Internal Validation of STRmix™ v2.6 (QIAGEN Investigator® 24plex QS with 3500xl)

Las Vegas Metropolitan Police Department

STRmix™ Internal Validation

This document describes the internal validation of STRmix™ version 2.6 at the Las Vegas Metropolitan Police Department Crime Laboratory (LVMPD) using 28 cycle QIAGEN Investigator® 24plex QS data analysed using 3500*xl* CE instrumentation.

Internal validation describes the activities the LVMPD laboratory has undertaken before the implementation of this updated version of STRmix™ into routine casework. Please refer to other the laboratory documents:

- 'Internal Validation of STRmix™ Las Vegas Metropolitan Police Department (v2.3 Identifiler™ Plus)' for a full validation of the software using a different amplification kit
- 'Internal validation of STRmix™ v2.4, Las Vegas Metropolitan Police Department Biology/DNA Detail (QIAGEN Investigator® 24plex QS)' for a similar validation in an earlier version of the software
- 'Internal Validation of STRmix™ v2.6.2 Las Vegas Metropolitan Police Department Biology/DNA Detail (ABI AmpFLSTR™ Identifiler™ Plus, 3130)' for a validation of Identifiler Plus data run on 3130xl CE instrumentation
- 'Internal Validation of STRmix™ v2.6 and Performance Check of STRmix™ v2.6.2 Las Vegas Metropolitan Police Department Biology/DNA Detail (QIAGEN Investigator® 24plex QS, 3130)' for a validation of data run on 3130xl CE instrumentation and the use of the multi-kit function for the interpretation of replicates from differing amplification chemistries/instrument platforms

Those documents follow the internal validation sections of the SWGDAM Guidelines for the Validation of Probabilistic Genotyping Systems [1]. This included the examination of known and non-probative evidence samples, investigations into reproducibility and precision, sensitivity and stochastic studies, and mixture studies.

As the LVMPD laboratory intends to upgrade to the use of 3500 CE instruments while maintaining STRmix™ v2.6 and the use of the QIAGEN Investigator® 24plex QS kit, a detailed but restricted internal validation has been undertaken and is reported here. Data analysis has been undertaken with the assistance of the STRmix™ training and support team, at ESR, Auckland, New Zealand. The present validation study focuses on analyzing mixtures of varying template and mixture proportion covering a range of contributors (including relatives), rates of degradation, and complex mixtures (section D). The sections where specific SWGDAM guidelines are discussed within this document are cross referenced in Appendix 2.

Note: The work undertaken and described below was carried out using STRmix™ version 2.6.3.

STRmix™ has previously been subjected to developmental validation. This involved, in part, the complete 'by hand' confirmation of the calculations behind the software. The results of the developmental validation are included in the STRmix™ User's Manual. In addition, a summary of the developmental validation is discussed in Bright et al. and Taylor et al. [2, 3]. A list of all papers describing the theory behind different aspects of STRmix™ is provided in Appendix 1 of this document.

The results of all experiments related to the internal validation of STRmix™ at the LVMPD laboratory are retained within the laboratory's quality system.

STRmix™ Parameters

The parameters described in the document 'Estimation of STRmix™ v2.6 Parameters (QIAGEN™ Investigator® 24plex QS with 3500xl)' were used for all internal validation checks of STRmix™ v2.6.3 presented in this report. All other run parameters have been optimized by the STRmix™ developers.

Section D: Sensitivity and specificity and mixtures

This section covers the following standards:

4.1.2. Hypothesis testing with contributors and non-contributors

4.1.6. Mixed specimens

4.1.6.1. Various contributor ratios (e.g., 1:1 through 1:20, 2:2:1, 4:2:1, 3:1:1, etc.)

4.1.6.2. Various total DNA template quantities

4.1.6.3. Various numbers of contributors. The number of contributors evaluated should be based on the laboratory's intended use of the software. A range of contributor numbers should be evaluated in order to define the limitations of the software.

4.1.6.5. Sharing of alleles among contributors

4.1.7. Partial profiles, to include the following:

4.1.7.1. Allele and locus drop-out

4.1.13. Sensitivity, specificity and precision, as described for Developmental Validation

As per previous internal validations, demonstration of sensitivity and specificity for a range of LVMPD Investigator® 24plex QS mixtures was undertaken as per Taylor [7]. With respect to interpretation methods, sensitivity is defined as the ability of the software to reliably resolve the DNA profile of known contributors within a mixed DNA profile for a range of starting DNA templates. The log(*Likelihood ratio [LR]*) for known contributors (H_p true) should be high and should trend to 0 as less information is present within the profile. 'Information' includes the amount of DNA from the contributor of interest, use of conditioning profiles (for example, the complainant's profile on intimate samples), the use of replicate amplifications, and decreasing the number of contributors (i.e., reduced profile complexity). Specificity is defined as the ability of the software to reliably exclude known non-contributors (H_d true) from a DNA profile for a range of starting DNA templates. The log(*LR*) should trend upwards to 0 as less information is present within the profile.

Specificity and sensitivity were tested by calculating the *LR* for both known contributors and known non-contributors for a number of two-, three-, four-, and five-person profiles. The plots presented in [7] have been reproduced with LVMPD Investigator® 24plex QS data, with the exception that the *LR*s calculated were plotted against average peak height (APH) rather than input template. This is discussed further below.

Another area explored within this study was the apparent number of contributors (*N*) within a mixture. By this we mean the N assigned by an analyst based on the electropherogram obtained, rather than the knowledge of the experimental design. Often these are the same, however sometimes when a donor is present in such small amounts they may not appear present or there may be stochastic effects which influence the assessment of *N*.

A significant number of mixtures were generated by the laboratory. A total of 348 'normal mixtures' were generated within the LVMPD laboratory following casework procedures and made available for this study. Please refer to the MS Excel™ workbooks 'EXPECTED PROFILES' and 'NOC for STRmix Runs' for a complete list of these mixtures including targeted mixture ratios, template amounts, the identities of the known contributors used to prepare the mixtures and the assigned *N*.

This study consisted of two-, three-, four-, and five-person experimentally designed mixtures. LVMPD undertook an assessment of the apparent number of contributors (*Apparent N*) within the mixture based on the electropherogram. These assignments can be found within the 'NOC for STRmix Runs' Excel file and within phase one of this study deconvolutions were only progressed for those samples with an 'x' in the columns associated with each mixture. Some mixtures were assigned an *Apparent N* which was the same as the experimental design N (*Exp N*), while sometimes these differed. For some cases where there was ambiguity in the apparent number of contributors there were two or more 'x' present for that sample indicating that the sample should be deconvoluted under different assumptions of *N*, similarly to provisions made during LVMPD casework. As such, this meant there were more deconvolutions than the 348 mixtures.

All the mixtures that could be progressed (dependant on computing memory) were deconvoluted using the apparent *N* (of which some happen to be same as experimental design *N*). The five-person mixtures which had an *Apparent N* of 5 were unable to be run to completion within STRmix™ due to insufficient computer memory.

Below is a summary of number of samples analyzed in phase one by comparing the *Exp N* and the *Apparent N*.

Table 1: Summary of samples and N used within phase one of the study.

		Experimental design N			
		2	*3*	*4*	*5*
Apparent N	*1*	**7**	**2**	**0**	**1**
	2	**203**	**11**	**15**	**3**
	3	**3**	**54**	**46**	**5**
	4	**0**	**6**	**18**	**6**
	5	**0**	**0**	**0**	**0**

This gave a total of 380 mixture deconvolutions. This includes an additional 12 duplicate amplifications which were included within the two-person mixtures.

The mixture proportions ranged from roughly equal amounts of DNA to major and minor components. As per previous studies a summary of the mixtures examined is provided in **Table 2**.

These profiles represent a spread of profile quality, including the 'worst' types of profiles likely to be encountered by the LVMPD laboratory in casework. The profiles are of varying DNA quantity and mixture proportions. The contributors include homozygous and heterozygous genotypes and there is varying amounts of allele sharing across the different loci (standard 4.1.6.5). Given the template amounts, allele and/or locus dropout was expected to occur within the profiles at the lower DNA amounts (standard 4.1.7.1).

Each of the 348 mixtures was analyzed in GeneMapper® *ID-X* within the LVMPD laboratory using the casework ATs (Blue 45 RFU, Green 55 RFU, Yellow 65 RFU, Red 75 RFU and Purple 45 RFU). Labels were retained for apparent allelic peaks and peaks falling in positions that are being modelled as stutter variants by LVMPD within STRmix™ v2.6, which were present above this AT.

The 380 *Apparent N* mixtures were each interpreted in STRmix™ v2.6.3. Following deconvolution, each profile was compared with a database of 1,019 individuals and an *LR* was calculated for each database individual. As with the previous studies this database contained the profiles of the 19 donors used to prepare the mixture sets as well as the profiles of 1,000 non-contributors that were simulated using the NIST Caucasian allele frequencies. Therefore, each mixture was compared with *N* known donors (H_p true) and 1,019 minus *N* non-contributors (H_d true), where *N* is the experimentally designed number of contributors to the mixture.

When assigning a likelihood ratio, the following propositions were considered:

H_p: The DNA originated from the database individual and *N*-1 unknown individuals

H_d: The DNA originated from *N* unknown individuals

The *LR*s were assigned using the Database Search functionality of STRmix™ with a theta value of 0.01 (1%) along with a factor of *N*!/sub-sub source correction. Note, however, that the STRmix™ Database Search functionality currently does not take into account sampling uncertainty (i.e., an HPD *LR* was not calculated in this study). The NIST Caucasian allele frequencies were used in these calculations.

In total, this constituted in excess of 380,000 individual *LR* comparisons to 380 mixtures, covering a very broad range of complexity.

Plots of log(*LR*) versus the average peak height (APH, in RFU) for the *Apparent N* one-, two-, three-, and four-contributor mixtures are given in **Figure 1**. The APH was approximated for each contributor considering any unmasked/unshared alleles from the known donor within each mixture. Where APH was unable to be calculated due to insufficient unmasked/unshared alleles being present from a contributor (typically due to allelic dropout), an APH of approximately half the lowest AT was applied (hence 22 RFU). The lowest APH calculated for the known donors to a mixture was used as the APH for all non-contributors compared. Exclusions (*LR*=0) have been plotted arbitrarily as log(*LR*)= -40. The results of all comparisons are provided in **Figure 1**.

Table 2: Summary of experimental design for specificity and sensitivity tests.

Mixture	Mixture ratios (M_r)	Total input template (pg) amplified	Comment	No. of STRmix™ decons
Two-person (n=204 experimental design)				
MIX3	1:1(A), 2:1(B), 3:1(C), 4:1(D), 5:1(E), 6:1(F), 8:1(G)	2000 (F[1] & G[1] only) 1500, 1000[2], 500, 200, 50	Limited allele sharing	43 (37 plus replicates)
MIX4	4:1(D), 5:1(E), 6:1(F), 8:1(G)	2000 (F[1] & G[1] only), 1500, 1000[2], 500, 200, 50	Some allele sharing	28 (22 plus replicates)
MIX5	1:1(A), 2:1(B), 3:1(C), 4:1(D), 5:1(E), 6:1(F), 8:1(G)	1500, 1000, 500, 200, 50	Some allele sharing	35
MIX6[3]	1:1(A), 2:1(B), 3:1(C), 4:1(D), 5:1(E)	1500, 1000, 500, 200, 50	Familial sharing	25
MIX7	1:1(A), 1:2(B1), 2:1(B2), 1:3(C1), 3:1(C2)	1500, 1000, 500, 200, 50	Degraded contributor 1	25
MIX8	As per MIX7	As per MIX7, expect no 500pg for B1	Degraded contributor 2	24
MIX9	As per MIX7	As per MIX7, expect no 1000pg for A	Both degraded	24
Three-person (n=64 experimental design)				
MIX12	1:1:1(A), 6:2:1(B), 9:2:1(C), 12:2:1(D), 3:2:1(H), 10:5:1(I)	1500, 1000, 500, 200, 50	Some allele sharing	30
MIX14	1:1:1(A), 6:2:1(B), 9:2:1(C), 12:2:1(D)[4], 15:2:1(E), 3:2:1(H), 10:5:1(I)	1500, 1000, 500, 200, 50	Some allele sharing	34

[1] MIX_3F & MIX_3G, MIX_4F & MIX_4G 2000pg all amplified in duplicate
[2] MIX_3F & MIX_3G, MIX_4F & MIX_4G 1000pg all amplified in triplicate
[3] MIX6 was from two related individuals. Furthermore, two additional relatives of the known donors were present within the 1000+ non-contributors that were subsequently compared with the mixtures constructed
[4] MIX_14D 200pg not present

Mixture	Mixture ratios (M_r)	Total input template (pg) amplified	Comment	No. of STRmix™ decons
Four-person (n=65 experimental design)				
MIX13	1:1:1:1(A), 6:1:1:1(B), 9:1:1:1(C), 12:1:1:1(D), 15:1:1:1(E), 4:3:1:2(H), 10:5:1:2(I)	1500, 1000, 500, 200, 50	Some allele sharing	35
MIX15	6:1:1:1(B), 9:1:1:1(C), 12:1:1:1(D), 15:1:1:1(E), 4:3:2:1(H)[5], 10:5:2:1(I)	1500, 1000, 500, 200, 50	High allele sharing	30
Five-person (n=15 experimental design)				
MIX16[6]	8:1:1:1:1(A), 12:1:1:1:1(B), 16:1:1:1:1(C)	1500, 1000, 500, 200, 50	Some allele sharing	15

Note: Total number of input text file entries to work with was 348. However, 380 mixture deconvolutions were initially undertaken due to the Apparent N values assigned.

The plots in **Figure 1** can help inform the limits of STRmix™, particularly the lower limit where an H_p true hypothesis may result in an *LR* below 1 (termed a 'Type I error' by SWGDAM) or where adventitious matches/false positives may arise (an *LR* greater than 1 where H_d is true, termed a 'Type II error' by SWGDAM).

As with previous validation studies utilizing these mixture sets, with the exception of the two-person MIX6 set (familial mixtures) and to a lesser extent the three-person MIX14 set and four-person MIX15 set, it was possible to successfully differentiate between true contributors and non-contributors down to low template, or equivalent low APH. Below this there were a number of H_d true results around or above 1. In addition, even when interpreting profiles with some allele sharing (e.g. the MIX5 set), known- and non-contributors were able to be distinguished at lower template/APH levels. Even with mixtures of related individuals being compared to non-contributors who are relatives of the true donors it is possible to distinguish known- from non-donors at high template.

