My Results are Unintuitive, Now What?

Casework Studies on Unintuitive STRmix™ Results and the Approaches Utilized for Reporting



Science Works | AAFS 75th Anniversary Conference

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Signature Science and Probabilistic Genotyping — A Timeline

Early 2017 July 2017

May 2018

April 2020

June 2022

Probabilistic genotyping:
Research and selection process begins

STRmix v2.5 license acquired STRmix – GlobalFiler system STRmix – Investigator 24plex QS system STRmix –New Formulation of Investigator 24plex QS system

Tune in on Friday for
Samantha Wandzek's
presentation on the
validation and evaluation of
Investigator 24plex QS New
Formulation

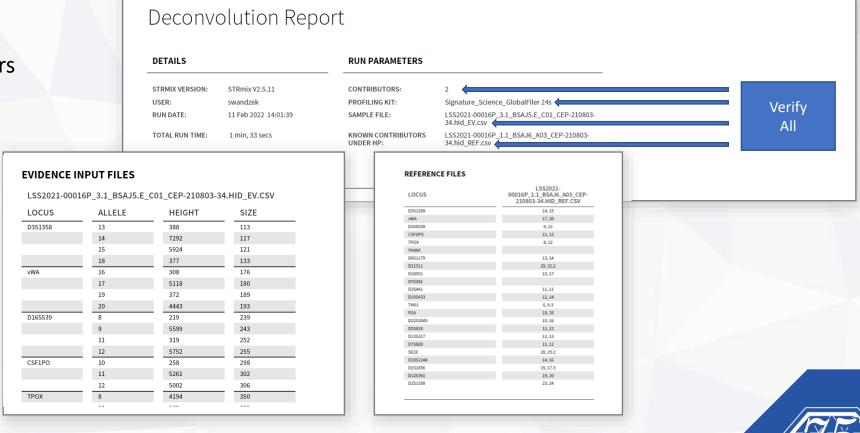


Evaluation Criteria

The Deconvolution Report contains information that will allow the analyst to evaluate if an appropriate deconvolution was performed. The following criteria must be reviewed by both the reporting analyst and the technical reviewer to ensure proper results were obtained through the software.

RUN PARAMETERS

- Number of contributors
- Profiling Kit
- Propositions
- Input file(s)

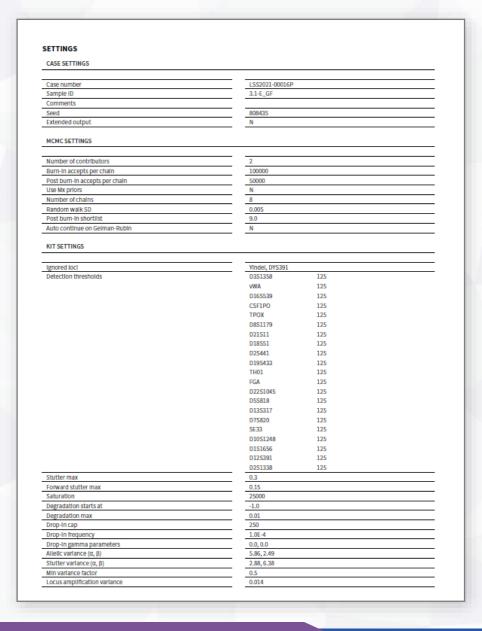




Evaluation Criteria

RUN SETTINGS

- Majority are default settings defined in SOP (unbolded)
 - Ignored Loci
 - Burn-in and Post burn-in accepts per chain





Evaluation Criteria

The presence of a single sub-optimal diagnostic is not always an indication that rework is required.

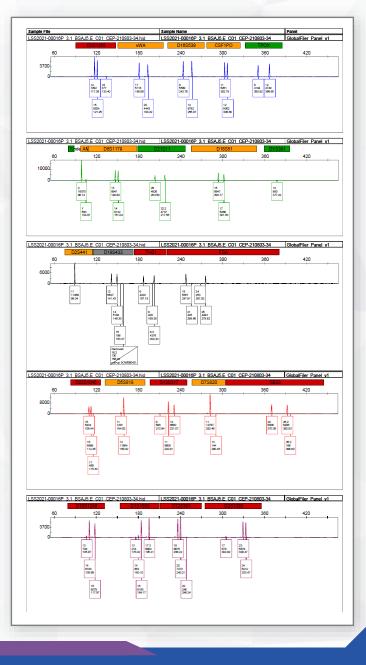
- POST BURN-IN SUMMARY
 - Total Iterations total number of post burn-in iterations that the MCMC has run during its analysis
 - Acceptance Rate Post burn-in accepts/Total Iterations
 - Effective Sample Size number of independent samples the MCMC has taken from the posterior distribution of all parameters
 - Gelman-Rubin Convergence Diagnostic ratio of the stationary distribution and within-chain variances; informs the user whether the MCMC analysis has likely converged
 - Log (likelihood) log of the average likelihood (or probability) value created at each of the post burn-in MCMC iterations
 - Allele Variance and Stutter Variance Constants average value for allele variance and stutter variance constants across the entire post burn-in MCMC analysis

OST BURN-IN SUMMARY			
Total iterations	2089102	Acceptance rate	1 in 5.22
Effective sample size	6703.03	log(likelihood)	57.07
Gelman-Rubin convergence diagnostic	1.04		
Allele variance (mode = 12.101)	11.465	Stutter variance (mode = 11.994)	22.34



Mixture Proportions – Is it Intuitive?

