

**Validation of the STRmix™ Software – Addendum****Five-person mixtures, Specificity testing using an expanded randomly generated 10K person Database, and GlobalFiler Drop-in Rate**

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*Introduction*

Additional validation studies were conducted to expand our capabilities to include running unconditioned 5-person DNA mixtures. As a part of those studies specificity testing using a database of ten thousand randomly generated DNA profiles was conducted. In addition, the drop-in rate previously used for STRmix was based upon our previous experience with the Identifiler Plus amplification kit. This rate was to serve until we could determine an appropriate value to use for the GlobalFiler amplification kit. With four months of casework experience using the GlobalFiler amplification kit, a study was undertaken to determine an appropriate drop-in rate for use within STRmix.

*Deconvolution of 5-person mixtures and associated likelihood ratios*

Five person mixtures are generally very complex, and require much consideration before interpreting. The number of contributors is difficult to determine in complex mixtures because of the potential for allele sharing. In higher order mixtures, the number of contributors is often underestimated (Reference 1). And while interpretation can still be done with a higher or lower number of contributors, there can be detrimental effects on the likelihood ratio (LR) when the number of contributors is incorrectly estimated (Reference 2). Additionally, when the number of contributors increases, the ability to distinguish between a true and a false proposition is reduced (Reference 3). However, recent studies acknowledge that the complexity of mixtures being encountered in casework is increasing, and work is being done to investigate interpretation of 5-person mixtures. Evidence suggests that true contributors could be distinguished from known non-contributors even in five person mixtures (Reference 4). Specifically, this study tests the deconvolution of 5 person mixtures and examines the distribution of LR values between known contributors and known non-contributors in a set of mixtures designed to test the limits of STRmix.

The deconvolution of complex mixtures requires more computing power. The validation of single source samples, 2-, 3- and 4- person mixtures was described in detail in the STRmix validation write-ups approved in October, 2015. At that time, computers limited the analysis of 5 person mixtures; they could only be completed if one of the contributors was assumed. Since that time, the lab acquired two computers that are able to deconvolute 5 person mixtures without assuming a contributor. Computational limits do still exist with these computers, and the results of twelve 5-person mixtures are described in this study.

*Purpose*

The goal of this study is to determine whether the STRmix deconvolution of 5-person mixtures result in inclusions of known-contributors and exclusions of known non-contributors when compared to a large number of subjects (specificity) and whether the deconvolution remain effective as template decreases (sensitivity). Using the LR of known contributors, and the LR of known non-contributors, the inconclusive range was also examined for interpretation purposes.

*Materials and Methods*

This study was designed to test several of the STRmix limits in the SDPD lab. The mixtures included two main types of mixture combinations and a range of template amounts so as to assess the MCMC in a variety of contexts (i.e. in the presence of dropout, in balanced mixtures, and in both high and low template samples). The construction of these samples utilized 4 different groups of five people amplified with the GlobalFiler kit at different target amounts (Table 1). Using both DNA quantification value and average peak height of the single source samples, input amounts were adjusted to create a set of 12 mixtures that fell within GlobalFiler validation recommendations (under 10,000 RFU). The lowest total input amount for the 5 person mixtures was ~150pg, and the highest was over 2ng. These two mixture combinations were chosen because we already know that STRmix handles samples with discreet contributor proportion (i.e., samples with less ambiguity 60:30:10) well from previous validation work, so only two mixture proportion types were used. One set of these mixtures was designed to have one contributor with about 6 times the amount of template DNA with the remaining contributors having roughly equal template amounts. The second set of mixtures was completely balanced. One of the limits being tested here is computational limits. Balanced contributor proportions increase the ambiguity, and therefore test these limits. See pages at the end for electropherograms.

**Table 1: Five person mixture design**

<b>Five Person Mixtures</b>			
<b>Ratio (% contribution)</b>	<b>Sample ID Target High level (3K-10K RFU)</b>	<b>Sample ID Target Mid level (1K-3K RFU)</b>	<b>Sample ID Target Low level (&gt;1.5K RFU)</b>
20:20:20:20:20	5-1	5-5	5-9
	5-2	5-6	5-10
60:10:10:10:10	5-3	5-7	5-11
	5-4	5-8	5-12

All 12 of these mixtures were evaluated with STRmix (casework settings) as 5 person mixtures. Each one was also run as a 5 person mixture with one of the contributors assumed in both H<sub>1</sub> and H<sub>2</sub>. The contributor that the mixture was being conditioned on was always a low level contributor (~10-20%). Over the course of this validation study, three different types of computers and two different STRmix software versions (2.3.06 and 2.3.07) were used to attempt

the deconvolution of these 5 person mixtures. Initially, the computers with 32 GB of RAM and dual core processor would only be able to finish mixture deconvolution if one of the known, low level contributors was assumed. The first set of custom computers with 128 GB and 8 cores. This computer was able to deconvolute more of the 5 person mixtures, but still ran into computational limitations. The current set of computers custom designed for our lab to run STRmix (STRMIX1 and STRMIX2) have 128 GB of RAM and 8 cores. See results for a full description of mixture deconvolution success.

Mixture deconvolution is not the only aspect of STRmix that is computationally intensive. Generating PDF reports for complex mixtures is also very computationally intensive. STRMIX1 and STRMIX2 have been set up to generate an HTML document that could be used as a substitute for the PDF document, if necessary. See Figure 1 for an example of the first page of the HTML document. Results below describe the time needed and size of PDFs generated for these mixtures on the STRMIX computers (Table 3).