The major difference within this study to previous studies undertaken within the LVMPD laboratory was the use of *Apparent N*. There are a number of *LR*s for true contributors (H_p true *LR*) which are well below 1 or that are even exclusions (*LR* = 0). In every instance (with one exception which will be discussed below) this occurred when the *Apparent N* was assigned either 1 or 2 contributors less than the experimental design, for example an experimentally designed three-person mixture which appeared to the analyst to be a two-person. These findings are to be expected. If the number of donors is reduced so are the number of allowable genotypes. In many instances the intended donor may well have dropped out due to the low input template, hence an *LR* supporting the defence hypothesis or an outright exclusion may actually be the 'correct' answer.

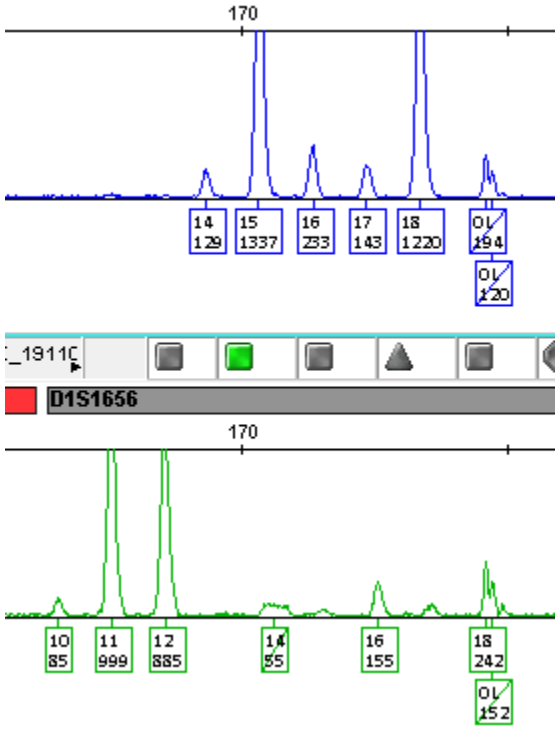
The single exception to the above relates to sample INV24-VAL23_IDEAL_1ng.2_(MIX_3F), where the *LR* to true donor 'GJ' gave an exclusionary *LR* where the *Apparent N* was the same as experimental design (two-person). This was an 'Ideal' template 1ng sample (6:1 mixture). This happens to be one of three/triplicate amps of the same sample.

To investigate this further an '*LR* from Previous' was undertaken using the deconvolution and a reference profile for this donor. This exclusion can be sourced to the D1S1656 locus alone. At the other loci the true donor is not excluded. The true donor is a 16,17 and should be in the minor position of this 6:1 proportion mixture. The input file contains a low 16 and a low 18 peak. The latter (at 242 RFU) is above the drop-in cap in place for LVMPD (225 RFU). STRmix is unable to explain this peak as anything other than allelic and hence all allowable genotype combinations contain at

[5] As per [4], sample INV24-VAL94_High_1.8ng.2_(MIX_15H) contained no data in the GeneMapper® text file
[6] All 5-person failed to run to completion on a high performance PC (256Gb RAM).

least one 18 peak, hence the true donor is excluded. A subsequent review of the electropherogram reveals that the 18-allele may be artifactual based on its morphology. A similarly shaped artifact is present at the D3S1358 locus in the blue channel, however, was edited due to falling off-ladder and therefore being less ambiguous.

It is important to note that should a similar situation occur during casework analysis, the *LR* of 0 at the single locus would act as a primary diagnostic for the review of the data. The artifact peak would be removed and STRmix re-run.



The other two amps of the same sample have 16 and 17 minor peaks and give high inclusionary *LR*s to same known donor.

The highest likelihood ratio for a known non-contributor (H_d true *LR*) in all the mixture sets (excluding the comparison of related H_d true individuals) was approximately 10^3. As previous studies have demonstrated, adventitious matches may occur where, by chance, an individual possesses alleles that are represented in the mixture, and may be exacerbated if dropout has been proposed.

As described in the validation documentation and previous validation studies, the MIX6 (two-person) set was constructed to represent the "worst case scenario", with the mixtures originating from two closely-related individuals. In addition, MIX14 (three-person) was constructed with individuals with similar genotypes and furthermore, MIX15 (four-person) contains two related individuals at low levels. This is further complicated by close relatives of the true donors being within the non-donors being compared. These samples drive a few H_d true *LR*s above 1, ranging in magnitude from 10^3 to 10^{13}. These types of mixtures have been discussed in detail in previous studies, but generally speaking, the affected samples were mainly LOW (500 pg total input) or VLOW (250 pg) mixtures. Furthermore, the samples typically had mixture ratios of 1:1, 2:1, or 3:1, that is, the mixtures were predominantly difficult to fully resolve. Given the low input amounts and limited differentiation in contributor amounts (i.e., close to 1:1 mixtures), STRmix™ has proposed numerous genotype combinations. Mixtures comprising DNA of related individuals and comparison of such mixtures to other related individuals is a known limitation of any DNA interpretation system. In this scenario, a close relative of the true contributor could be included purely by the spread of genotype combinations and the fact they are likely to possess some of the same alleles as the true donor(s).

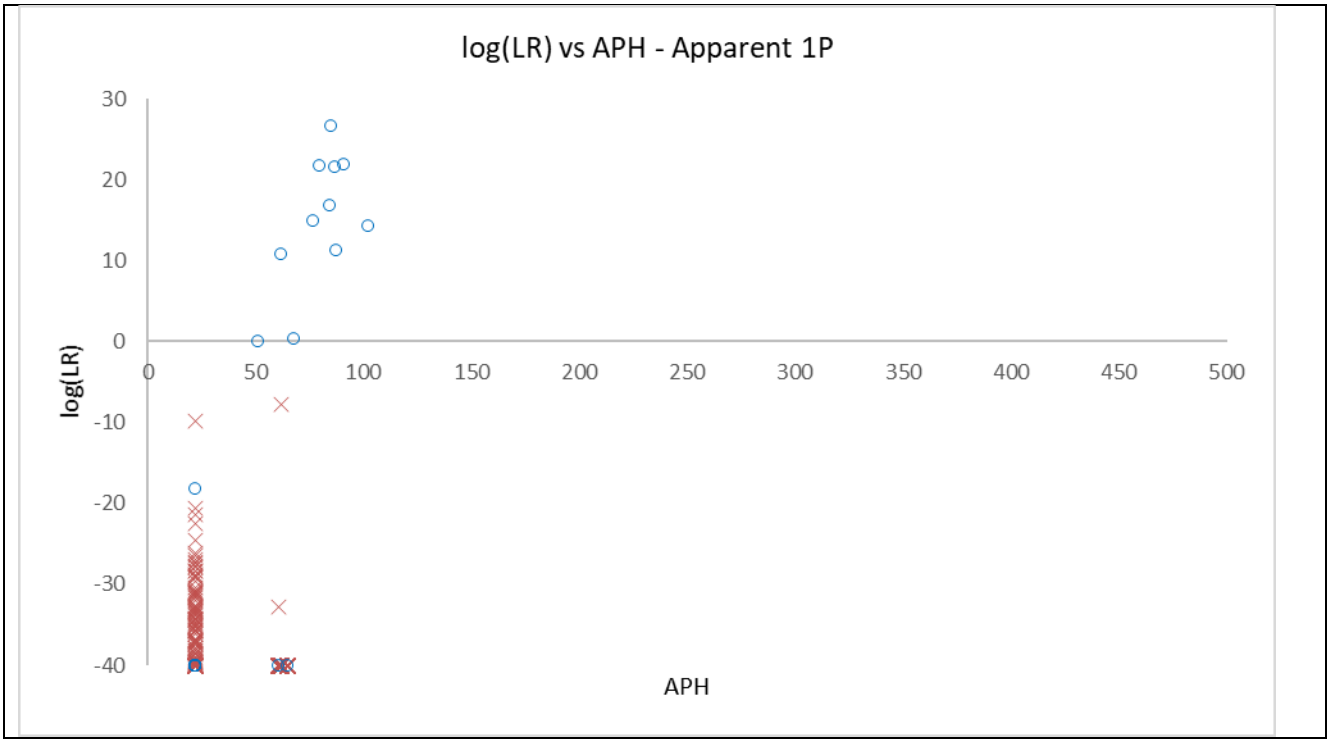
It is recommended that caution be exercised if the case information suggests that relatives may be a consideration and where the profiles recovered are of low template/peak height. Nevertheless, STRmix™ was able to reliably exclude related non-contributors when interpreting profiles with high APH and greater distinction in mixture ratio.

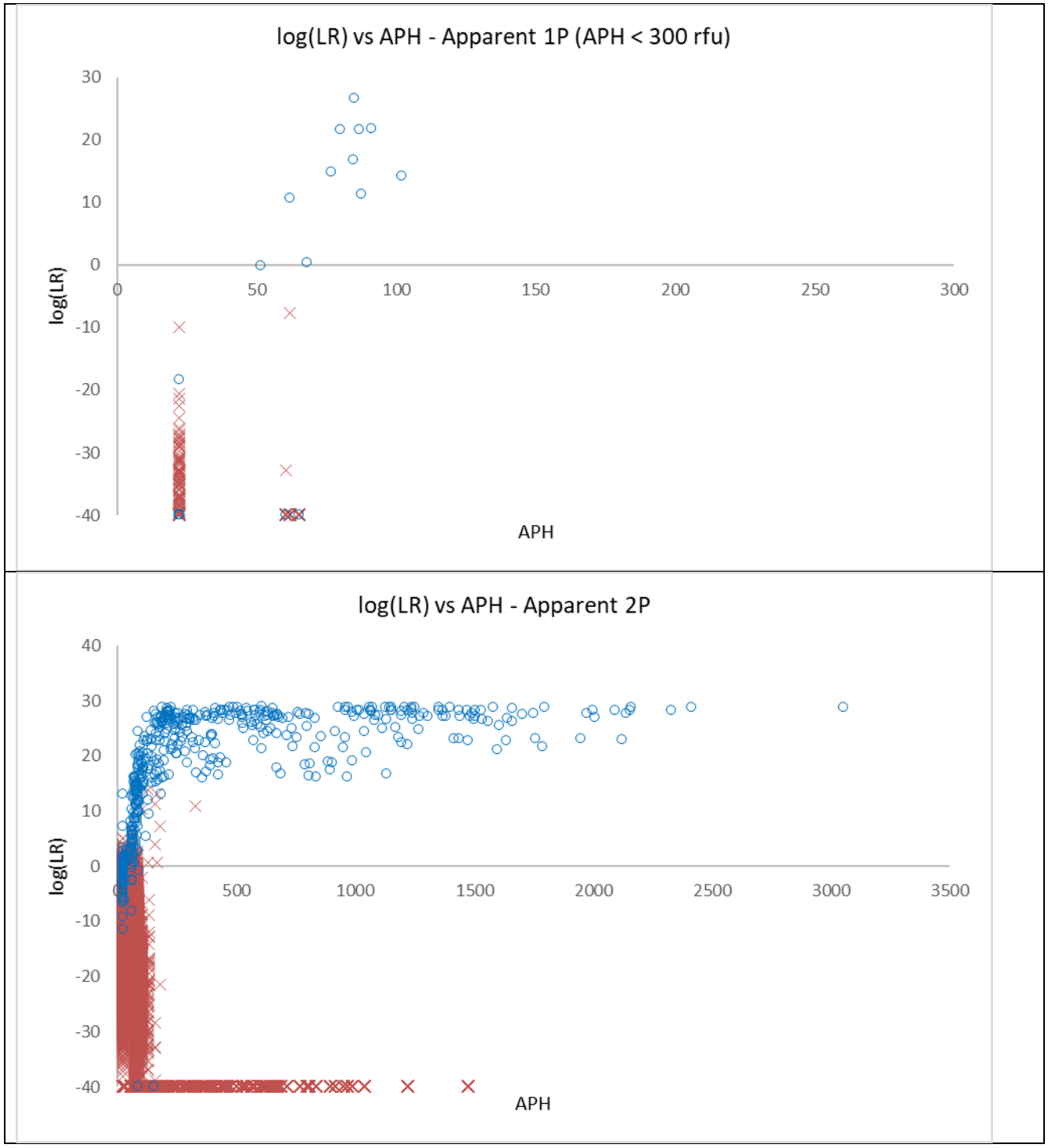
With the modelling of additional stutter products in STRmix™ v2.6, interpretations can become more complex and hence require greater computing power. The five-person mixtures where the *Apparent N* was also assigned as 5 failed to run using the greater computing power available at ESR, New Zealand (128 to 256Gb RAM). This may represent a limiting factor when considering the type of mixtures to interpret in casework. A standard desktop PC may lack the computing power to interpret such complex profiles. The use of 'Low Memory Mode' within STRmix™ may assist in some instances. The use of conditioning profiles during interpretation may also assist, as these substantially decrease the complexity of the interpretation.

Overall, this study has shown that STRmix™ v2.6 is able to correctly distinguish between true and false donors for a range of mixtures where there is high template (or peak heights) from the donors, regardless of whether relatives are considered as alternative contributors.

Replicate studies were not undertaken within this present study. The effect of replicates was explored in detail within the LVMPD laboratory's validation of STRmix™ version 2.4. Regardless of the version of STRmix™ used, the use of amplification replicates is expected to improve the ability of the software to distinguish between true and false donors.

Figure 1: Log(*LR*) versus APH (RFU) for apparent one-, two-, three-, and four-person Investigator® 24plex QS mixtures amplified within the LVMPD laboratory and run on a 3500 CE instrument. H_p true *LR*s are plotted as blue circles whilst H_d true *LR*s are plotted as red crosses. In each instance, the first plot shows all results whilst the second plot is a zoom to better illustrate the data below an APH of 300 RFU. For the two-, three-, and four-person mixtures, additional plots are provided omitting *LR*s to known relatives of the true donors.

