Examining the Risk Associated with Qualitative Exclusions in Forensic DNA Casework

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Opening Question

The location depicted to the right is part of what appears to be a two-person STR DNA mixture. If this was the only location that had four peaks, would you qualitatively exclude a person of interest who is a 17,25.2 at this location?

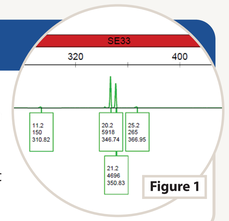

Figure 1

Abstract

Based on practical experience, assessing the number of contributors in a forensic DNA sample can be confounded by multiple factors including allele sharing, the presence of peaks below the laboratory's stochastic threshold, stochastic effects that can result in peak height imbalance, elevated stutter, and the possibility of allelic drop-in. As a result, a single locus that appears to have full representation of all contributors under certain assumptions, and therefore suitable for exclusionary assessments, may be affected by one or more of these factors. This can result in a false exclusion of an individual of interest. Many of the concerns that would make profiles uninterpretable for inclusionary purposes are likewise applicable and can be equally concerning when utilizing exclusionary criteria during manual interpretation efforts (i.e., interpretation without the use of a forensic expert software). Profiles, or portions of profiles, that are determined to be uninterpretable often present with incomplete or limited data. Additionally, profiles in which an accurate determination of number of contributors cannot be made or when the number of apparent contributors exceeds the laboratory's validation parameters may lead to an inconclusive result for comparison to known reference samples.

With the advent and implementation of probabilistic genotyping, available software can more conclusively determine an inclusion or exclusion for complex DNA profiles using statistics and considering phenomenon (e.g., drop-in, drop-out, stutter by allele including longest uninterrupted stretch) that analysts are aware of but do not have the ability to incorporate during qualitative exclusions. In addition, when the software is applied correctly by a trained analyst, assessing performance diagnostics can give additional insight into the number of contributors present in the profile.

Validation data including known single source and two-person mixtures of varying templates were used to assess drop-in, stutter, and allelic modeling with the addition of simulated drop-in alleles above and below the laboratory's drop-in cap. Limitations were further explored by varying peak heights of the alleles for the known donors (i.e., modifying major and minor peak heights to evaluate stutter where the peak in stutter position was consistent with the minor donor). In some cases, for the mixture samples, a false manual exclusion could be made based on the apparent number of contributors without accounting for stutter and/or drop-in, whereas STRmix™ was able to account for these phenomena and/or had diagnostic flags (e.g., LR=0 at one locus) that would warrant additional scrutiny by the analyst. Overall, the results of the study demonstrate how readily a false manual exclusion can occur when an analyst does not consider all variables (e.g., elevated stutter, drop-in).

Materials and Methods

Single source and two-person mixtures from validation data utilizing the QIAGEN Investigator® 24plex QS PCR Amplification Kit were run utilizing STRmix™. Artificial drop-in peaks as well as modified stutter ratios/peak heights were used to examine when a false exclusion may occur in a set of four experiments.

Parameter	Value
Drop-in cap	200
Drop-in rate parameter	0.0001
Drop-in parameters	0,0

Table 1: *STRmix drop-in parameters utilized.*

See individual experiments for detailed Materials and Methods.

Experiment #1

Materials & Methods for Experiment #1
Simulated Drop-in Peak Below the Drop-In Cap (Single Source)

- Three single source profiles at total inputs of 1ng and 0.1ng
- Four drop-in alleles were added *below* the drop-in cap at 150 RFU
- A low molecular weight locus (D10S1248) and a high molecular weight locus (SE33) with two distinct drop-in alleles evaluated for each donor
- Included both homozygous and heterozygous loci

Results

Simulated Drop-in Peak Below the Drop-In Cap (Single Source)

High template (1ng) samples:

- The artificial peak was modeled by STRmix™ as drop-in
- Peak did not reasonably pair with the donor's true allele(s)

Low template (0.1ng) samples:

- STRmix™ modeled the artificial peak as both drop-in and a true allelic peak
- The true donor alleles were at a similar level as the simulated drop-in peak

Drop-In Peak	Donor M1		Donor M3		Donor M4	
	Genotype	Weight	Genotype	Weight	Genotype	Weight
D10-9	[9,14]	0.99897234	[9,12]	0.99952838	[13,14]	1
D10-16	[14,14]	0.00102766	[12,12]	0.00047162		
SE33-11.2	[14,16]	0.99960445	[12,16]	0.99980982	[13,14]	1
SE33-19	[14,14]	0.00039555	[12,12]	0.00019018		

Table 2: *Genotype weights – Artificial drop-in peak at 150RFU, low template samples (donor true genotype in red)*

- The likelihood ratios most impacted by the modeling of the drop-in peak were for donors M1 and M3
- A false exclusion was not observed in this simulated set