STRmix V2.3.07 - User: FBuser					
Analysis run:	25 February 2016 23:58				
Case number:	5 prsn				
Sample ID:	5-10 cond				
Comments:	93				
SUMMARY OF INPUT DATA					
Kit Used	SDPD GlobalFiler				
Number of Contributors	5				
Input Files	02_G02_5-10.csv				
Known contributors under Hp	93.csv				
Known contributors under Hd	93.csv				
SUMMARY OF CONTRIBUTORS					
<b>Contributor</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>
DNA Amounts	188	288	228	192	136
Mixture Proportions	18%	28%	22%	19%	13%
Degradation starting at	85.0bp	0.450 rfu/bp	0.558 rfu/bp	0.305 rfu/bp	0.252 rfu/bp
	0.159 rfu/bp				
RUN INFORMATION					
Total iterations	1.8005330332E10		Gelman-Rubin convergence diagnostic 1.56		
Inter replicate efficiency	• PCR 1 - 100.00%		Allele variance	7.20	
Effective sample size	16476.99		Stutter variance	15.30	
Average (log) likelihood	37.76		Seed value	684160	
Mx prior mean	n/a		Mx prior variance	n/a	

Figure 1 – First section of the HTML document of a conditioned 5 person balanced mixture.

Once the 5-person mixtures were successfully deconvoluted, a database search was used, with the minimum LR set to 0. This was a database of 76 profiles containing all five known contributors and 71 known non-contributors. This was designed to provide a lot of information about how known contributor LRs compare to known non-contributor LRs.

A LR from a database search does not result in the same number as when that profile compared to a previous analysis directly. Each of the individuals in the database are considered as a potential contributor in turn to the mixture under the following two hypotheses:

H<sub>p</sub>: Database individual and N - 1 unknown contributors

H<sub>d</sub>: N unknown contributors

where N is the number of contributors under consideration, as set by the analyst in the STRmix mixture analysis. The likelihood ratios in this feature are run without the Balding and Nichols adjustments to improve run time.

Each of the profiles of the known contributors was available for an individual comparison to the mixture. Data from this LR calculation will be presented below. Even though the database search LRs are not exactly the same as the LR following an individual comparison, they are not unrelated to the individual comparison LRs. Database search LRs and individual LRs are presented from a subset of the 5 person mixtures below.

### *Results and Discussion*

#### Run time and statistics of 5 person mixtures

Five person mixtures have been attempted with STRmix for over a year. Until recently, computers (RAM and processors) have limited their successful deconvolution. Table 2 details the type of mixture, characteristics and overall success of run.

A subset of the mixtures has been run with both the previous version of STRmix (2.3.06) and the current version (2.3.07) to verify reproducibility. Results were reproducible between the two different versions. Six of the mixtures (no conditioning) were not completed in the SDPD lab. There is no consistent characteristic (ex: dropout, balanced contribution level vs. one person contributing the majority of the DNA, lower RFU peaks vs. higher RFU peaks) that accurately predicts success of the run. See electropherograms following this document for peak heights observed in each mixture.

**Table 2 – Summary of 5 person mixture deconvolutions**

Mixture number and scenario	STRmix version	Successful completed run?	Contributor description	Dropout?
5_1	Both	Yes	all balanced	No
5_1 conditioned	2.3.07	Yes		
5_2	Both	No	all balanced	No
5_2 conditioned	2.3.06	Yes		
5_3	Both	Yes	one 60% contributor	No
5_3 conditioned	2.3.06	Yes		
5_4	2.3.06	Yes	one 60% contributor	Yes
5_4 conditioned	2.3.07	Yes		
5_5	Both	No	all balanced	Yes
5_5 conditioned	2.3.07	Yes		
5_6	Both	Yes	all balanced	Yes
5_6 conditioned	2.3.07	Yes		
5_7	Both	Yes	one 60% contributor	Yes
5_7 conditioned	2.3.07	Yes		
5_8	2.3.07	No	one 60% contributor	Yes
5_8 conditioned	2.3.07	Yes		
5_9	2.3.07	No	all balanced	Yes
5_9 conditioned	2.3.07	Yes		
5_10	2.3.07	Yes	all balanced	Yes
5_10 conditioned	2.3.07	Yes		
5_11	2.3.07	No	one 60% contributor	Yes
5_11 conditioned	2.3.06	Yes		
5_12	2.3.07	No	one 60% contributor	Yes
5_12 conditioned	2.3.07	Yes		

The mixtures with no conditioning were attempted using several different computers. Two computers (STRMIX1 and STRMIX2) have now replaced the previous computers, and these computers have version 2.3.07 of STRmix. Table 3 lists the mixtures that were run on these two computers, and details the run time and file generation by these computers. Mixtures that were not successful with version 2.3.07 on these computers always terminated before 4 days with an error message suggesting that maximum computing capacity had been reached (a java heap space reference). In some cases, PDF generation was terminated early because an HTML document was created. The mixtures ranged from 7 to 25 hours of computing time without conditioning on one contributor. Only one of these mixtures 5-1 (duplicate) had a PDF, which took ~46 hours to generate, and was over 2,000 pages long. The mixtures that were conditioned took three to seven hours to complete the deconvolution, and their PDFs took considerably shorter time to generate (with the exception of mixture 5-10). This table does not detail every type of 5 person mixture that will be encountered, but gives an idea as to how long analysis may take.

