Mean?
2974
2975 2016 – Document Examination Specialist Advisory group, Melbourne 2017 - Logical
2976 Reporting for Forensic Handwriting and Signature Examinations
2977
2978 2015– International Society of Forensic Genetics symposium in Krakow, Poland 2015
2979 workshop – Interpretation of complex DNA profiles using a continuous model – an
2980 introduction to STRmix™
2981
2982 2014 – International Symposium on Advances in Legal Medicine – Fukuoka Japan – Invited
2983 to speak on the topic of Advances in DNA evidence interpretation
2984
2985 2013 – International Society of Forensic Genetics symposium in Melbourne 2013 – Lectured
2986 at the Basic and Advanced DNA interpretation workshops on population genetics, continuous
2987 DNA interpretation systems and implementation of continuous DNA interpretation systems
2988
2989 2013 – Australian Association of Crown Prosecutors in Adelaide – Invited to speak on
2990 Familial Searching and STRmix™
2991
2992 2013 – Magistrates Judicial Development – Invited to speak on STRmix™
2993
2994 2012 – Australian Association of Crown Prosecutors in Darwin – Invited to speak on
2995 Population genetics
2996
2997 **Positions held**
2998 2021 – present: Member of the Australasian working group for activity level reporting
2999
3000 2021 – present: Expert and Assessor for the NRGD (Netherlands Register for Judicial
3001 Experts) in the field of DNA Activity Level evaluations
3002

3003 2020 – 2021: Associate Investigator member of the Australian Research Council Centre of
3004 Excellence for Mathematical and Statistical Frontiers (ACEMS)
3005
3006 2019 – present: Editorial board member of Forensic Science International Genetics journal
3007
3008 2016 – 2019: Member of the International Society of Forensic Genetics working group on
3009 evidence interpretation
3010
3011 2016: member of the ANZPAA-NIFS working group on evaluative reporting.
3012
3013 2015 – 2016: Member of the US Scientific Working Group on DNA Analysis Methods
3014 (SWGDAM) group formed to evaluate Y-STR evidence.
3015
3016 2014 – present: Member of the Australian and New Zealand Statistical Scientific Working
3017 Group
3018
3019 2013 – present: Associate Professor at Flinders University in Biological Sciences
3020
3021 2012 – present: Ad hoc university student thesis examiner and journal article reviewer
3022
3023 2012 – 2014: Chair of the Australian and New Zealand Statistical Scientific Working Group
3024
3025 2010 – 2012: Vice Chair of the Australian and New Zealand Statistical Scientific Working
3026 Group – An international group of statistical experts tasked with developing and reviewing
3027 statistical methodologies to be used by Forensic Laboratories throughout Australia and New
3028 Zealand.
3029
3030 **Awards and recognitions**
3031 2021 – Awarded the Public Service Medal in the Australia Day Honours January 26th 2021
3032
3033 2020 & 2021 – Identified by in the compilation of the World's Top 2% Scientists by Stanford
3034 University (DOI: 10.13140/RG.2.2.18594.45767)
3035
3036 2020 – Identified as the field leader in forensic science in Australia in '*the Australian:*
3037 *RESEARCH*' 2020, edited by Tim Dodd.
3038
3039 2018 – New Zealand Prime Minister's Science Award – Awarded to the STRmix™ team
3040
3041 2018 – Flinders University Distinguished Alumni Award
3042
3043 2017 – SA Science Excellence Award winner in STEM Professional Category 2017
3044
3045 2015 – KiwiNet Research Commercialisation Award – finalist in PwC Commercial Deal
3046 category

3047
3048
3049 **Publication awards from the National Institute of Forensic Science (NIFS)**
3050 NIFS Best paper award 2021 – Best Paper – Capability Enhancement and Innovation –
3051 "Validation of a top-down DNA profile analysis for database searching using a fully
3052 continuous probabilistic genotyping model"
3053
3054 NIFS Best paper award 2021 – Highly commended in Best Technical Article or Note –
3055 "Probabilistic interpretation of the Amelogenin locus"
3056
3057 NIFS Best paper award 2020 – Best Paper – Capability Enhancement and Innovation –
3058 "Using Bayesian networks to track DNA movement through complex transfer scenarios"
3059
3060 NIFS Best paper award 2020 – Best Paper – Forensic Fundamentals – "Investigating the
3061 position and level of DNA transfer to undergarments during digital sexual assault"
3062
3063 NIFS Best paper award 2020 – Best New Publisher in a Refereed Journal – "Examining the
3064 additivity of peak heights in forensic DNA profiles"
3065
3066 NIFS Best paper award 2019 – Best Technical Article or Note – "Inter-sample contamination
3067 detection using mixture deconvolution comparison"
3068
3069 NIFS Best paper award 2019 – Highly commended in Best paper in a refereed journal –
3070 "Likelihood ratio development for mixed Y-STR profiles"
3071
3072 NIFS Best paper award 2019 – Highly commended in Best literature review – "Evaluation of
3073 forensic genetics findings given activity level propositions: a review"
3074
3075 NIFS Best paper award 2018 – Best paper in a refereed journal – "Internal validation of
3076 STRmix™ – A multi laboratory response to PCAST"
3077
3078 NIFS Best paper award 2018 – Highly commended in Best paper in a refereed journal – "A
3079 template for constructing Bayesian networks in forensic biology cases when considering
3080 activity level propositions."
3081
3082 NIFS Best paper award 2018 – Highly commended in Best case study – "Likelihood ratio
3083 formulae for disputed parentage when the product of conception is trisomic"
3084
3085 NIFS Best paper award 2017 – Best paper in a refereed journal – "Teaching artificial
3086 intelligence to read electropherograms"
3087
3088 NIFS Best paper award 2017 – Best Technical Article or Note – "Observations of DNA
3089 transfer within an operational Forensic Biology Laboratory"
3090

NIFS Best paper award 2015 – Best paper in a refereed journal – “Testing likelihood ratios produced from complex DNA profiles”

NIFS Best paper award 2015 – Highly commended in Best paper in a refereed journal – “Toward male individualization with rapidly mutating Y-chromosomal short tandem repeats”

NIFS Best paper award 2014 – Best paper in a refereed journal – “The interpretation of single source and mixed DNA profiles”

NIFS Best paper award 2014 – Highly commended in Best Technical Article or Note – “Searching mixed DNA profiles directly against profile databases”

Employment

I have been employed at Forensic Science SA since 2005 and currently hold the position of Chief Scientist in Forensic statistics within the biology group. I have appeared in court to present expert evidence on approximately 100 occasions in the Magistrates, District and Supreme Courts in states around Australia. These include appearance as a prosecution witness and a defence witness.

I work on various criminal matters including sexual assaults, homicides, cold cases and coronial investigation, involving both Autosomal and Y-Chromosome STR data, and activity level evaluations. I have conducted familial searches for several matters and provided informational sessions for stakeholders. I provide activity level reports for prosecution and defence council around Australia and have provided reports for international innocence project matters. I have also carried out calculations for complex kinship scenarios. I have carried out DNA database analysis for various organisations and have analysed population datasets of DNA allele frequencies, generated by the forensic laboratories across Australia.

Within Forensic Science SA I developed methodology, validated, wrote standard operating procedures and implemented the following:

- Probabilistic Genotyping using STRmix™
- Standardised and semi-automated reporting of DNA results
- Familial searching
- Searching of mixed DNA profiles against the searchable DNA database
- Activity level evaluation and reporting
- Complex kinship calculation
- Mixture to mixture analyses and reporting