Experiment #2

Materials & Methods for Experiment #2
Simulated Drop-in Peak Above the Drop-In Cap (Single Source)

- The same three single source profiles and same loci were utilized as in experiment #1
- The same four drop-in alleles were added *above* the drop-in cap at 250 RFU

Results

Simulated Drop-In Peak Above the Drop-In Cap (Single Source)

Donor heterozygous (M1-SE33, M3-SE33, M4-D10 and M4-SE33):

- Deconvolution *failed* as STRmix™ was unable to explain the locus as originating from one individual

Donor was homozygous (M1-D10 and M3-D10):

- A *false exclusion* was obtained for the true donor as the drop-in peak was modeled as an obligate allele

Drop-In Peak	Donor M1 (D10 = 14,14)				Donor M3 (D10 = 12,12)			
	0.1ng		1ng		0.1ng		1ng	
D10-9	9	250	9	250	9	250	9	250
	14	529	13	1765	12	525	11	794
			14	12805			12	11008
D10-16	14	529	13	1765	12	525	11	794
	16	250	14	12805	16	250	12	11008
			16	250			16	250

Table 3: *Peak heights (RFU) for allelic and stutter peaks and simulated drop-in peak (in red)*

Experiment #3

Materials & Methods for Experiment #3
Simulated Drop-In Peak Below the Cap (Two-person Mixture)

- Four replicates of a two-person mixture profile (two replicates at 10:1 mixture ratio and two replicates at a 20:1 mixture ratio). DNA inputs for all replicates were at 1ng
- A drop-in allele was added *below* the drop-in cap at 150 RFU at four distinct loci (D2S1338, D3S1358, SE33, and D21S11)
- Included loci where minor donor was both homozygous and heterozygous as well as with a minor donor peak in and not in stutter position to the major donor's allele
- Fifty replicate runs of one of the 20:1 mixtures was performed based on results

Results

Simulated Drop-In Peak Below the Cap (Two-person Mixture)

D2S1338 (minor homozygous, not in stutter position):

- The artificial drop-in peak (14) was modeled as >99% allelic for all replicates with some weight given to minor donor's true genotype
- Allele pairing with the simulated drop-in peak at 150 RFU would be considered intuitive given peak heights

D3S1358 (minor homozygous, in stutter position):

- The artificial drop-in peak (10) was modeled as allelic >90% for all replicates with some weight given to minor donor's true genotype
- Simulated drop-in peak at 150 RFU may be considered intuitive for the 20:1 mixture replicates given peak heights

SE33 (minor heterozygous, not in stutter position):

- When both minor obligate alleles were present, the artificial drop-in peak (11.2) was modeled as drop-in 100%
- When dropout of the minor donor was observed with only one allele above the analytical threshold detected, modeling of the artificial drop-in peak with the obligate minor allele occurred at >99%.
  - *An exclusionary LR (LR=0) was obtained*
  - The allele pairing with the simulated drop-in peak at 150 RFU would be considered intuitive.
- 50 replicate runs → In 24 of the 50 replicates, dropout was considered with low weighting
  - When dropout was considered, a likelihood ratio supporting inclusion was obtained under the propositions considered

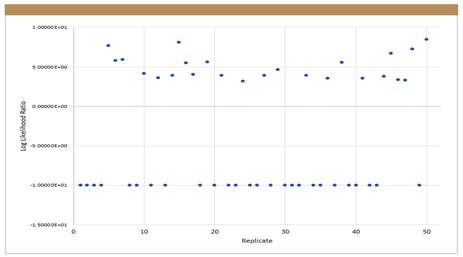

Figure 2: *Log Likelihood Ratio for Minor Donor Across Replicates (LR=0 plotted as -10)*

D21S11 (minor heterozygous, in stutter position):

- 10:1 mixture ratio
  - The artificial drop-in peak (27.2) was modeled as drop-in 100% of the time for one replicate
  - For the second replicate, some weight was given to the drop-in peak paired with the 29 allele with the 32.2 allele modeled as stutter
- 20:1 mixture ratio
  - The obligate minor donor alleles are present at lower levels
    - Results in increasing the potential for stutter modeling for the 32.2 allele
  - At this mixture ratio, the drop-in allele was modeled >99% of the time as allelic with some weight given to minor donor's true genotype
  - The simulated drop-in peak at 150 RFU would be considered intuitive

Experiment #4

Materials & Methods for Experiment #4
Modifying Drop-In/Stutter Percentage/Parent Peak Height

- Fifty replicate runs of one of the simulated 10:1 mixtures was performed three times
- An artificial drop-in peak (27.2) was inserted at D21S11 at 150 RFU
- The minor donor at this locus was heterozygous (29,32.2) with one peak (32.2) in stutter position to the major donor's allele. Stutter and parent peak height were modified for each of the replicate series