**Table 3 – Mixtures that were run on the STRMIX1 or STRMIX2 computer**

<b>Run time and file generation with STRMIX1 and STRMIX2 computers</b>				
Mixture number and scenario	PDF created?	Run time (rounded to closest hour)	PDF # pages	PDF file size (MB)
5_1	No	12		
5_1 (duplicate)	Yes	12 (plus PDF: 46)	2018	7.9
5_1 conditioned	Yes	7 (plus PDF: 16)	774	3.2
5_4 conditioned	Yes	3 (plus PDF: 2)	466	2.1
5_5 conditioned	Yes	7 (Plus PDF: 3)	722	3
5_6	No	7		
5_6 conditioned	No	5		
5_7	No	25		
5_7 conditioned	Yes	4 (Plus PDF: 2)	547	2.4
5_8 conditioned	Yes	4 (Plus PDF: 5)	636	2.7
5_9 conditioned	No	11		
5_10	No	13		
5_10 conditioned	Yes	6 (Plus PDF: 26)	2536	9.7
5_12 conditioned	Yes	7 (Plus PDF:2)	603	2.6

### Likelihood ratios

The LRs generated during this study for each of the mixtures were very informative about the quality of the deconvolution and the inclusion of known contributors and exclusion of known non-contributors. Where this statement breaks down is indicative of some of the STRmix interpretation limits. One of the two methods used to generate LR values was to search a database consisting of 76 individuals which include the 5 known contributors in each mixture. Table 4 lists the LR values of the 5 known contributors to each mixture. This table demonstrates strong support for inclusion of all contributors for most of the mixtures. In the mixtures with a 60% contributor, that person was always included with an extremely high LR (at least  $10^{25}$ ). In some cases, even the low level contributors were included with a very strong LR. However, much uncertainty is introduced with stochastic effects. This is evident starting in mixture 5-4, with one contributor that has some alleles dropping out. One of the contributors has 7 instances of known dropout. This same contributor has an LR of 0.003. With the current Likelihood Ratio and Verbal Wording scale, that would suggest limited support for exclusion of this contributor. And almost all of the low level mixtures (even with conditioning) have at least one contributor falling into the inconclusive range, because of the amount of dropout.

**Table 4 – Likelihood ratios for the 5 known contributors in each mixture using the 76 person database search**

<b>5_1</b>	<b>5_1 conditioned</b>	<b>5-2 conditioned</b>	<b>5_3</b>	<b>5-3 conditioned</b>	<b>5_4 (2.3.06)</b>	<b>5_4 conditioned</b>
2.13E+13	7.41E+24	1.06E+28	2.91E+27	7.86E+29	1.06E+28	2.34E+32
3.55E+09	1.26E+20	2.21E+18	7.75E+10	3.59E+27	4.45E+12	1.06E+28
5.89E+07	1.92E+11	2.64E+10	2.03E+10	3.36E+13	1.27E+11	5.28E+15
1.32E+07	5.37E+09	6.46E+08	9.53E+06	3.84E+09	4.13E+08	4.05E+12
8.79E+05	4.10E+09	2.98E+08	1.70E+06	3.80E+09	0.003	3.35
<b>5_5 conditioned</b>	<b>5_6</b>	<b>5_6 (2.3.06)</b>	<b>5_6 conditioned</b>	<b>5_7</b>	<b>5_7 (2.3.06)</b>	<b>5_7 conditioned</b>
3.77E+26	9.55E+11	9.74E+11	1.06E+28	1.62E+27	1.66E+27	7.86E+29
7.41E+24	2.57E+11	3.01E+11	3.52E+13	2.89E+12	2.27E+11	2.41E+27
5.56E+11	1.32E+08	1.14E+08	5.47E+12	2.24E+09	3.63E+08	5.49E+17
9.48E+07	3.37E+07	9.83E+07	1.03E+09	3.39E+08	1.71E+08	1.65E+11
1.60E+07	3.04E+06	9.70E+05	9.35E+07	1.32E+08	3.89E+06	2.67E+09
<b>5-8 conditioned</b>	<b>5-9 conditioned</b>	<b>5-9 (duplicate) conditioned</b>	<b>5_10</b>	<b>5_10 conditioned</b>	<b>5-11 conditioned</b>	<b>5-12 conditioned</b>
2.34E+32	1.57E+22	1.57E+22	1.45E+15	1.06E+28	7.86E+29	2.34E+32
1.03E+28	3.68E+05	3.84E+05	7.49E+07	1.81E+16	3.61E+27	9.61E+27
9.53E+16	72.87	52.16	1.13E+07	2.83E+08	6.95E+08	1.02E+14
2.86E+10	12.72	23.75	524.52	4.83E+07	1.88E+07	2.72E+12
7.58	0.60	2.25	95.93	311.39	1.76E+05	1.52
Limited support for exclusion			Inconclusive	Limited support for inclusion		

Four of the mixtures (5-4, 5-8 conditioned, 5-9 conditioned, and 5-12 conditioned) had known non-contributors with LR values above one or more of the known contributors. The highest LR that a known-non contributor had been 363 in mixture 5-9 conditioned. With the current Likelihood Ratio and Verbal Wording scale, that would suggest limited support for inclusion of this known non-contributor. Figure 2 below shows Mixture 5-1 as an example of the majority of the results. All 5 known contributors (red dots) were included, and all 71 other people were excluded (LR = 0 is not shown on these graphs). Three other mixtures are shown here to give some context for the LR values that other known non-contributors had in relation to the five known contributors that are considered inconclusive (or excluded). The LRs of the known non-contributors hover around the same order of magnitude as the lowest known contributor, or have LR values closer to 0. Mixture 5-8 conditioned is the only one of these 4 not shown in Figure 2. It had only one known non-contributor with a LR value of 20. The 5 known contributors in all other mixtures had the top 5 highest LR values using the results from this 76 person database search, and are listed in Table 4.