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log(LR) vs APH - Apparent 2P
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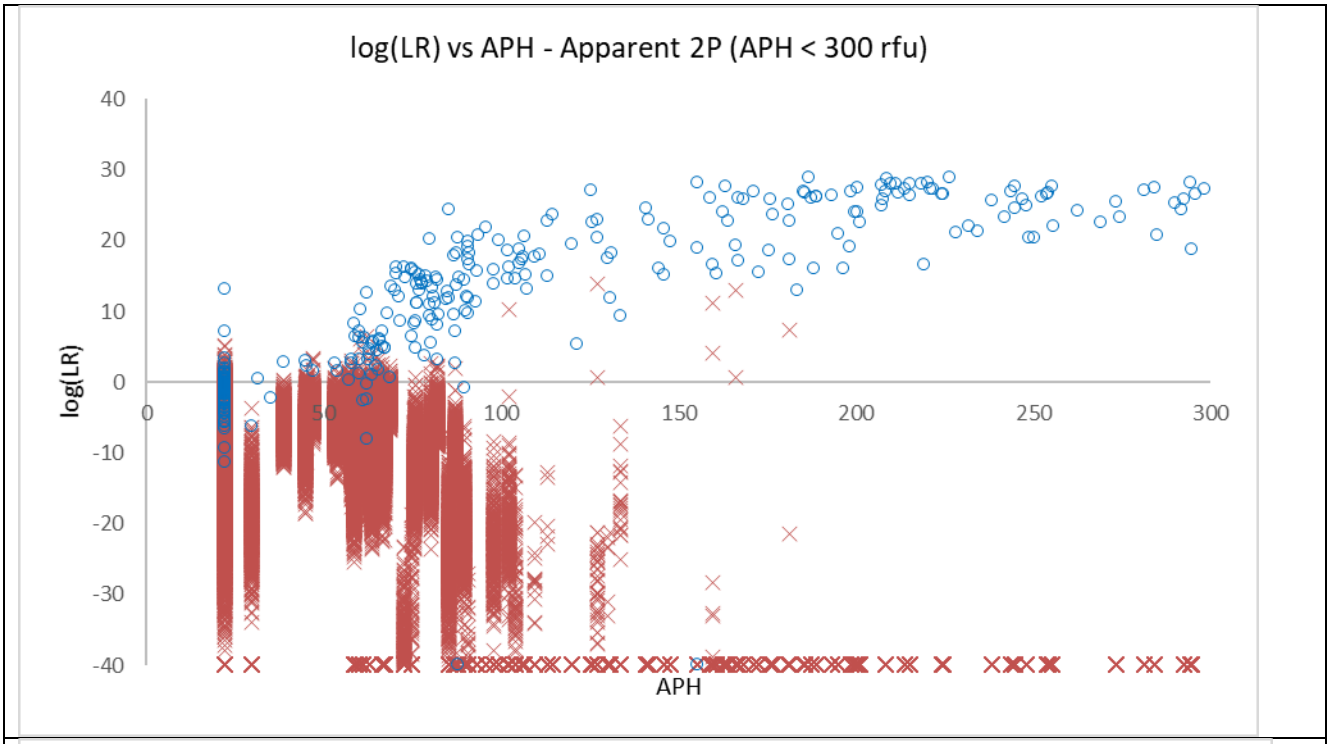
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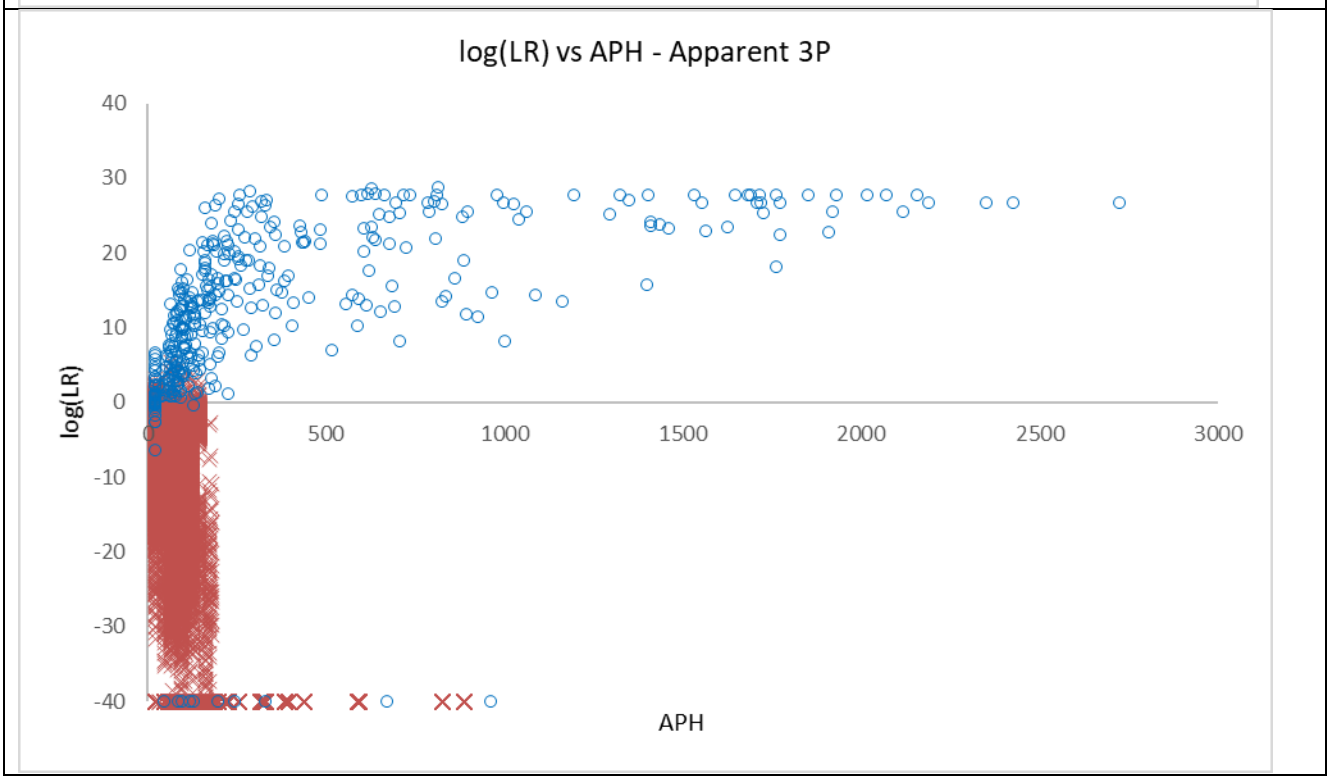
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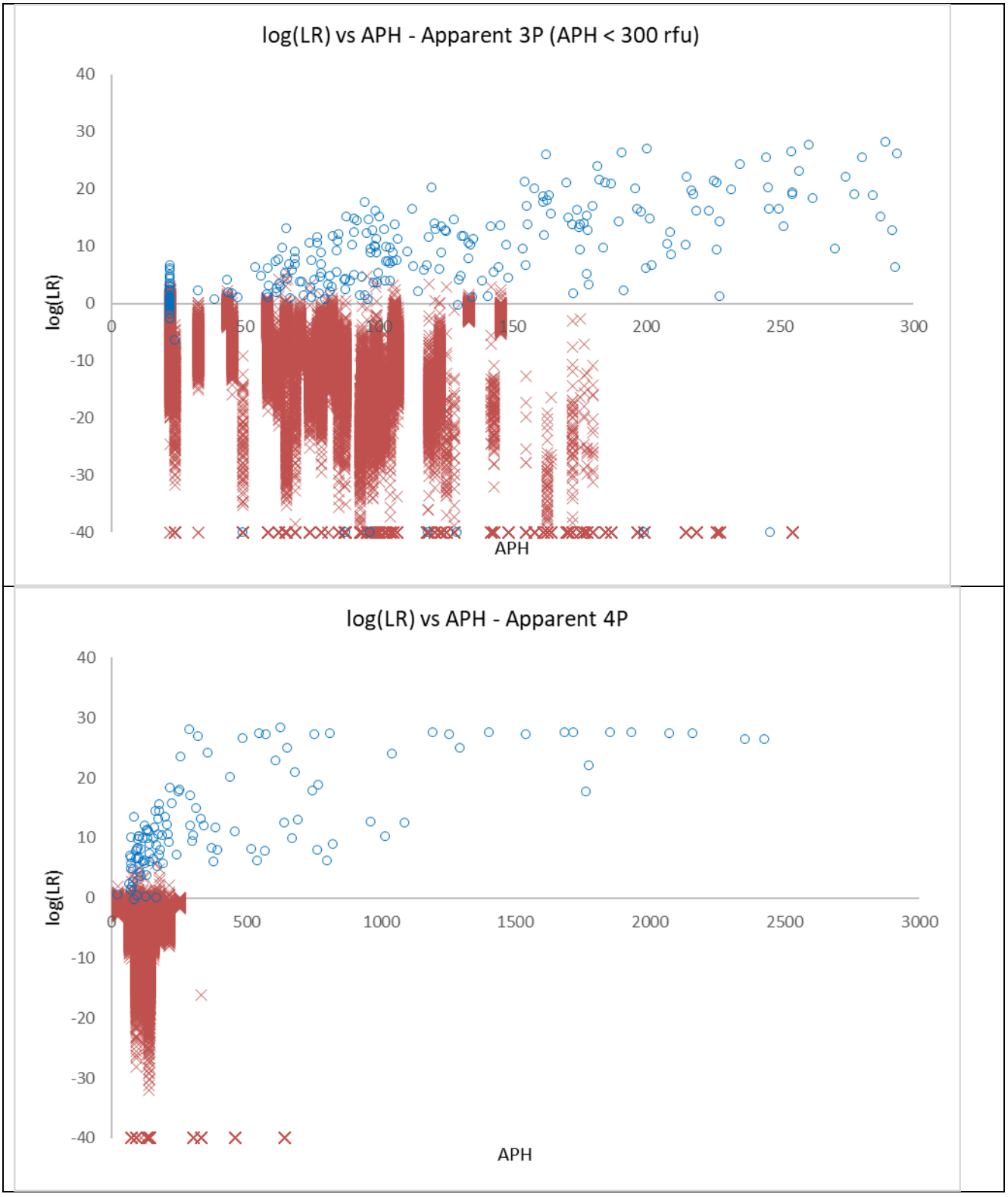
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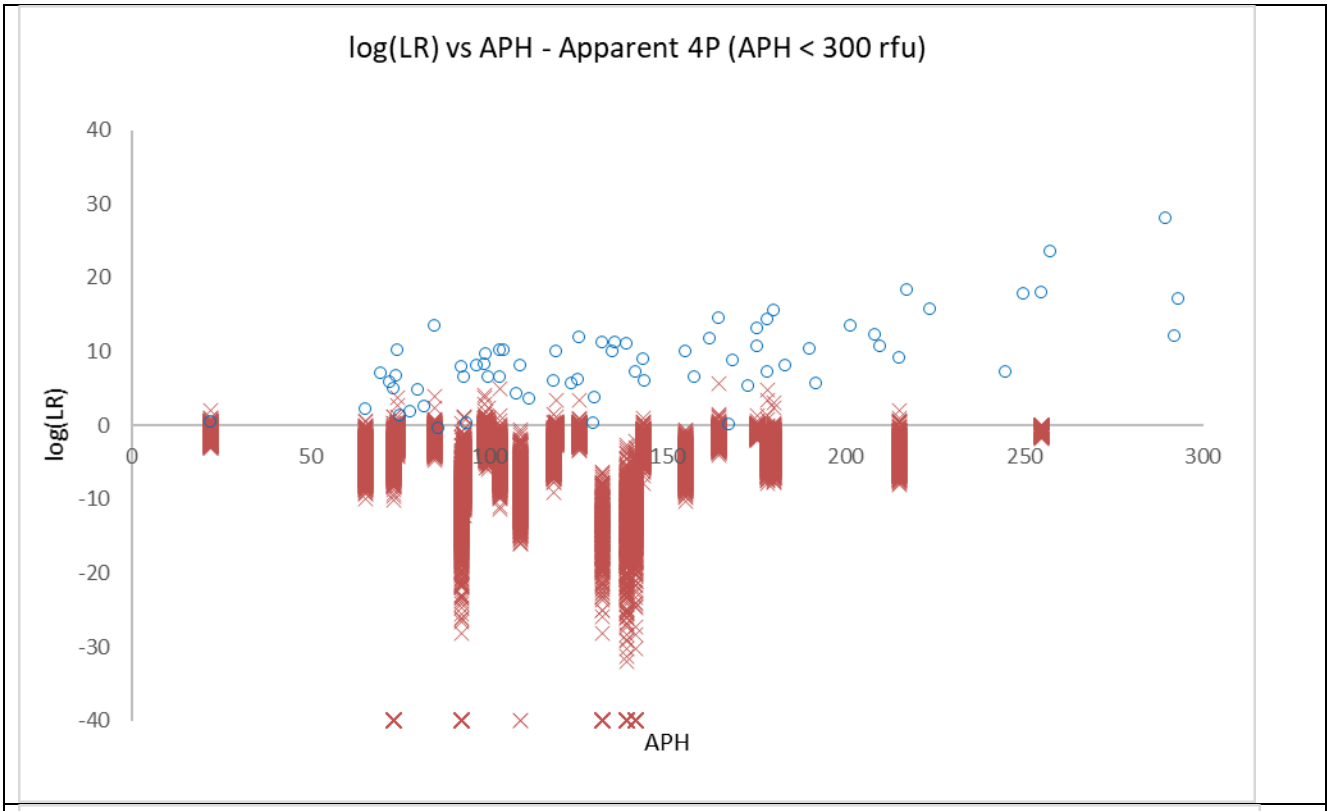
log(LR) vs APH - Apparent 4P (APH < 300 rfu)
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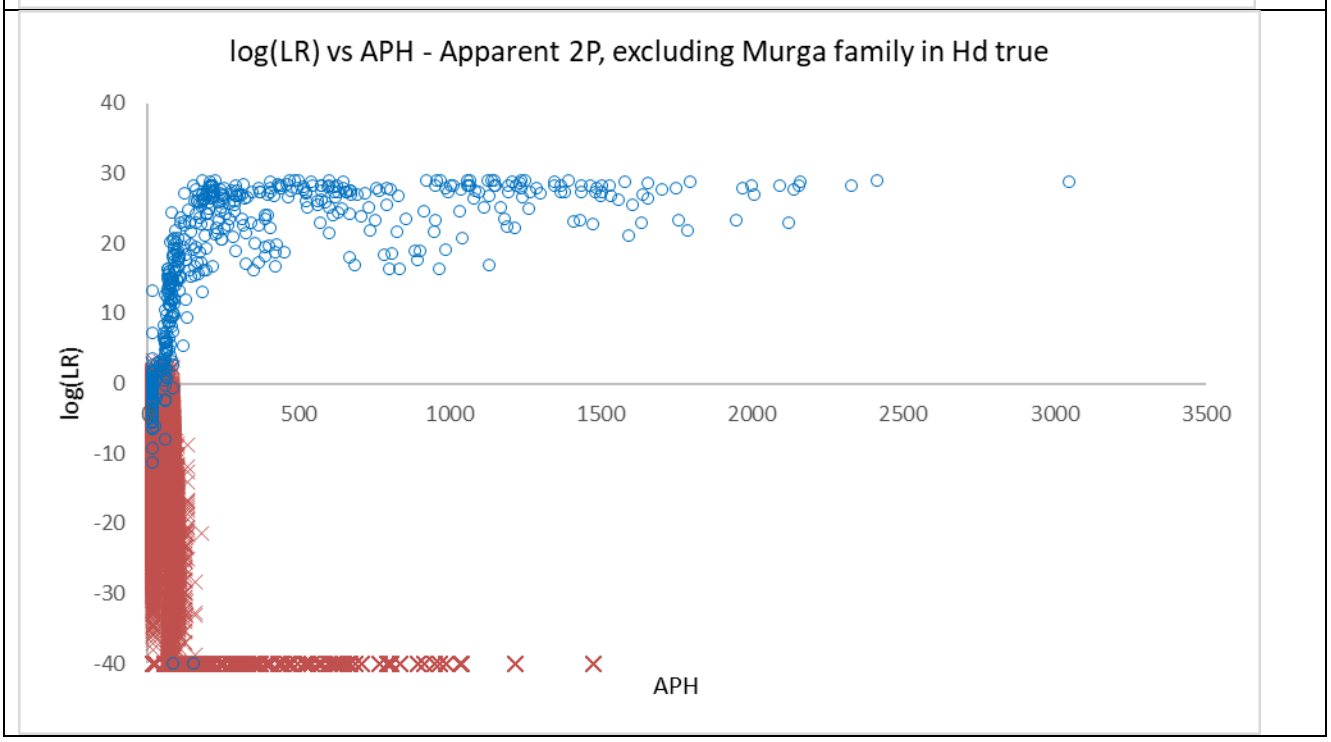