Results

Modifying Drop-In/Stutter Percentage/ Parent Peak Height

- Fifty replicates using true validation peaks and heights plus artificial drop-in peak (27.2) inserted at D21S11 at 150 RFU
  - 27.2 was modeled as drop-in 100% of the time

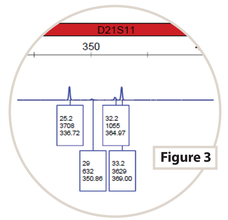

Figure 3

D21S11 from validation shown on right, 32.2 is 29% of the parent peak

- Fifty additional replicate runs were performed with the 32.2 stutter peak at 25% of the parent peak → the 32.2 peak was modified to 908 RFU
  - 27.2 was modeled as drop-in 100% of the time
- Fifty additional replicate runs were performed with the 32.2 stutter peak at 29% of the parent peak with the parent peak height lowered → the 32.2 peak was modified to 1055 RFU and the RFU for the 33.2 peak was modified to 2224 RFU
  - The likelihood ratio for the minor donor was not affected
  - The 27.2 was modeled as allelic in 31 out of 50 replicates

Contributor 1		Contributor 2		Drop-In	Weight
25.2	33.2	27.2	29	31.2	0.0001%
25.2	33.2	27.2	29	31.2	0.0002%
25.2	33.2	27.2	29	31.2	0.0003%
25.2	33.2	27.2	29	31.2	0.0005%
25.2	33.2	27.2	29	31.2	0.0008%
25.2	33.2	27.2	29	31.2	0.0009%
25.2	33.2	27.2	29	31.2	0.0014%
25.2	33.2	27.2	29	31.2	0.0018%
25.2	33.2	27.2	29	31.2	0.0019%
25.2	33.2	27.2	29	31.2	0.0021%
25.2	33.2	27.2	29	31.2	0.0021%
25.2	33.2	27.2	29	31.2	0.0025%
25.2	33.2	27.2	29	31.2	0.0026%
25.2	33.2	27.2	29	31.2	0.0031%
25.2	33.2	27.2	29	31.2	0.0031%
25.2	33.2	27.2	29	31.2	0.0031%
25.2	33.2	27.2	29	31.2	0.0036%
25.2	33.2	27.2	29	31.2	0.0036%
25.2	33.2	27.2	29	31.2	0.0038%
25.2	33.2	27.2	29	31.2	0.0041%
25.2	33.2	27.2	29	31.2	0.0044%
25.2	33.2	27.2	29	31.2	0.0046%
25.2	33.2	27.2	29	31.2	0.0048%
25.2	33.2	27.2	29	31.2	0.0049%
25.2	33.2	27.2	29	31.2	0.0050%
25.2	33.2	27.2	29	31.2	0.0069%
25.2	33.2	27.2	29	31.2	0.0083%
25.2	33.2	27.2	29	31.2	0.0085%
25.2	33.2	27.2	29	31.2	0.0113%
25.2	33.2	27.2	29	31.2	0.0135%
25.2	33.2	27.2	29	31.2	0.0185%

Table 4: *Weights for Simulated Drop-in Peak modeled as Allelic*

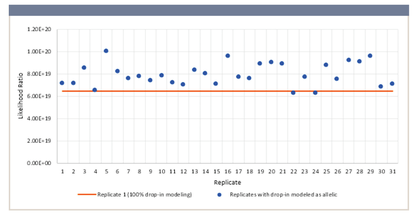

Figure 4: *Likelihood Ratio (most conservative 99% 1-sided HPD LR) for Minor Donor*

Conclusions

Back to the opening question …
The true minor donor at this location is a 17,25.2 while the 11.2 is a simulated drop-in peak.

A qualitative exclusion for this individual would have been false.

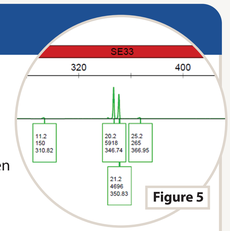

Figure 5

Even when utilizing probabilistic software such as STRmix™, care must be taken to carefully evaluate all results to see if they are intuitively supported by the associated evidence and reference electropherograms.

What to watch out for:

- Extremely low genotype weight(s) (e.g., <1%) in comparison to the person of interest at one or more loci; however, an inclusionary statistic is generated
- LR=0 at one locus

The results of this experiment highlight the complexity associated with drop-in modeling when considered in conjunction with other stochastic effects such as drop-out and stutter variability. It should be noted that this body of work only evaluated mixtures with two contributors. Mixtures comprised of more contributors (i.e., three or more) would add an even greater level of complexity when evaluating an unknown evidence sample. It is prudent to consider all of these factors when assessing the potential number of contributors to a DNA profile and whether or not a qualitative comparison for the determination of exclusion is appropriate and supported by sound scientific judgement.

Tests were performed by Alicia M. Cadenas, Validation Coordinator at Signature Science Forensic DNA Laboratory, Austin, Texas • For additional information, please contact swandzek@signaturescience.com