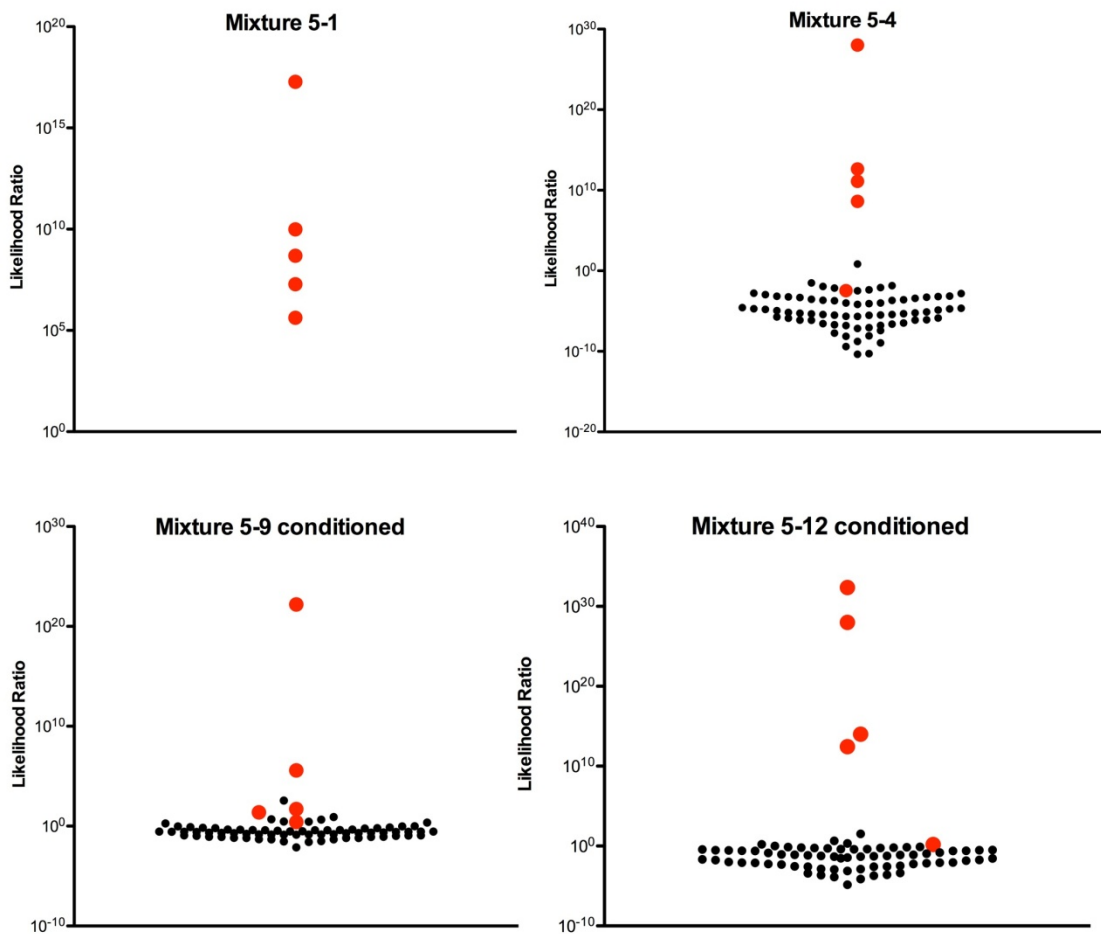


Figure 2 – Likelihood ratios from 76 person database search; 5 known contributors are highlighted in red.

Database search LRs provide a lot of insight about the inclusion of known contributors and known non-contributors for validation work. It is also important to examine the LRs generated individually using the “LR from previous analysis” feature in STRmix, because those LRs are calculated differently. These LRs incorporate theta, and they use the Balding and Nichols adjustment. “LR from previous analysis” can provide a point LR as well as the 99% lower HPD. The HPD incorporates a measure of allele and MCMC variance. Four representative examples were chosen to show all of these numbers side by side in Table 5 for a comparison. Within each of the mixtures, the difference in LR strength *between* the contributors is consistent in each LR measure. For example, the contributor with the highest HPD LR also has the highest point LR and database search LR, but the magnitudes of those numbers change between LR calculations. This is important to take into account for interpretation, because using the HPD value, even more of the known contributors would be inconclusive, or even excluded, because it is a more statistically conservative calculation.