log(LR) vs APH - Apparent 2P, excluding Murga family in Hd true
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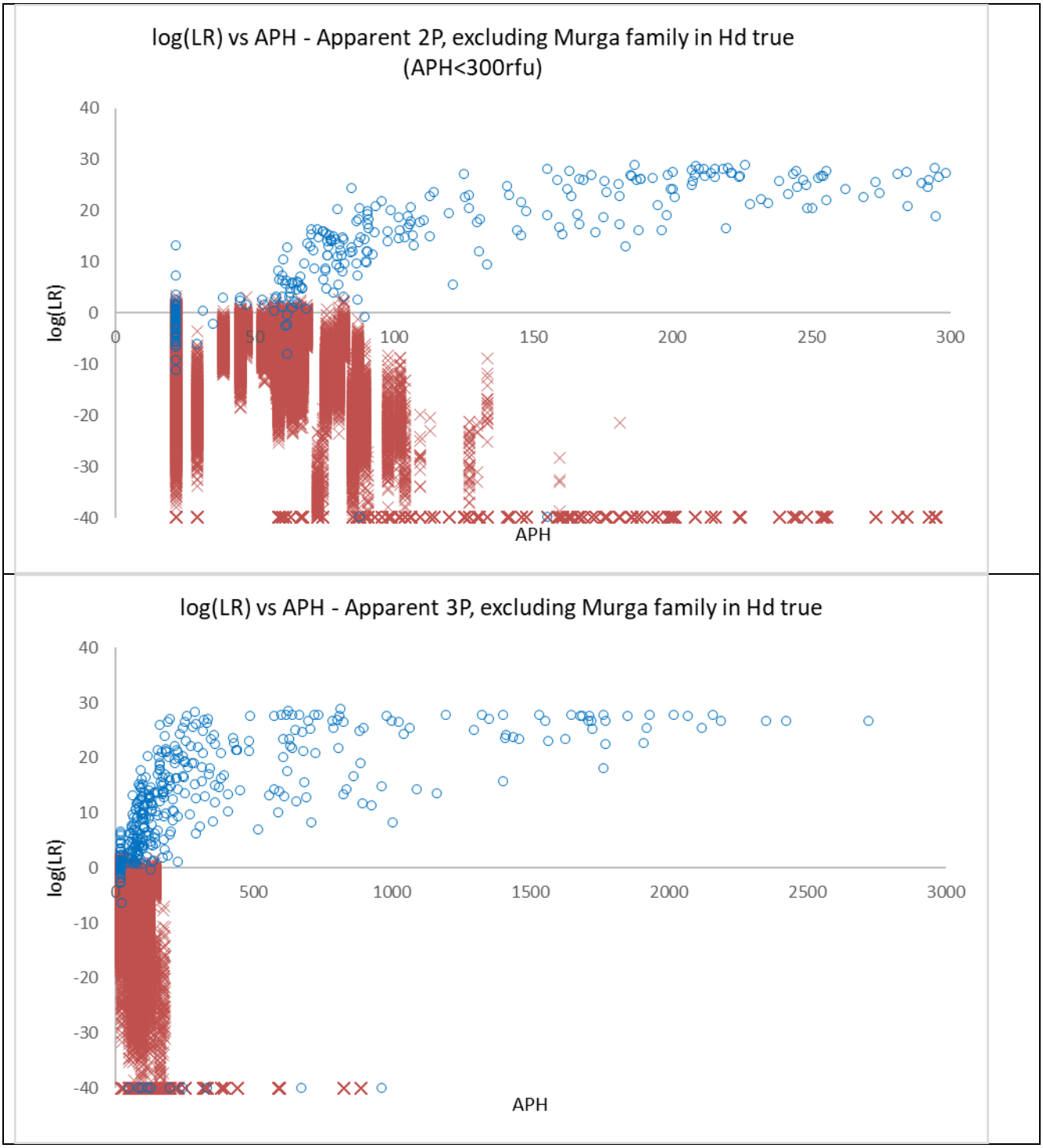
log(LR) vs APH - Apparent 2P, excluding Murga family in Hd true
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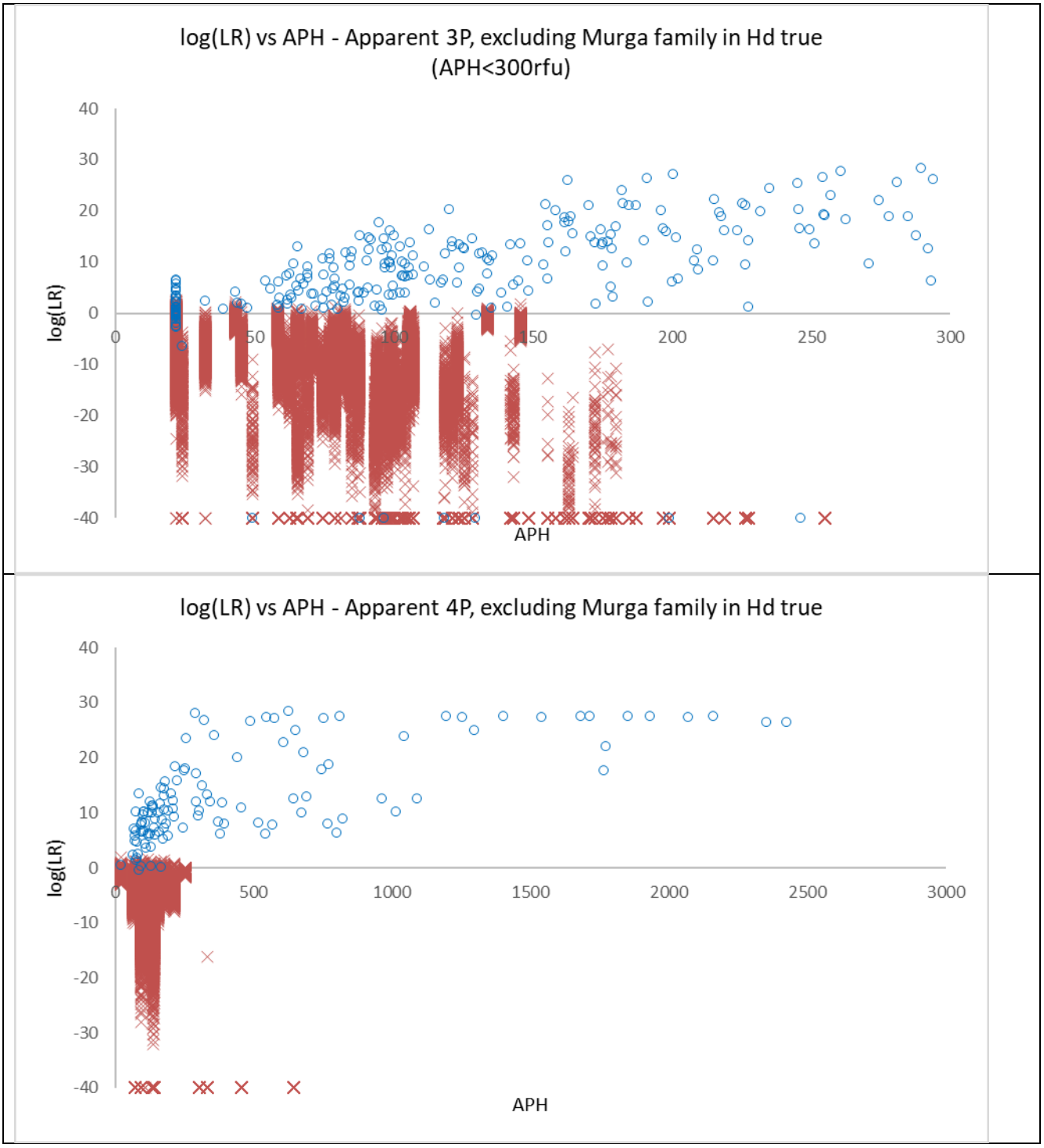
log(LR) vs APH - Apparent 3P, excluding Murga family in Hd true
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log(LR) vs APH - Apparent 4P, excluding Murga family in Hd true
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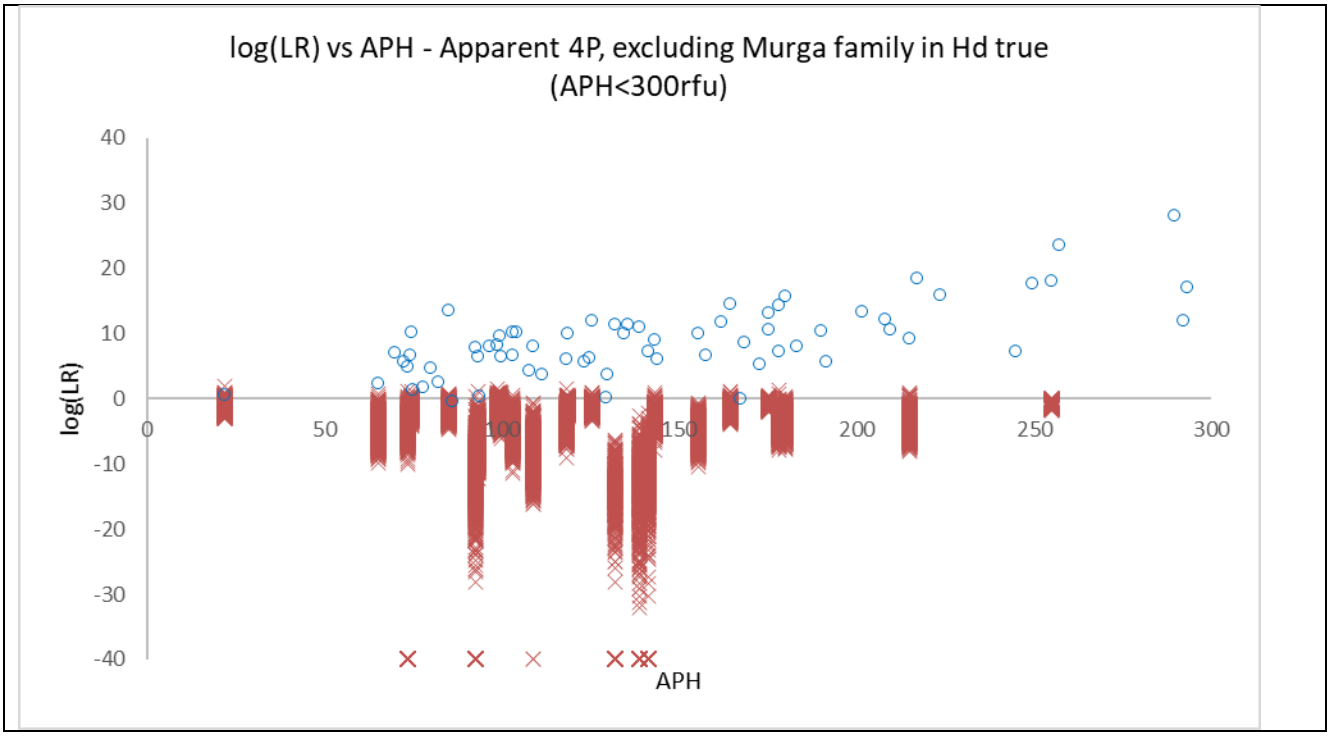
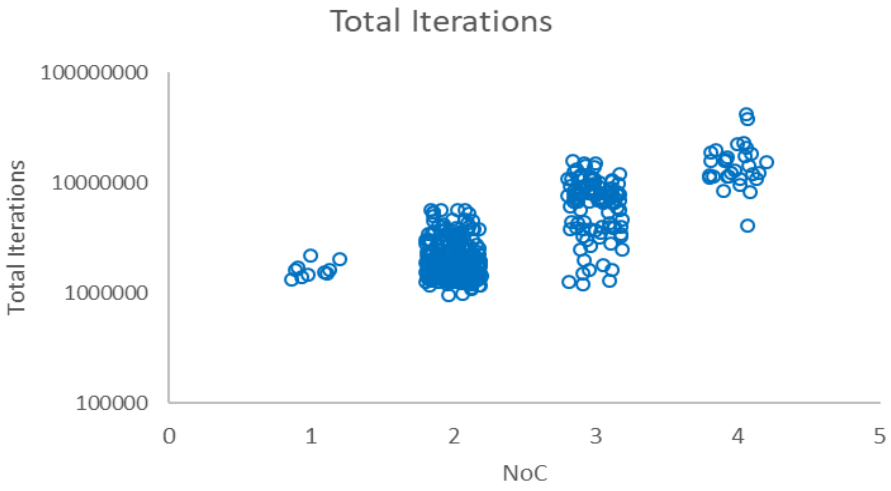