**Table 5 – Three different LR<sub>s</sub> for the same contributor in 4 different mixtures**

Mixture	Contributor	HPD	Point LR	DB search LR
5_1 27% 23% 20% 18% 13%	1	2.37E+06	2.19E+07	3.55E+09
	2	3.58E+04	1.23E+05	8.79E+05
	3	9.37E+04	2.51E+05	1.32E+07
	4	2.24E+11	5.36E+11	2.13E+13
	5	1.51E+07	3.95E+07	5.89E+07
5_4 75% 8% 7% 5% 5% (2.3.06)	1	1.05E+26	2.38E+26	1.06E+28
	2	8.27E+09	1.56E+10	1.27E+11
	3	3.57E+06	1.34E+07	4.45E+12
	4	1.96E+07	4.69E+07	4.13E+08
	5	1.31E-03	3.20E-02	3.39E-03
5_7 59% 14% 11% 9% 7%	1	3.97E+25	1.55E+26	1.62E+27
	2	1.69E+07	3.52E+07	1.32E+08
	3	2.94E+06	1.25E+07	2.24E+09
	4	2.66E+06	1.30E+07	3.39E+08
	5	3.02E+08	9.08E+08	2.89E+12
5_10 31% 24% 19% 16% 10%	1	3.60E+03	1.22E+04	1.13E+07
	2	1.08E+04	4.43E+04	7.49E+07
	3	3.73	16.50	95.93
	4	1.23E+10	5.04E+10	1.45E+15
	5	22.30	125.00	524.52

*Conclusion*

This study supports the interpretation of 5 person mixtures, with several caveats. Interpretation of some 5 person mixtures will still be prevented due to the computational requirements. There are no consistent characteristic that would indicate a successful run or not, so it would be difficult to triage 5 person mixtures in advance.

Since these were mixtures designed and created in the lab, this study did not test the effects of analyzing these mixtures using an alternate number of contributors. In casework, more ambiguity will be associated with designating a mixture as a 5 person mixture. The work by Coble et al (Reference 1) describes the very large probability that the number of contributors will be understated. The probability of understating the number of contributors increases for higher order mixtures (especially mixtures of 4 or more contributors, and especially if dropout is occurring). This is true even with the additional discriminatory loci that GlobalFiler affords and taking peak height balance into account.

Likelihood ratios for contributors in higher order mixtures can still provide considerable weight to the evidence in many cases, but don't always, especially when dropout is apparent. The magnitude of the likelihood ratio should also be considered, especially when this LR is between

0.001 and 1000. The conclusions made here are supported by data published in the field as well as data collected from within the SDPD lab.

### *References*

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### *Specificity Testing of STRmix Deconvolutions using a Database of 10,000 Randomly Generated DNA Profiles.*

#### *Introduction*

##### *Purpose*

The goal of this study is to consider the inconclusive range for 2- to 5-person mixtures given likelihood ratios of known contributors and known non-contributors.

##### *Materials and Methods*

Nineteen of each: single source, 2 person, 3 person, 4 person and 5 person mixtures were used in this study. The mixtures spanned a wide range of mixture proportions, total DNA input, and included mixtures with and without allelic dropout. Additional samples with extreme dropout were also used. All mixtures were previously evaluated with STRmix, using software version 2.0, 2.3.06 or 2.3.07. An important dataset in determining an accurate inconclusive range is LR values of many non-contributors. Using STRmix version 2.3.07, each of these deconvolutions was searched against a database of 10,000 randomly generated profiles. These profiles were generated randomly, reflecting the NIST African American allele frequencies. The minimum LR for the database search was set to 0, so that each profile searched had an LR value associated with it. As a result there were 190,000 LR values to sort through for each single source, 2, 3, 4, and 5-person mixture (a total of 760,000 LRs).

##### *Results and Discussion*

Likelihood ratios from the 10,000 random profile database were compiled. The vast majority of the results were 0 in every sample searched against this database. Robust single source samples and two person mixtures did not have any known non-contributors with LR values other than 0. When locus information was severely dropped out, Type 1 errors (false positives) started to occur. For example, when alleles were present at only 7 loci of a single source profile, three profiles in the random profile database had LR values above 100: the highest was 2794 and the lowest was 284. When this is considered together with the information that the LR of the known contributor was only 1090, the false matches are understandable and to be expected at that level of inclusion (and ambiguity). LR values of known non-contributors also increased to between 1 and 100 for two person mixtures under circumstances where only one or two non-stacking alleles from a second contributor were present in the mixture.

The top values for 3, 4 and 5 person mixtures are presented in Tables 5-7.

**Table 6– 10,000 random profile database search: highest 5 LR for each 3 person mixture**

<b>3_1</b>	<b>3_4</b>	<b>3_28</b>	<b>3_64</b>	<b>3_5</b>	<b>3_9</b>	<b>3_17</b>
0	1.54E-06	0	6.61E-08	9.77E-05	0	2.27E-17
0	7.79E-07	0	2.64E-11	3.59E-07	0	2.36E-18
0	4.01E-07	0	1.85E-11	4.32E-08	0	1.11E-19
0	1.51E-07	0	1.23E-11	1.66E-08	0	6.12E-20
0	1.05E-07	0	1.11E-11	1.52E-10	0	4.23E-20
<b>3_21</b>	<b>3_29</b>	<b>3_33</b>	<b>3_37</b>	<b>3_49</b>	<b>3_53</b>	<b>3_25</b>
0	0	3.46E-10	0	3.29E-14	0.07	790.01
0	0	4.01E-12	0	1.15E-16	6.30E-03	2.71
0	0	8.52E-13	0	1.09E-18	3.36E-03	1.94
0	0	7.51E-13	0	1.78E-20	2.73E-03	1.66
0	0	7.72E-14	0	4.50E-21	2.56E-03	0.97
<b>3_41</b>	<b>3_57</b>	<b>3_65</b>	<b>3_45</b>	<b>3_61</b>		
6.69	1.52	13.78	83.45	125.76		
3.22E-08	0.39	2.20	8.10	11.09		
2.39E-09	1.87E-02	1.31	6.82	5.34		
9.47E-11	1.32E-02	0.60	4.04	1.92		
7.90E-12	1.30E-03	0.53	0.51	0.47		