Additional Review of Mixtures:

The STRmix™ output contains a number of run diagnostics to assist the user with evaluating the results and to give confidence that the interpretation has run as expected. The primary diagnostics are the genotype weights, mixture proportions and, where calculated, the individual locus *LR*s. There are also a number of secondary diagnostics which can help give confidence the deconvolution has progressed as expected and these should be reviewed.

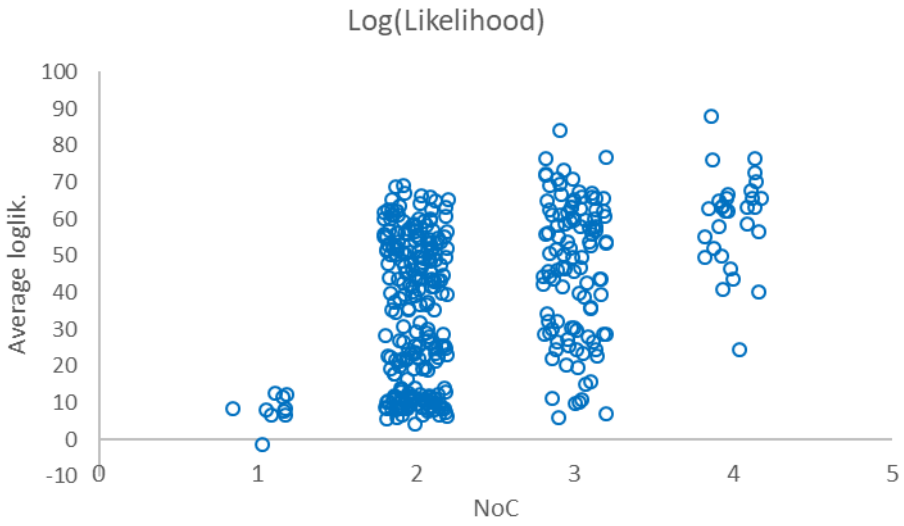
Diagnostic values for all of the 380 mixtures interpreted were collated. A summary of this information is provided in the following figures and discussed further below.

Figure 2: Plot of the total number of iterations required to achieve 400,000 post burn-in accepts (50,000 accepts per chain) for all interpretations, categorized according to the apparent number of contributors assigned. The values have been jittered to sit around the x-axis point to better show the density.



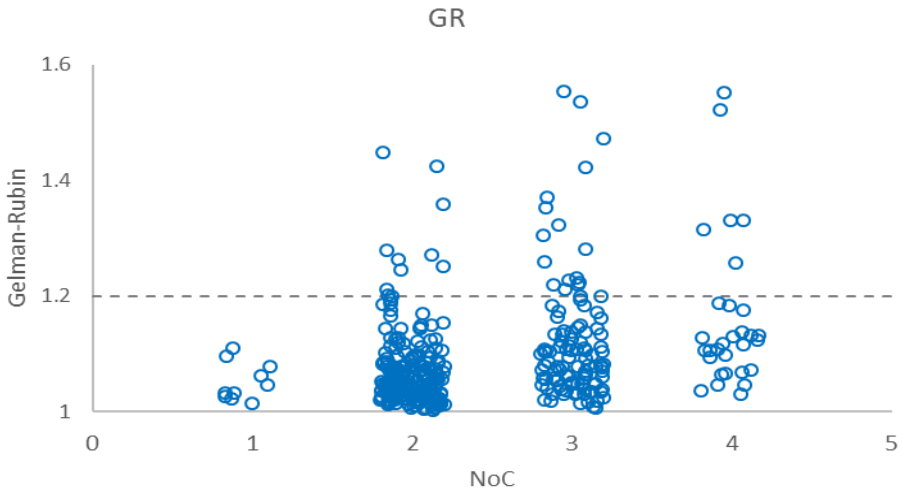
Inspection of **Figure 2** shows the expected increase in the number of iterations required for higher order mixtures. In general, the more complex the mixture, the greater the number of iterations required to achieve the user-defined number of accepts.

Figure 3: Plot of the average log(likelihood) from the post burn-in phase, produced for all interpretations, categorized according to the apparent number of contributors.



Inspection of **Figure 3** shows a spread of average log(likelihood) or probability density values, ranging from approximately -1.2 to 88. It is expected that a range of log(likelihood) values will be observed when interpreting casework profiles and, broadly speaking, larger values are desirable as this can indicate that STRmix™ has been able to model the data well. However, it is important to note that low or even negative numbers may be produced for some profiles and do not necessarily indicate unreliable results and could be due to the low number of peaks in a profile. The negative value for the apparent single source sample could be driven by peak imbalances due to the true additional contributors.

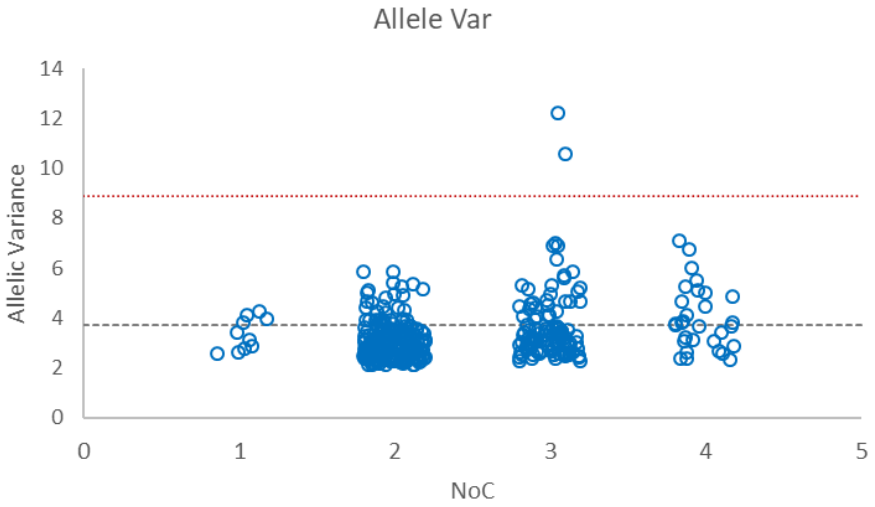
Figure 4: Plot of the Gelman-Rubin convergence diagnostic (GR) produced for all interpretations, categorized according to the apparent number of contributors. The dashed line represents a GR value of 1.2. The "Auto-Continue" feature was not utilized during the validation runs.



Inspection of **Figure 4** shows a spread of GR values for the runs, with the majority (~91%) being below 1.2 and a maximum observed value of 1.84 obtained from one of the four-person mixtures. The orignators of this diagnostic indicate a value below 1.2 suggests likely convergence of the MCMC chains. However, a value greater than 1.2 does

not necessarily mean the results are invalid. It is anticipated that for some complex mixtures this value may be greater than 1.2. Where an elevated GR value is seen, if all of the remaining primary (weights, M_x and *LR*) and secondary diagnostics appear typical, then there is increased confidence the results are likely suitable for use. If any of the other diagnostics are not intuitive or are atypical, it is recommended that the interpretation be repeated using the same or an increased number of MCMC accepts. The LVMPD utilizes the "Auto-Continue" feature in STRmix v2.6. In the event the GR value is greater than 1.2 at the completion of the initial post-burn-in stage, an additional 50,000 accepts, per chain, will automatically be added to the MCMC process. This will be noted on the final STRmix Summary Report.

Figure 5: Plot of the average allelic peak height variance parameter proposed by STRmix™ during the post burnin accepts compared to the apparent number of contributors. The grey dashed line represents the mode of LVMPD's prior distribution for allelic peak height variance. The red dotted line represents the 95th percentile of the prior gamma distribution.



Inspection of **Figure 5** shows a spread of allelic peak height variance values for the samples run. The bulk of the data clusters around the mode of the prior distribution (the dashed line). There are two outliers within the apparent three-person mixtures. These both relate to relatively high template experimentally design four-person mixtures which were run as three (INV24-VAL80_HIGH2_1.5ng.1_(MIX_13H) & INV24-VAL94_HIGH2_1.5ng.1_(MIX_15H)). It is possible the true fourth donor is masked and causing elevated peak imbalances.

The mode is a useful reference point as is a plot of the prior gamma distribution (provided below) in order to gauge where a given posterior mean value sits. In the context of **Figure 6** below, most the above data points (which range from approximately 2 to 12) sit within the body of the prior distribution.

Figure 6: Plot of the allelic peak height variance prior gamma distribution for Investigator® 24plex QS data within the LVMPD laboratory, over-laid with the (above) posterior allele variance from the STRmix™ output.

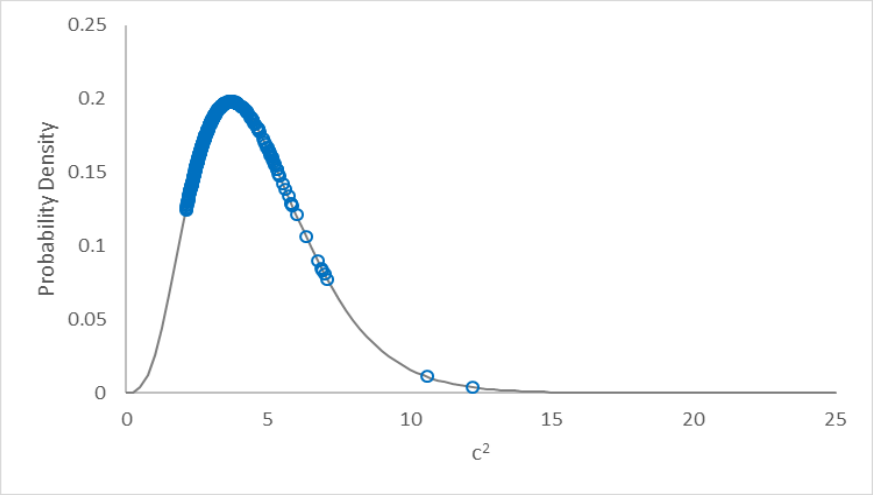


Figure 7: Plot of the average back stutter (-1,0) peak height variance values during the post burnin accepts compared to the apparent number of contributors. The grey dashed line represents the mode of LVMPD's prior gamma distribution for back stutter peak height variance whilst the red dotted line represents the 95th percentile of this distribution.

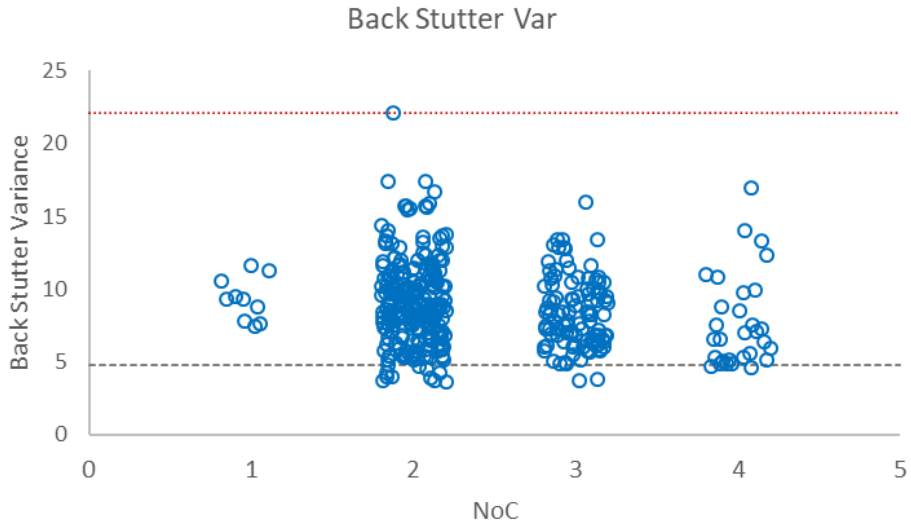


Figure 8: Plot of the back stutter variance prior gamma distribution for Investigator® 24plex QS data within the LVMPD laboratory, over-laid with the (above) posterior back stutter variance values from the STRmix™ output.

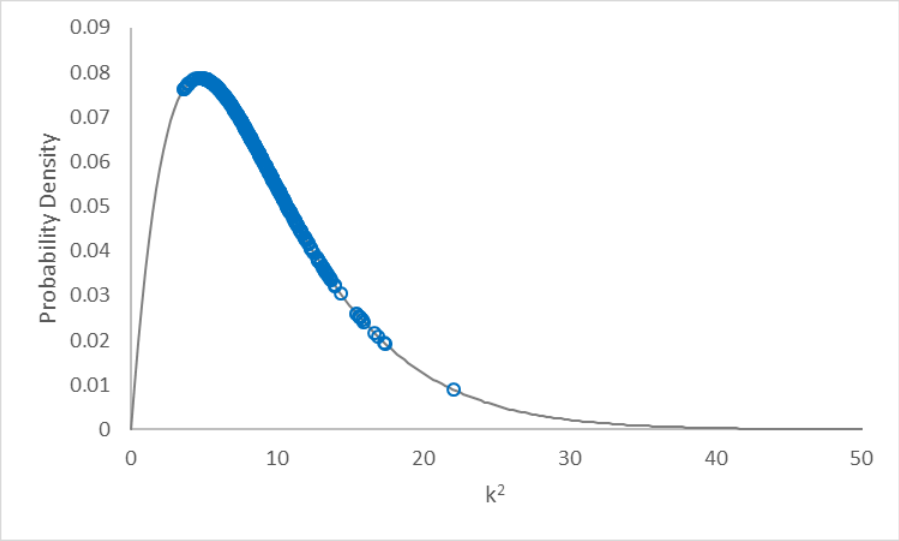


Figure 9: Plot of the average forward stutter (1,0) peak height variance value during the post burnin accepts compared to the apparent number of contributors. The grey dashed line represents the mode of LVMPD's prior distribution for forward stutter peak height variance whilst the red dotted line represents the 95th percentile of this distribution.

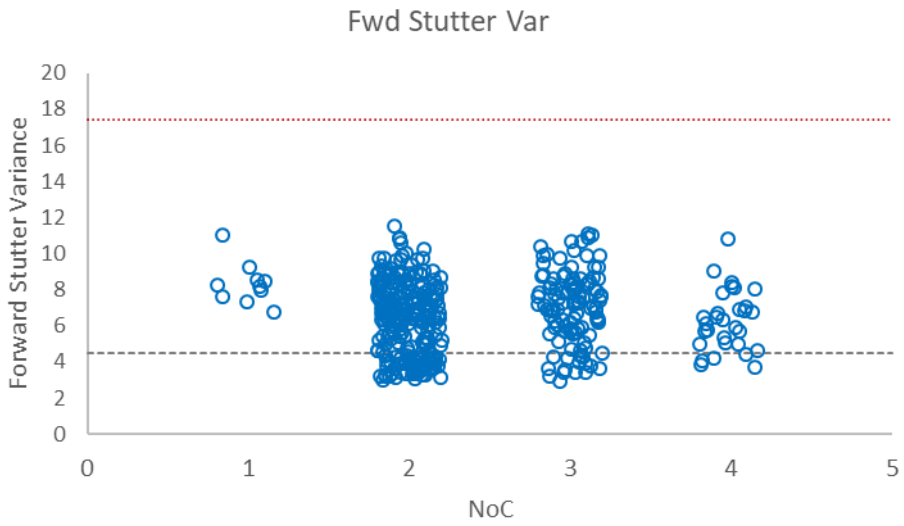


Figure 10: Plot of the forward stutter peak height variance prior gamma distribution for Investigator® 24plex QS data within the LVMPD laboratory, over-laid with the (above) posterior forward stutter variance values from the STRmix™ output.