>100
>1, <100

Table 1 lists the top LR values of the random profiles when compared to 3-person mixtures. Of the 190,000 LR values from the random profiles, only two were higher than 100. Mixture 3-61 has a contributor breakdown of 63:19:12. There were 8 instances of dropout in this mixture. The LR values of the known contributors were:  $1.31 \times 10^{31}$ ,  $5.85 \times 10^{13}$ , and  $1.53 \times 10^{-3}$ , respectively. With so little information about whether the third contributor is included in the mixture, and after 10,000 comparisons to random profiles, it is reasonable for even more of the random profiles to have LR values well over 100.

Mixture 3-25 is very similar to 3-61 in the sense that there is one contributor responsible for the majority of the signal, and the other two minor contributors are relatively balanced. Mixture 3-25 is a 77:12:11 mixture. There were 6 instances of dropout. The LR values of the known contributors were:  $4.03 \times 10^{28}$ ,  $3.87 \times 10^{11}$ , and  $1.15 \times 10^7$ .

Table 2 lists the top LR values of the random profiles when compared to 4-person mixtures. Even with some dropout and a few balanced, low level contributors, there are no Type 1 errors where a known non-contributor is included in the mixture. Referencing the 76 person database search results, the highest LR of a known-non contributor was 37. Searching against this random profile database provided further support for an inconclusive range of 0.01 to 100 for mixtures of 4 people or less.

**Table 6– 10,000 random profile database search: highest 5 LR's for each 4 person mixture**

<b>4_3 (2)</b>	<b>4_7</b>	<b>4_11</b>	<b>4_15</b>	<b>4_19</b>	<b>4_23</b>	<b>4_23 (2)</b>
0	0	2.33E-06	5.24E-07	0	1.74E-09	3.71E-08
0	0	2.21E-06	2.34E-12	0	8.85E-13	2.77E-08
0	0	4.74E-07	1.98E-12	0	1.29E-13	2.96E-11
0	0	4.00E-07	6.74E-14	0	5.08E-14	2.39E-11
0	0	3.19E-07	1.93E-15	0	2.95E-18	5.71E-12
<b>4_27</b>	<b>4_27 (b)</b>	<b>4_27 (2)</b>	<b>4_31</b>	<b>4_35</b>	<b>4_35 (2)</b>	<b>4_35 (NR)</b>
1.14E-04	6.28E-05	5.53E-04	0	3.86E-04	1.10E-03	3.12E-06
1.25E-07	3.13E-09	3.59E-09	0	1.71E-04	1.31E-04	2.93E-07
1.34E-09	0	2.59E-13	0	1.12E-05	2.80E-06	8.06E-08
9.01E-10	0	1.76E-16	0	4.57E-06	1.36E-06	3.80E-08
7.79E-10	0	6.21E-18	0	1.12E-06	1.16E-06	9.63E-09
<b>4_39</b>	<b>4_39 (NR)</b>	<b>4_43</b>	<b>4_43 (2)</b>	<b>4_47</b>		
0	0	0	3.61E-17	0.46		
0	0	0	1.06E-17	1.87E-02		
0	0	0	3.45E-19	4.68E-03		
0	0	0	2.90E-22	6.24E-04		
0	0	0	6.02E-23	3.89E-04		

>100
>1, <100

Table 3 lists the 5 person mixtures that were searched against the database, and the top 5 LR's from the random profiles.

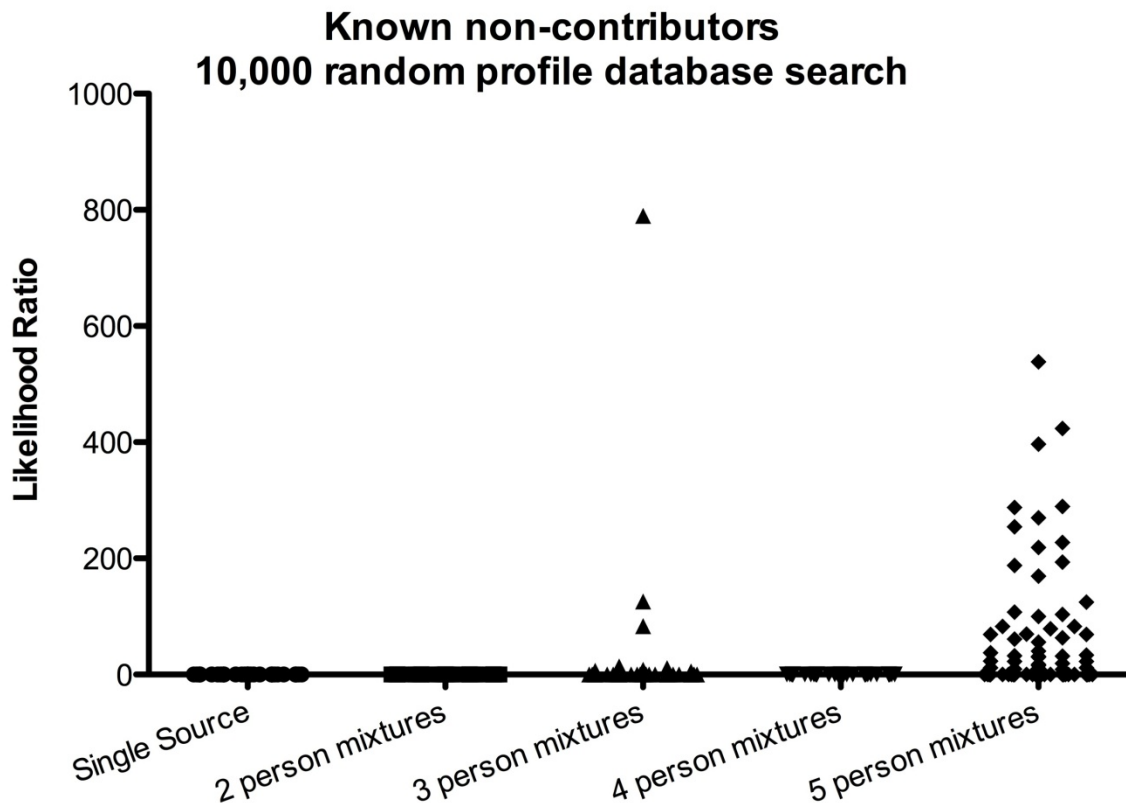
Table 7– 10,000 random profile database search: highest 5 LR for each 5 person mixture

<b>5-1 (2.3.07)</b>	<b>5-1 (2.3.06)</b>	<b>5-1 conditioned</b>	<b>5-2 conditioned</b>	<b>5_3</b>	<b>5-3 conditioned</b>	<b>5_4</b>
3.13E-05	6.08E-07	1.15E-18	0	0.51	0.00	538.62
2.47E-06	8.12E-12	4.91E-20	0	0.01	1.02E-04	69.40
1.71E-06	2.28E-13	3.70E-20	0	0.01	1.32E-05	31.46
1.32E-06	7.06E-15	1.33E-23	0	0.00	4.23E-06	15.75
1.67E-07	2.55E-15	1.49E-27	0	0.00	1.76E-06	12.03
<b>5-4 conditioned</b>	<b>5-5 conditioned</b>	<b>5_6</b>	<b>5-6 conditioned</b>	<b>5_7</b>	<b>5-7 conditioned</b>	<b>5-8 conditioned</b>
287.67	7.73	1.82E-06	1.39E-16	107.33	0.01	289.33
254.18	0.07	2.37E-07	1.80E-17	32.47	1.77E-04	61.03
78.67	0.07	2.12E-07	2.53E-19	0.54	1.35E-05	40.63
69.49	0.03	3.39E-08	0	0.04	1.47E-06	29.87
63.42	0.02	1.47E-08	0	0.01	8.16E-07	22.55
<b>5-9 conditioned</b>	<b>5_10</b>	<b>5-10 conditioned</b>	<b>5-11 conditioned</b>	<b>5-12 conditioned</b>		
396.86	423.64	33.53	100.24	227.54		
124.91	270.11	19.08	68.92	218.85		
55.88	193.67	17.67	37.43	188.18		
32.08	169.47	9.61	22.62	103.77		
23.34	83.11	9.17	11.74	83.07		

>100
>1, <100

Searching 5 person mixtures against the random profile database yielded 16 LR's greater than 100 for some randomly generated profiles. There are many more LR's that fall between 1 and 100. Results from the deconvolution of 5 person mixtures demonstrate that known contributors are included, except in mixtures with one or more stochastic level contributors and dropout. As the overall peak height of a known contributor decreases, the LR associated with their inclusion also decreases.

When looking at the number of Type I errors that occur between 2 to 5 person mixtures, it is clear that 5 person mixtures have more than any other type Figure 3.



**Figure 3– 10,000 random profile database search: LR values from all mixtures tested.**

Results from 5 person mixtures demonstrate that while most known non-contributors have exclusionary LR values, the mixtures with a lot of ambiguity more consistently have LR values above 100, when compared to 2, 3 and 4 person mixtures. With the current LR interpretation scale/verbal wording, some of those known non-contributors would be included. This suggests that the inconclusive range should be expanded for 5 person mixtures. The highest LR of a known non-contributor from the 76 person database was 363, and the highest LR from the 10,000 random profile database was 539. An inconclusive range of 0.001 to 1000 would increase the range an order of magnitude for 5 person mixtures, which reflect the LR values that were collected as part of this study. There were many comparisons done over the course of this study, which results in the overall chance of seeing an LR of a known non-contributor over 100 being very low. Even though the chance of a non-contributor having an LR over 100 is low, the ambiguity of the 5 person mixtures results in much different pattern of LR values between 100 and 1000 than the other orders of mixtures. Expanding the inconclusive range for these mixtures would serve to minimize the chance of falsely including a person in a 5 person mixture.

Expanding the inconclusive range does have trade-offs. One of the implications is the LR value at which a contributor is included with limited, moderate and strong support. Referencing the 5 person mixture study, this would mean that several of known contributors would shift from limited support for inclusion to inconclusive or excluded.

### *Conclusion*

The data collected as part of this study suggests that the current Likelihood Ratio and verbal wording scale for single source samples, 2-, 3-, and 4-person mixtures should remain the same, with an inconclusive range of 0.01 to 100. Once the interpretation of 5 person mixtures begins, the inconclusive range for these types of mixtures will be 0.001 to 1000. The Limited Support for inclusion/exclusion category will be eliminated and the Moderate Support (1000-10,000 or 10-3-10-4) will remain the same as the other types of mixtures as will the Strong Support and Excluded categories.