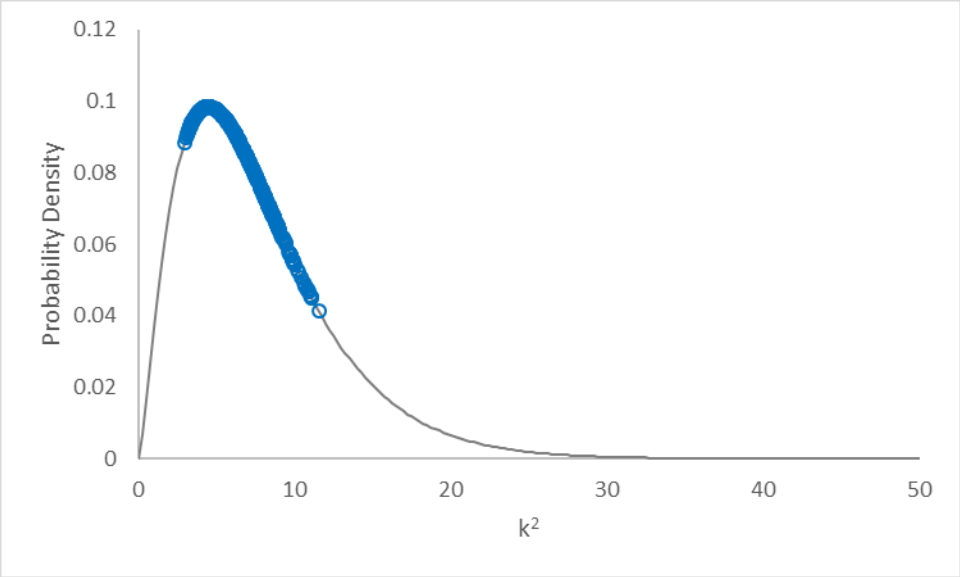


Figure 11: Plot of the average double back stutter (-2,0) peak height variance values of the post burnin accepts compared to the apparent number of contributors. The grey dashed line represents the mode of LVMPD's prior gamma distribution for double back stutter peak height variance whilst the red dotted line represents the 95th percentile of this distribution.

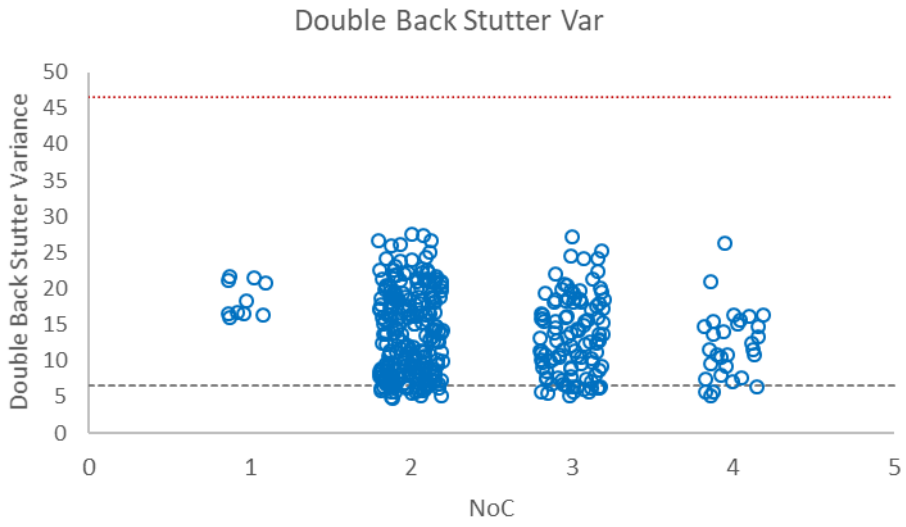


Figure 12: Plot of the double back stutter peak height variance prior gamma distribution for Investigator® 24plex QS data within the LVMPD laboratory, over-laid with the (above) posterior double back stutter variance values from the STRmix™ output.

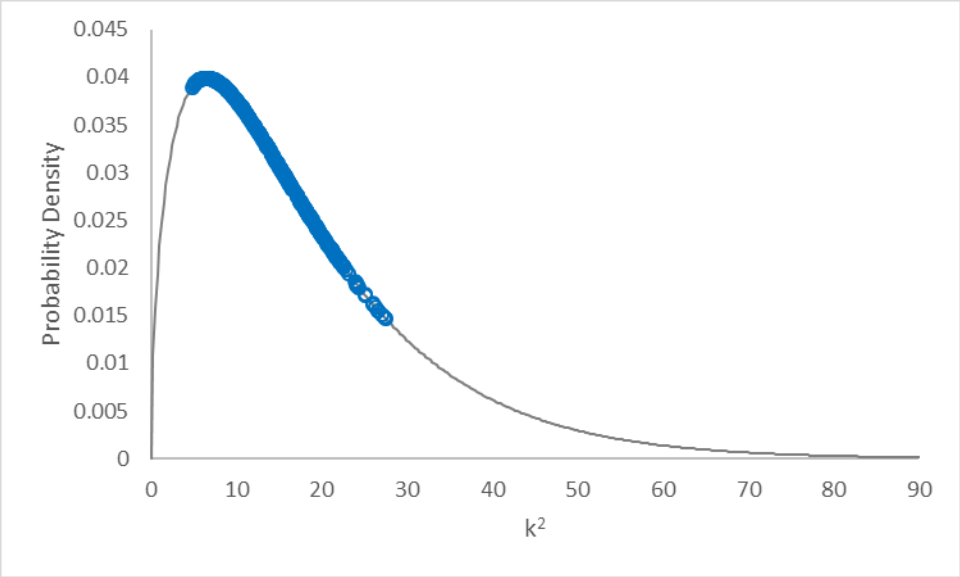


Figure 13: Plot of the average half back/minus 2 base pair stutter (0,-2) peak height variance values of the post burn-in accepts compared to the apparent number of contributors for the D1S1656 and SE33 loci. The grey dashed line represents the mode of LVMPD's prior gamma distribution for half back/minus 2 base pair stutter peak height variance whilst the red dotted line represents the 95th percentile of this distribution.

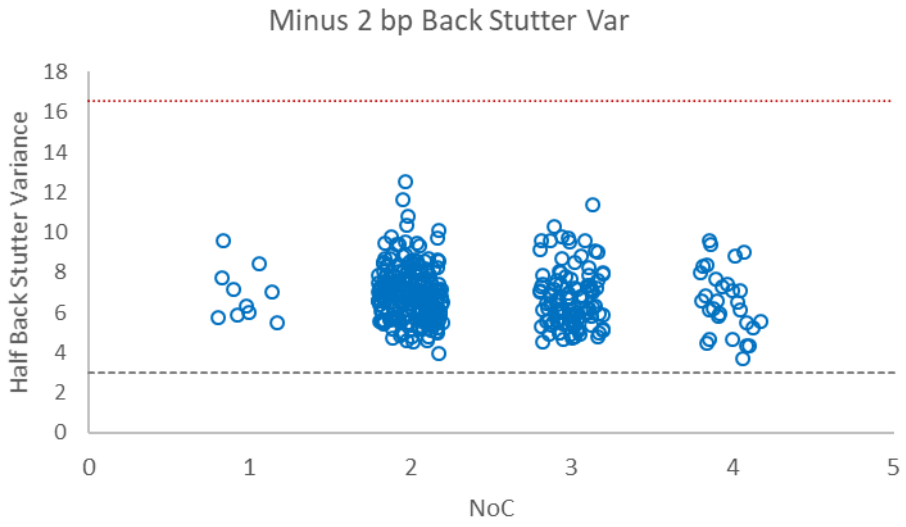
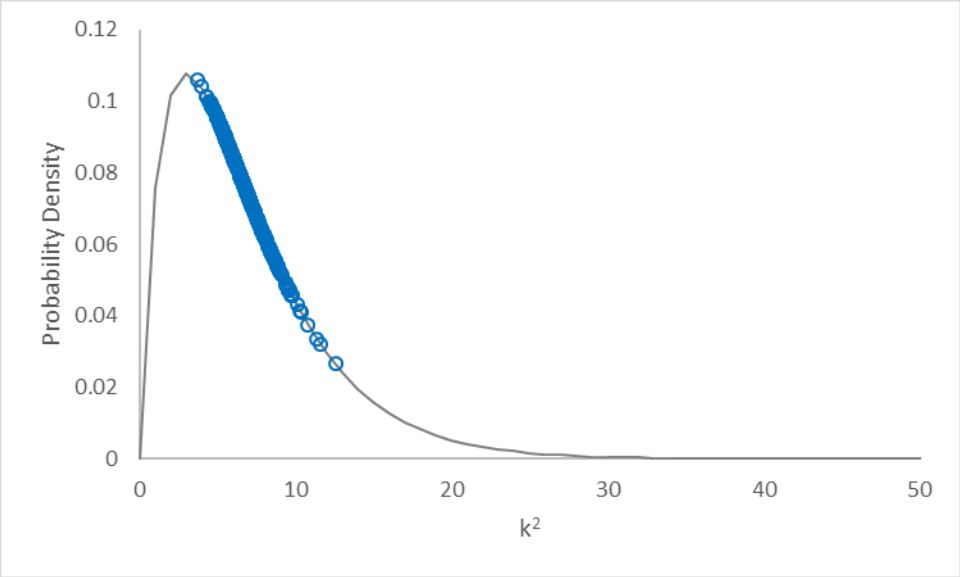


Figure 14: Plot of the half back/minus 2 base pair stutter peak height variance prior gamma distribution for Investigator® 24plex QS data within the LVMPD laboratory, over-laid with the (above) posterior 2 base pair or half back stutter variance values from the STRmix™ output.



Inspection of **Figure 7** to **Figure 14** shows a spread of stutter variance values for the 380 interpretations carried out. Some samples were observed to give slightly elevated variance values compared to the mode of the prior distributions. However, reference to plots of the prior gamma distributions (**Figures 8, 10, 12**, and **14**) revealed that the vast majority of the elevated stutter variance values observed sat within the body of these distributions.

Impact of Over- or Under-Assigning the Number of Contributors

In an extension of the phase one study above, a further study was undertaken to review the impact of over- or under-assigning the number of contributors compared to the experimental design.

Where the *Apparent N* differed from *Exp N* the *LR*s (and hence the mixture deconvolutions) obtained under *Apparent N* and *Exp N* were compared. There were a number of samples where the *Exp N* was not undertaken in phase one and so all these were re-deconvoluted using *Exp N* to provide the baseline for comparison. This constituted 58 additional deconvolutions and can be determined by a review of the Excel document 'NOC for STRmix Runs' where there is no 'x' within the column for the true *N*. It was not possible to run the five person-mixtures as 5 and so no further comparison of those is included here.

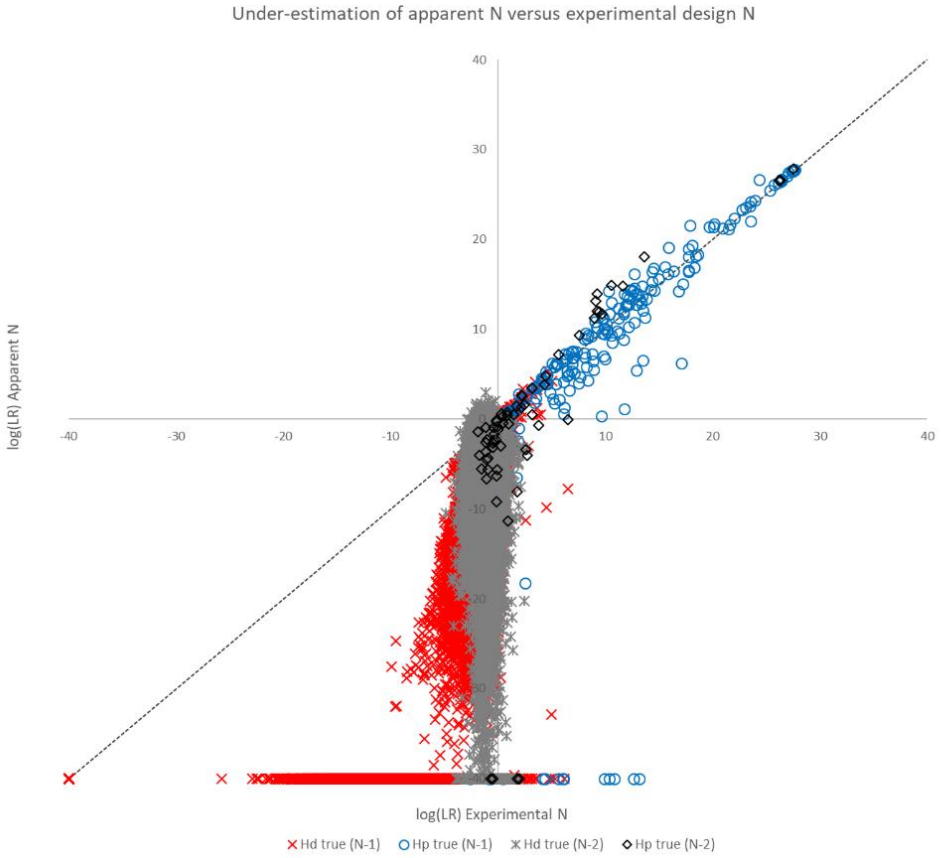
Within phase one there were 380 deconvolutions progressed (from 348 available samples). Each had a deconvolution and *LR*s under the *Apparent N,* which for some samples was also the *Exp N.* The additional 58 deconvolutions described above ensured each sample had at least one run as *Exp N*.

Therefore, in total 438 mixtures were deconvoluted within phase one and two.

However, only those samples where experimental *N* and apparent *N* differed (n=90) are studied here. There were 64 runs where the *Apparent N* was assigned one under the *Exp N* design (*N*-1), 17 where this was two under (*N*-2) and 9 where it was over-assigned (*N*+1).

Using the *LR*s obtained a comparison of the deconvolutions under *Apparent N* and *Exp N* can be seen in **Figure 15** and **Figure 16**.