### *References*

6. STRmix v2.3 Users Manual. Issued by Institute of Environmental Science and Research Limited; Date of Issue: 8 October 2015
7. An assessment of the information content of likelihood ratios derived from complex mixtures. Marsden CD, Rudin N, Inman K, and Lohmueller KE. *Forensic Science International: Genetics* 22 (2016) 64-72.
8. Searching mixed DNA profiles directly against profile databases. Jo-Anne Bright, Duncan Taylor, James Curran, John Buckleton. *Forensic Science International: Genetics* 9 (2014) 102–110.

### *GlobalFiler Drop-in Rate*

STRmix provides the option of considering the possibility of accounting for the possibility of drop-in during mixture deconvolution. DNA is present at low levels in the environment, and the sensitivity of the DNA typing methods being employed make it possible to detect very small amounts of DNA. Drop-in is defined as one or two unexplained peaks observed within a profile. Drop-in is on a continuum of contamination with unattributable DNA types (drop-in) on one end and a full profile contaminant that can be attributed to a specific source on the other.

The presence of an extra or unexpected allele detected in a sample was observed using our previous amplification methods (i.e., Identifiler Plus at 28 cycles). In the first part of the STRmix validation, the drop-in rate assumed for the GlobalFiler amplification kit was based on historical data from the use of the Identifiler Plus amplification kit. This rate was to serve until such time as we had sufficient data that we could establish a drop-in rate for the GlobalFiler kit within our laboratory.



To obtain an estimate of drop-in, the parameters of number of observations of drop-in out of the total number of possibilities (loci x sample number) and the heights of those peaks are required. The height of a drop-in peak should follow a gamma distribution (1).

For the SDPD GlobalFiler data, peaks attributable to drop-in were assessed from the validation, training and casework results from the validation period as well as the forensic casework completed from October 2015 through February 2016 (a five month period). The number of times unattributable types were detected in reagent blanks, positive or negative amplification controls, and validation/training samples were counted.

From the sample set described, the drop-in rate was too low to perform the maximum likelihood estimation that would produce the  $\alpha$  and  $\beta$  variables of the gamma distribution. Therefore, the flat rate of the drop-in frequency will be used for STRmix. The data indicated that 9 instances of drop-in were observed from a total of 686 sample (16,464 loci). This equates to a drop-in rate of 0.000546. The maximum height of the observed drop-in peaks was 271RFU. The drop-in cap should be set at a value slightly higher than the highest observed drop-in peak. For that reason the drop-in cap will be set at 300 RFU. This rate is higher than that determined for the Identifiler Plus kit (0.000363), which is to be expected given the extra PCR cycle employed by the testing kit procedure.

We will continue to monitor the drop-in rate as additional data becomes available. This will be accomplished through the use of the unexpected results summaries.

1. Puch-Solis R. A dropin peak height model. Forensic Science International: Genetics. 2014;11:80-4.

#### *Summary of the additional validation work*

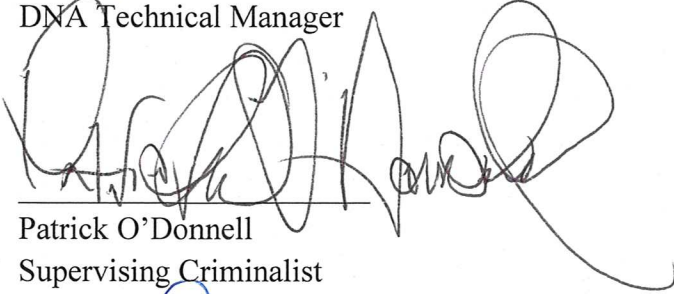
5-person mixtures can be deconvoluted using STRmix. Provided the computers have enough memory to produce a result, the results obtained from STRmix on 5-person mixtures provided accurate inclusions. For some mixtures known contributors were determined to be inconclusive or had results slightly favoring exclusions. This was due to the occurrence of drop-out as well as the general ambiguity of the results for those contributors.

Further studies using a randomly generated database of ten thousand DNA profiles indicates that 5-person mixtures result in data that is generally more ambiguous and can lead to the potential inclusion of known non-contributors. The specificity study led to the conclusion that known non-contributors were more likely to be included with likelihood ratios over our current inconclusive range of 100, but all had likelihood ratios less than 1000. For this reason, it was decided that our verbal scale for 5-person mixtures would be expanded to  $10^{-3}$  to  $10^3$  while remaining unchanged for mixtures of four or fewer people. This effectively eliminates the “limited support” category for 5-person mixtures.

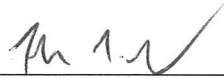
We will change our drop-in rate and cap for STRmix to that calculated for that observed for the five months of use since implementation of the GlobalFiler amplification kit in casework. This rate is higher than that observed for the Identifiler Plus kit, which is to be expected given the extra PCR cycle employed by the testing kit procedure. The observed drop-in rate was calculated to be 0.000546 and the new drop-in cap will be set at 300 RFU.



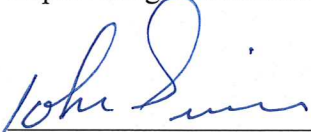
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DNA Technical Manager



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Supervising Criminalist



Frank Healy  
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John Simms  
Quality Manager