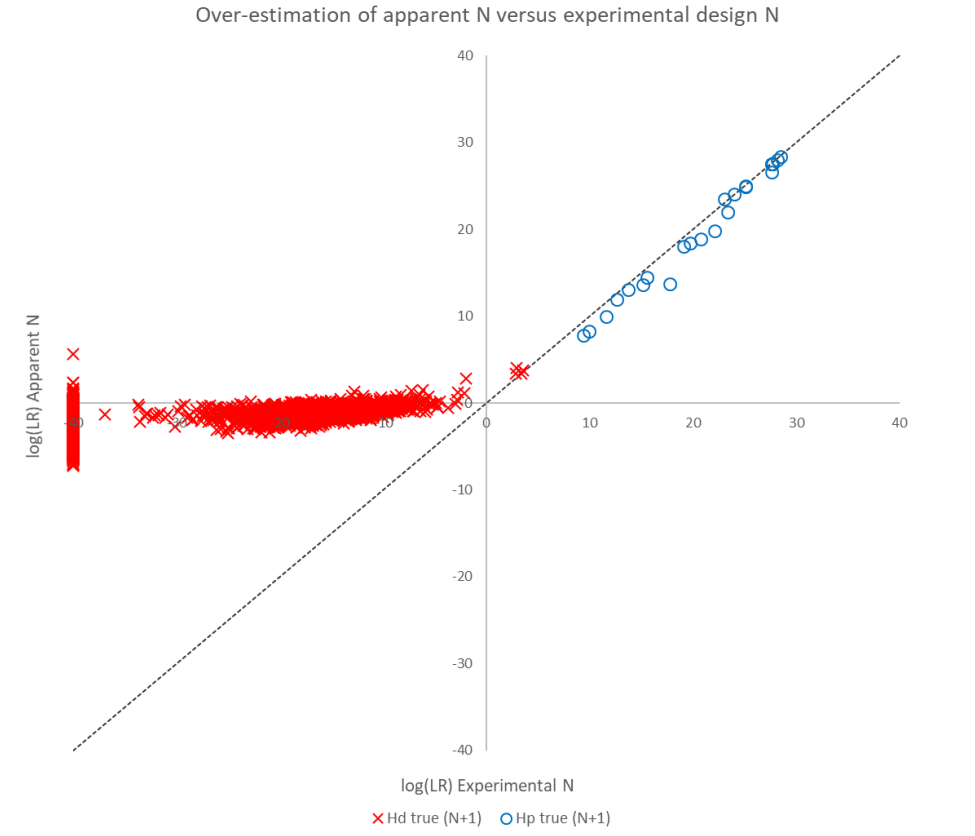
Figure 15: Comparison of *LR*s calculated by STRmix™ when the *Apparent N* is under-assigned, compared to *Exp N*. *LR*s for known donors (H_p true) have been plotted using blue circles (*N*-1) or black diamonds (*N*-2). *LR*s for known non-contributors (H_d true) have been plotted using red crosses (*N*-1) or grey stars (*N*-2). Where identical *LR*s are produced between both versions, the data points fall on the $y = x$ line (indicated as a grey dashed line).



Overall, this plot demonstrates that the *LR* to a true contributor who is contributing a reasonable amount of DNA to a sample does not differ regardless of the under-assignment of *N*. Whilst for non-contributors the power to distinguish increases (i.e. the *LR*s tend more towards exclusion).

It is important to maintain perspective that during casework we would not have the luxury of knowing the *Exp N* so these studies are crucial in understanding the impact of assigning the value of *N* in casework. Very broadly speaking either the 'true' N is assigned (here this would be the equivalent or comparable to *Exp N*) or the outcome of under-assigning tends to be conservative.

Figure 16: Comparison of *LR*s produced by STRmix™ when the *Apparent N* is over-assigned, compared to *Exp N*. *LR*s for known donors (H_p true) have been plotted using blue circles. LRs for known non-contributors (H_d true) have been plotted using red crosses. Where identical LRs are produced between both versions, the data points fall on the y = x line (indicated as a grey dashed line).



Overall this plot demonstrates that the *LR* to a true contributor does not differ greatly regardless of the over-assignment of *N*. However, on the whole these are slightly lower when over-assigning, as we spread some more weight across other contributor genotypes.

The opposite effect can be seen for the H_d true *LR*s. As more donors and hence more genotypes are allowed the power to distinguish reduces. The group of H_d true *LR*s sitting above 1 relates to the family studies.

Impact of Under- or Over-Assigning NOC with COSTaR

The COSTaR workbook utilizes a STRmix deconvolution settings file to automate the determination of CODIS-eligible profiles for each contributor by using trimming thresholds applied to the allelic weights. A MME calculation that is automatically performed within the workbook determines how much trimming is necessary to determine the highest level of CODIS a profile would be suitable for. As noted in the previous studies, under-assigning the number of contributors to a mixture may constrict the genotypes which may be considered by STRmix and lead to false exclusions of true donors. Conversely, the over-estimation of the number of contributors during deconvolution allows STRmix to consider more genotypes as possible explanations for the DNA data, therefore resulting in the possibility for some low-level positive LRs for adventitious known non-contributors.

In order to determine the impact of under- or over-assigning the number of contributors during STRmix deconvolution has on the ability of COSTaR to identify CODIS-eligible profiles which would target the true donors, each STRmix run deconvoluted using a different number of contributors than the experimental design was run through COSTaR. All resultant CODIS-eligible components were then visually compared to the DNA profiles of the

true donors of the mixtures in order to determine whether they would be targeted during a subsequent CODIS search. Refer to **Table 3** thru **Table 5** below.

In general, the results of the COSTaR runs demonstrate that when a reasonable amount of overall template exists, the significant contributors to the mixtures would be targeted during a CODIS search, even when the number of contributors used during deconvolution is under-assigned. However, there are several instances in which CODIS-eligible DNA components were identified using COSTaR, however these components would not target the true contributors to the mixtures if searched (listed as "N" in each "True Contributor Targeted" column). In these instances, the use of COSTaR could result in the entry of a profile into the "Forensic mixture" index of CODIS which would not target the true donors during moderate stringency searching. This is likely due to the constriction of genotypes associated with the under-assignment of the number of contributors to the mixture during deconvolution and typically occurs for the lower-level contributors to three and four-person mixtures.

When the number of contributors used during deconvolution is over-assigned, CODIS-eligible DNA components identified using COSTaR were able to target the true donors of the mixture. However, the additional non-contributor considered during deconvolution generated no CODIS-eligible DNA components (listed as "---" across the "CODIS Level" and "True Contributor Targeted" columns). This is likely due to the consideration of a high number of genotypes and weightings containing Q-alleles to account for the additional contributor. During the COSTaR process, these additional genotypes would result in increased trimming beyond eligible thresholds.

The LVMPD utilizes STRmix to perform the match dispositioning of potential CODIS hits. STRmix deconvolutions are searched against all CODIS candidate matches using the Database Search Function in STRmix. The resultant LR must be greater than 50,000 in order to be considered for disposition as a hit. In the event an LR between 50,000 and 100,000 is identified during this database search, an LR from Previous calculation is undertaken in order to take into consideration the additional sampling uncertainty associated with the 99% 1-sided lower HPD interval calculation prior to dispositioning the candidate match.

As such, low-quality DNA profiles identified by COSTaR as eligible for the "Forensic Mixture" index are not expected to return LR stringencies which surpass the disposition threshold for true non-contributors to the mixtures.

Table 3: Results of COSTaR runs for experimentally designed two-person mixtures interpreted as single source (N-1) and three-person (N+1)

Experimental Design: Two-Person					
NOC used in STRmix: 1 N-1					
Mixture Name	Template input	COSTaR contributor	CODIS level	True Contributor Targeted? 1	2
3E 5:1	50pg	C1	National	Y	N
3G 8:1	50pg	C1	National	Y	N
4E 5:1	50pg	C1	National	Y	N
6B 2:1	50pg	C1	National	Y	N
6C 3:1	50pg	C1	National	Y	N
6D 4:1	50pg	C1	National	Y	N
6E 5:1	50pg	C1	National	Y	N

NOC used in STRmix: 3 N+1					
Mixture Name	Template input	COSTaR contributor	CODIS Level	True Contributor Targeted? 1	2
8B2 2:1	200pg	C1	National	Y	N
		C2	State	Y	Y
		C3	---	---	---
9B2 2:1	200pg	C1	State	Y	Y
		C2	State	Y	Y
		C3	---	---	---
9C2 3:1	200pg	C1	Local	Y	Y
		C2	Local	N	Y
		C3	---	---	---

Table 4: Results of COSTaR runs for experimentally designed three-person mixtures when interpreted as single source (N-2), two-person (N-1), and four-person (N+1)

Experimental Design: Three-Person

NOC used in STRmix: 1
N-2

Mixture Name	Template input	COSTaR Contributor	NDIS level	True Contributor Targeted? 1	2	3
14E 15:2:1	50pg	C1	National	Y	N	N
14H 3:2:1	50pg	C1	National	N	N	N

NOC used in STRmix: 3
N-1

Mixture Name	Template input	COSTaR contributor	NDIS level	True Contributor Targeted? 1	2	3
12B 6:2:1	50pg	C1	State	Y	N	N
		C2	---	---	---	---
12C 9:2:1	50pg	C1	---	---	---	---
		C2	---	---	---	---
12D 12:2:1	200pg	C1	National	Y	N	N
		C2	State	N	N	N
12D 12:2:1	50pg	C1	State	Y	N	N
		C2	---	---	---	
12I 10:5:1	50pg	C1	Local	Y	N	N
		C2	---	---	---	---
14B 6:2:1	50pg	C1	---	---	---	---
		C2	---	---	---	---
14C 9:2:1	50pg	C1	---	---	---	---
		C2	---	---	---	---
14D 12:2:1	200pg	C1	---	---	---	---
		C2	---	---	---	---
14D 12:2:1	50pg	C1	---	---	---	---
		C2	---	---	---	---
14E 15:2:1	200pg	C1	National	Y	N	N
		C2	---	---	---	---
14E 15:2:1	50pg	C1	---	---	---	---
		C2	---	---	---	---
14H 3:2:1	200pg	C1	National	Y	N	N
		C2	National	N	N	N

NOC used in STRmix: 4
N+1

Mixture Name	Template input	COSTaR contributor	NDIS level	True Contributor Targeted? 1	2	3
14B 6:2:1	1.5ng	C1	National	Y	N	N
		C2	National	N	Y	Y
		C3	State	N	Y	Y
		C4	---	---	---	---
14C 9:2:1	1.5ng	C1	National	Y	N	N
		C2	State	N	Y	Y
		C3	Local	N	Y	Y
		C4	---	---	---	---
14C 9:2:1	1ng	C1	National	Y	N	N
		C2	State	Y	Y	Y
		C3	Local	Y	Y	Y
		C4	---	---	---	---
14E 15:2:1	1.5ng	C1	National	Y	N	N
		C2	Local	N	Y	Y
		C3	Local	N	Y	Y
		C4	---	---	---	---
14I 10:5:1	1.5ng	C1	National	Y	N	N
		C2	National	N	Y	N
		C3	State	N	N	Y
		C4	---	---	---	---
14I 10:5:1	1ng	C1	National	Y	N	N
		C2	National	N	Y	N
		C3	---	---	---	---
		C4	---	---	---	---

Table 5: Results of COSTaR runs for experimentally designed four-person mixtures when interpreted as two-person (N-2) and three-person (N-1)

True 4-Person

NOC used in STRmix: 2
N-2

Mixture Name	Template input	COSTaR Contributor	CODIS Level	True Contributor 1	2	3	4
13A 1:1:1:1:	50pg	C1	---	---	---	---	---
		C2	---	---	---	---	---
13B 6:1:1:1	50pg	C1	---	---	---	---	---
		C2	---	---	---	---	---
13C 9:1:1:1	50pg	C1	Local	Y	N	N	N
		C2	---	---	---	---	---
13D 12:1:1:1	200pg	C1	National	Y	N	N	N
		C2	State	N	N	N	N
13D 12:1:1:1	50pg	C1	Local	Y	N	N	N
		C2	---	---	---	---	---
13E 15:1:1:1	200pg	C1	National	Y	N	N	N
		C2	---	---	---	---	---
13E 15:1:1:1	50pg	C1	State	Y	N	N	N
		C2	---	---	---	---	---
13H 4:3:1:2	50pg	C1	Local	N	Y	N	N
		C2	---	---	---	---	---
13I 10:5:1:2	50pg	C1	---	---	---	---	---
		C2	---	---	---	---	---
15B 6:1:1:1	50pg	C1	---	---	---	---	---
		C2	---	---	---	---	---
15C 9:1:1:1	50pg	C1	---	---	---	---	---
		C2	---	---	---	---	---
15D 12:1:1:1	50pg	C1	State	Y	N	N	N
		C2	---	---	---	---	---
15E 15:1:1:1	200pg	C1	National	Y	N	N	N
		C2	Local	N	N	N	N
15E 15:1:1:1	50pg	C1	---	---	---	---	---
		C2	---	---	---	---	---
15H 4:3:2:1	50pg	C1	---	---	---	---	---
		C2	---	---	---	---	---

NOC used in STRmix: 3
N-1

Mixture Name	Template input	COSTaR Contributor	CODIS Level	True Contributor 1	2	3	4
13A 1:1:1:1	50pg	C1	---	---	---	---	---
		C2	---	---	---	---	---
		C3	---	---	---	---	---
13B 6:1:1:1	1.5ng	C1	National	Y	N	N	N
		C2	State	N	N	N	N
		C3	Local	N	Y	Y	Y
13B 6:1:1:1	1ng	C1	National	Y	N	N	N
		C2	State	N	Y	N	Y
		C3	Local	N	Y	Y	Y
13B 6:1:1:1	500pg	C1	National	Y	N	N	N
		C2	Local	N	Y	Y	N
		C3	Local	N	N	Y	N
13B 6:1:1:1	200pg	C1	National	Y	N	N	N
		C2	Local	N	N	N	N
		C3	---	---	---	---	---
13C 9:1:1:1	50pg	C1	National	Y	N	N	N
		C2	Local	Y	Y	Y	N
		C3	Local	N	Y	Y	Y
13C 9:1:1:1	1ng	C1	National	Y	N	N	N
		C2	Local	N	Y	Y	Y
		C3	Local	N	Y	Y	Y
13C 9:1:1:1	500pg	C1	National	Y	N	N	N
		C2	Local	N	Y	N	Y
		C3	Local	N	Y	N	Y
13C 9:1:1:1	200pg	C1	National	Y	N	N	N
		C2	---	---	---	---	---
		C3	---	---	---	---	---
13D 12:1:1:1	1.5ng	C1	National	Y	N	N	N
		C2	Local	Y	Y	N	Y
		C3	Local	N	Y	N	Y

NOC used in STRmix: 3
N-1

Mixture Name	Template input	COSTaR Contributor	CODIS Level	True Contributor 1	2	3	4
13D 12:1:1:1	1ng	C1	National	Y	N	N	N
		C2	Local	N	Y	Y	Y
		C3	Local	N	Y	Y	Y
13D 12:1:1:1	500pg	C1	National	Y	N	N	N
		C2	Local	N	N	Y	Y
		C3	---	---	---	---	---
13E 15:1:1:1	1.5ng	C1	National	Y	N	N	N
		C2	State	N	N	Y	N
		C3	Local	N	N	Y	N
13E 15:1:1:1	1ng	C1	National	Y	N	N	N
		C2	Local	N	Y	N	N
		C3	Local	N	Y	N	N
13E 15:1:1:1	500pg	C1	National	Y	N	N	N
		C2	Local	N	N	Y	N
		C3	---	---	---	---	---
13H 4:3:1:2	1.5ng	C1	National	Y	N	N	N
		C2	State	N	Y	N	N
		C3	State	N	N	N	N
13H 4:3:1:2	200pg	C1	Local	Y	Y	N	Y
		C2	Local	Y	Y	N	Y
		C3	Local	Y	N	N	N
13I 10:5:1:2	1.5ng	C1	National	Y	N	N	N
		C2	National	N	Y	N	N
		C3	National	N	N	N	N
13I 10:5:1:2	1ng	C1	National	Y	N	N	N
		C2	National	N	Y	N	N
		C3	State	N	N	N	N
13I 10:5:1:2	500pg	C1	National	Y	N	N	N
		C2	State	N	Y	N	N
		C3	Local	N	Y	Y	Y

NOC used in STRmix: 3
N-1

Mixture Name	Template input	COSTaR Contributor	CODIS Level	True Contributor 1	2	3	4
13I 10:5:1:2	200pg	C1	State	Y	N	N	N
		C2	Local	Y	Y	N	N
		C3	---	---	---	---	---
15B	1.5ng	C1	National	Y	N	N	N
		C2	National	N	N	Y	Y
		C3	National	N	N	N	N
15B 6:1:1:1	1ng	C1	National	Y	N	N	N
		C2	National	N	N	Y	Y
		C3	State	N	Y	Y	Y
15B 6:1:1:1	500pg	C1	National	Y	N	N	N
		C2	Local	Y	N	Y	Y
		C3	State	N	Y	N	Y
15B 6:1:1:1	200pg	C1	National	Y	N	N	N
		C2	Local	Y	N	N	N
		C3	---	---	---	---	---
15C 9:1:1:1	1.5ng	C1	National	Y	N	N	N
		C2	National	N	Y	Y	Y
		C3	State	N	Y	Y	Y
15C 9:1:1:1	500pg	C1	National	Y	N	N	N
		C2	---	---	---	---	---
		C3	Local	Y	N	Y	Y
15C 9:1:1:1	200pg	C1	National	Y	N	N	N
		C2	---	---	---	---	---
		C3	---	---	---	---	---
15D 12:1:1:1	1.5ng	C1	National	Y	N	N	N
		C2	State	N	Y	N	Y
		C3	State	N	Y	Y	Y
15D 12:1:1:1	1ng	C1	National	Y	N	N	N
		C2	Local	Y	Y	Y	Y
		C3	Local	Y	Y	Y	Y

NOC used in STRmix: 3 N-1							
Mixture Name	Template input	COSTaR Contributor	CODIS Level	True Contributor 1	2	3	4
15D 12:1:1:1	500pg	C1	National	Y	N	N	N
		C2	---	---	---	---	---
		C3	---	---	---	---	---
15D 12:1:1:1	200pg	C1	National	Y	N	N	N
		C2	---	---	---	---	---
		C3	---	---	---	---	---
15D 12:1:1:1	50pg	C1	---	---	---	---	---
		C2	---	---	---	---	---
		C3	---	---	---	---	---
15E 15:1:1:1	1.5ng	C1	National	Y	N	N	N
		C2	Local	N	N	Y	Y
		C3	Local	N	N	Y	Y
15E 15:1:1:1	1ng	C1	National	Y	N	N	N
		C2	Local	Y	Y	N	N
		C3	Local	Y	Y	N	N
15E 15:1:1:1	500pg	C1	National	Y	N	N	N
		C2	---	---	---	---	---
		C3	---	---	---	---	---
15H 4:3:2:1	1.5ng	C1	State	Y	Y	Y	Y
		C2	State	Y	Y	Y	Y
		C3	State	Y	Y	Y	Y
15H 4:3:2:1	1ng	C1	State	Y	Y	N	Y
		C2	State	Y	Y	Y	Y
		C3	State	Y	Y	Y	Y
15H 4:3:2:1	500pg	C1	State	Y	Y	Y	N
		C2	State	Y	Y	Y	N
		C3	State	Y	Y	Y	N
15H 4:3:2:1	200pg	C1	Local	Y	Y	Y	N
		C2	Local	Y	Y	Y	N
		C3	Local	Y	Y	Y	N

NOC used in STRmix: 3 N-1							
Mixture Name	Template input	COSTaR Contributor	CODIS Level	True Contributor 1	2	3	4
15I 10:5:2:1	1.5ng	C1	National	Y	N	N	N
		C2	State	N	Y	Y	Y
		C3	State	N	Y	Y	Y
15I 10:5:2:1	1ng	C1	National	Y	Y	N	N
		C2	State	N	Y	Y	N
		C3	State	N	Y	Y	N
15I 10:5:2:1	500pg	C1	National	Y	N	N	N
		C2	Local	Y	Y	N	N
		C3	State	N	N	N	N
15I 10:5:2:1	200pg	C1	National	Y	N	N	N
		C2	---	---	---	---	---
		C3	---	---	---	---	---
15I 10:5:2:1	50pg	C1	---	---	---	---	---
		C2	---	---	---	---	---
		C3	---	---	---	---	---

Summary:

This validation study has shown that:

- The new parameters established for 28 cycle, QIAGEN Investigator® 24plex QS data run on a 3500*xl* CE instrument for version 2.6.3 of STRmix™ appear fit for purpose.
- Additional stutter types (double back stutter at all loci and minus 2 base pair stutter at SE33 and D1S1656) will now be modelled in STRmix™ within the Biology/DNA Detail and therefore peak labels should be retained for these stutter types during analysis.
- Within the present study, STRmix™ has been able to successfully distinguish true from false contributors at a range of input templates/APHs, from high APH down to relatively low APH per-contributor for two-, three-, and four-person mixtures. Five-person mixtures were not successfully interpreted due to insufficient computing power during these studies. It is however noted that the Biology/DNA Detail *will not interpret* samples which appear to have originated from five-person mixtures during casework.
- As with previous validations, caution is required when relatives are a consideration within certain mixture types, particularly when the mixtures are at a low level and the contributors are in roughly equal proportions.
- As anticipated, as the input information (such as peak heights) decreases, the *LR* tends towards 1 (inconclusive) for both known contributors and known non-contributors.

Conclusion:

This document describes the LVMPD laboratory's internal validation activities for STRmix™ v2.6 with 28 cycle QIAGEN Investigator® 24plex QS data analyzed using an ABI 3500*xl* CE instrument. The findings demonstrate that STRmix™ is suitable for its intended use for the interpretation of DNA profiles generated from crime scene samples within the LVMPD laboratory.

Based on the results of this study, the following recommendations are made for implementation of the STRmix™ v2.6 software into LVMPD casework:

- The software may be used in the deconvolution and calculation of *LR*s for two-, three-, and four-person mixtures. The use of this software is *not approved* for use with mixtures that exhibit signs of being from five contributors.
- The Multi-Kit function may be considered when the same sample has been amplified using different amplification chemistries or validated platforms such as the 3130xl or 3500xl. This function is restricted to profiling results generated from amplifications of the same original DNA extract. It is not intended for use in combining results from different samples.
- With the exception of full single source profiles where all homozygous loci are above the qualitative 400 RFU stochastic threshold and all heterozygous loci are above the 225 RFU drop-in cap, DNA profiles will be deconvoluted in STRmix prior to the calculation of an LR.
- In the event the number of contributors to a profile is ambiguous, profiles should be run in STRmix using the lowest number which can most reasonably explain the DNA profile.
- STRmix will be used to individually assess reference standards to determine whether they are included, excluded, or inconclusive as a contributor to the evidence profile. Manual comparisons for exclusions may only be performed when two or more loci resolve to 100% weight following STRmix deconvolution.
- The LVMPD will consider an LR with an exponent falling between 10^3 and 10^{-3} as uninformative and will be reported as such. If an individual is determined to be uninformative, this individual will not be considered in combination with other individuals in alternative propositions
- The point estimate sub-sub source LR will be used to report inclusions to full single source profiles
- The 99% 1-sided lower HPD interval will be used to report inclusions to partial single source, two, three, or four-person mixtures
- At minimum, STRmix interpretation must be attempted on the following sample types:
    - **Single source DNA profiles:** contain at least one allele above the dye-specific analytical threshold at **6 or more loci** (not to include Amelogenin or the DYS391 locus)
    - **Conditioned mixture DNA profiles:** contain at least one minor/foreign allele above the dye-specific analytical threshold at **6 or more loci** (not to include Amelogenin or the DYS391 locus)
    - **Non-conditioned mixture DNA profiles:** contain at least one allele above the dye-specific analytical threshold at **8 or more loci** (not to include Amelogenin or the DYS391 locus)

Signatures

Jessica Lehrner #14785 022020

Jessica Lehrner, DNA Technical Leader, LVMPD

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[7] Taylor D. Using continuous DNA interpretation methods to revisit likelihood ratio behaviour. Forensic Science International: Genetics. 2014;11:144-53.

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[9] Bright J-A, Taylor D, Curran J, Buckleton J. Searching mixed DNA profiles directly against profile databases. Forensic Science International: Genetics. 2014;9:102-10.

[10] Bright J-A, Stevenson KE, Curran JM, Buckleton JS. The variability in likelihood ratios due to different mechanisms. Forensic Science International: Genetics. 2015;14:187-90.

[11] Taylor D, Bright JA, Buckleton J, Curran J. An illustration of the effect of various sources of uncertainty on DNA likelihood ratio calculations. Forensic Science International: Genetics. 2014;11:56-63.

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Appendix 1: List of papers that support STRmix™

The following is a list of papers that directly support STRmix™.

1. D. Taylor, J.-A. Bright and J.S. Buckleton, The interpretation of single source and mixed DNA profiles. Forensic Science International: Genetics, 2013 7(5): 516-528 **(Core maths paper)**
2. J.-A. Bright, D. Taylor, J.M. Curran and J.S. Buckleton, Developing allelic and stutter peak height models for a continuous method of DNA interpretation. Forensic Science International: Genetics, 2013. 7(2): 296-304 **(Core models paper)**
3. J.-A. Bright, D. Taylor, J.M. Curran and J.S. Buckleton, Degradation of forensic DNA profiles, Australian Journal of Forensic Sciences, 2013. 45(4): 445-449
4. D. Taylor. Using continuous DNA interpretation methods to revisit likelihood ratio behaviour. Forensic Science International: Genetics, 2014. 11: 144-153
5. J.-A. Bright, D. Taylor, J.M. Curran and J.S. Buckleton, Searching mixed DNA profiles directly against profile databases. Forensic Science International: Genetics, 2014. 9: 102-110
6. D. Taylor, J.-A. Bright, J.S. Buckleton, J. Curran, An illustration of the effect of various sources of uncertainty on DNA likelihood ratio calculations. Forensic Science International: Genetics, 2014. 11: 56–63
7. J.-A. Bright, J.M. Curran and J.S. Buckleton, The effect of the uncertainty in the number of contributors to mixed DNA profiles on profile interpretation. Forensic Science International: Genetics, 2014. 12: 208-214
8. J.-A. Bright, K.E. Stevenson, J.M. Curran and J.S. Buckleton, The variability in likelihood ratios due to different mechanisms. Forensic Science International: Genetics, 2015. 14:187-190
9. D .Taylor, J.-A. Bright and J.S. Buckleton, Considering relatives when assessing the evidential strength of mixed DNA profiles. Forensic Science International: Genetics, 2014. 13: 259-263
10. D. Taylor, J-A. Bright and J.S. Buckleton. Interpreting forensic DNA profiling evidence without specifying the number of contributors. Forensic Science International: Genetics, 2014. 13: 269-280

The following is a subset of other papers that support the theory within STRmix™:

1. J.-A. Bright, J.M. Curran. Investigation into stutter ratio variability between different laboratories. Forensic Science International: Genetics, 2014. 13: 79-81
2. C. Brookes, J.-A. Bright, S.A. Harbison, and J.S. Buckleton, Characterising stutter in forensic STR multiplexes. Forensic Science International: Genetics, 2012. 6(1): 58-63
3. H. Kelly, J.-A. Bright, J.M. Curran, and J.S. Buckleton Identifying and modelling the drivers of stutter in forensic DNA profiles. Australian Journal of Forensic Sciences, 2014. 46(2): 194-203
4. J.-A. Bright, S. Neville, J.M. Curran, and J.S. Buckleton. Variability of mixed DNA profiles separated on a 3130 and 3500 capillary electrophoresis instrument. Australian Journal of Forensic Sciences, 2014. 46(3): 304-312
5. J.-A. Bright, K.E. Stevenson, M.D. Coble, C.R. Hill, J.M. Curran, and J.S. Buckleton Bright, Characterising the STR locus D6S1043 and examination of its effect on stutter rates. Forensic Science International: Genetics, 2014. 8(1): p. 20-23.
6. D. Taylor, J.S. Buckleton. Do low template DNA profiles have useful quantitative data? Forensic Science International: Genetics, 2015. 16: 13-16.

The following is a subset of other papers that support the validation and use of STRmix™:

1. J.-A. Bright, I.W. Evett, D. Taylor, J.M. Curran and J.S. Buckleton, A series of recommended tests when validating probabilistic DNA profile interpretation software. Forensic Science International: Genetics, 2015. 14: 125-131
2. T.W. Bille, S.M. Weitz, M.D. Coble, J.S. Buckleton, J.-A. Bright. Comparison of the performance of different models for the interpretation of low level mixed DNA profiles. ELECTROPHORESIS. 2014;35:3125-33.
3. S.J. Cooper, C.E. McGovern, J.-A. Bright, D. Taylor, J.S. Buckleton. Investigating a common approach to DNA profile interpretation using probabilistic software. Forensic Science International: Genetics, 2014. 16: 121-131.

Appendix 2: Cross reference for document sections and SWGDAM recommendations

Standard	Text	Refer section
4.1	Test the system using representative data	Preamble
4.1.1	Specimens with known contributors	Preamble
4.1.2	Hypothesis testing with contributors and non-contributors	D
4.1.3	Variable DNA typing conditions	Preamble
4.1.6	Mixed specimens	D
4.1.6.1	Various contributor ratios	D
4.1.6.2	Various total DNA template quantities	D
4.1.6.3	Various numbers of contributors	D
4.1.6.5	Sharing of alleles among contributors	D
4.1.7	Partial profiles	D
4.1.7.1	Allele and locus drop-out	D
4.1.12	In-house parameters	Preamble
4.1.13	Sensitivity, specificity and precision	D