CONTRIBUTORS	1	2	
Template (rfu)	6000	306	
Mixture Proportion	95%	5%	
Degradation starts at 84bp (rfu/bp)	4.272	0.962	
CONTRIBUTOR ORDER GIVING HIGHEST L	R		
All Populations	LSS2021-00016P_1.1_BSAJ6_A03_CEP- 210803-34.hid_REF.csv	Unknown	

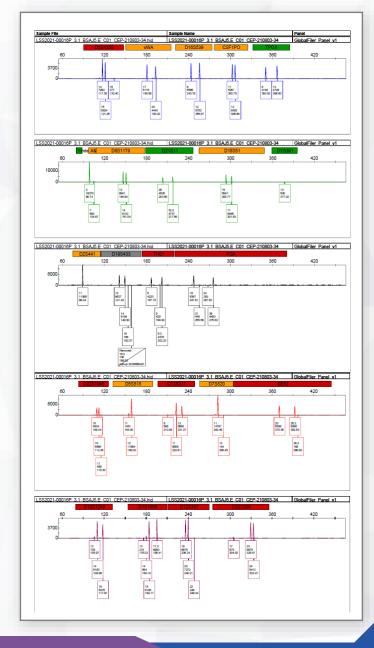




Component Interpretation – Is it Intuitive?

May also be evaluated against Genotype Probability Distribution

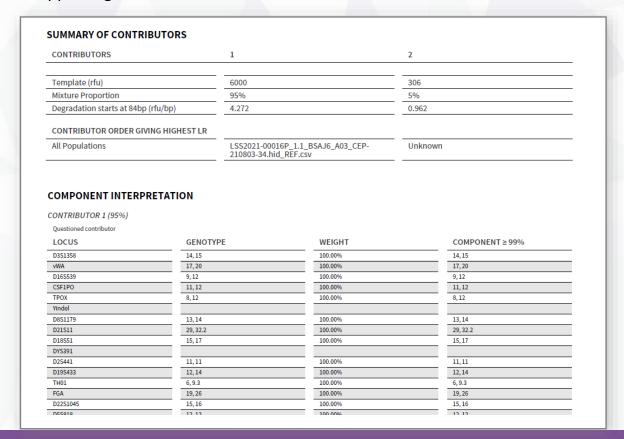
COMPONENT INTER	RPRETATION		
CONTRIBUTOR 1 (95%)			
Questioned contributor			
LOCUS	GENOTYPE	WEIGHT	COMPONENT ≥ 99%
D3S1358	14, 15	100.00%	14, 15
vWA	17, 20	100.00%	17,20
D16S539	9, 12	100.00%	9,12
CSF1PO	11, 12	100.00%	11, 12
TPOX	8, 12	100.00%	8,12
Yindel			
D8S1179	13, 14	100.00%	13, 14
D21S11	29, 32.2	100.00%	29, 32.2
D18S51 DYS391	15, 17	100.00%	15, 17
DYS391 D2S441		100.00%	
D19S433	11,11	100.00%	11,11
D195433 TH01	12,14	100.00%	12,14
FGA	6, 9.3 19, 26	100.00%	6, 9.3 19, 26
D22S1045	15,16	100.00%	15, 16
D5S818	12, 12	100.00%	12, 12
D13S317	11,13		
D7S820	11,13	100.00%	11,13
SE33	20, 25.2	100.00%	20, 25.2
D10S1248	14, 16	100.00%	14, 16
D1S1656	15, 17.3	100.00%	15, 17.3
D12S391	19, 20	100.00%	19, 20
D2S1338	23,24	100.00%	23,24
0231330	25,24	100.00%	25,24
CONTRIBUTOR 2 (5%)			
Questioned contributor			
	GENOTYPE	WEIGHT	COMPONENT - 000/
LOCUS		WEIGHT	COMPONENT ≥ 99%
D3S1358	14, 18	48.61%	18, F
	18, 18	25.17%	
	15, 18	25.06%	
	Q, 18	0.83%	
	13,18	0.34%	
vWA	17,17	34.65%	F, F

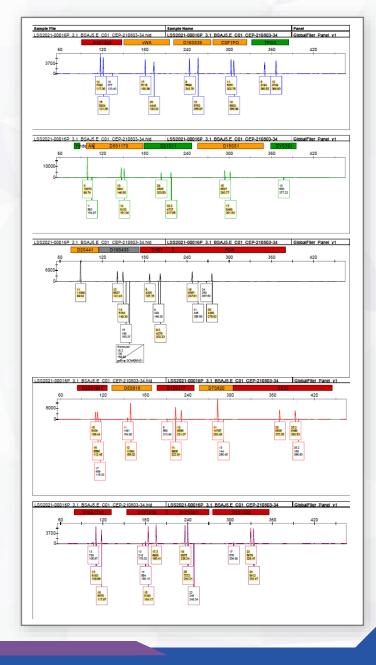




Contributor Assignment – Is it Intuitive?

- Red flags are observed at one or more loci for the POI
 - Low weights for POI genotypes in component interpretation
 - Contributor assignment is not intuitive when POI is compared to EPG
- Familial component
 - First degree relatives assigned to same contributor with a likelihood ratio supporting inclusion

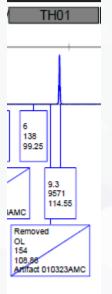






Unintuitive Mixture Proportions and/or Genotype weights – Case example 1

- Only indication for the minor donor was TH01-6, robust major donor (5K-10K rfu) was noted at all other loci
- STRmix deconvolution
 - Mixture proportions C1 94% / C2 6%
 - Genotype weights for C1 not intuitive



uestioned contributor			
ocus	GENOTYPE	WEIGHT	COMPONENT ≥ 99%
H01	9.3, 9.3	100.00%	9.3, 9.3
351358	15, 15	100.00%	15, 15
WA	15, 17	95.38%	F,F
	15, 15	2.45%	
	17, 17	2.17%	
21511	30, 31	95.22%	F,F
	31, 31	3.44%	
	30, 30	1.34%	
POX	11, 11	100.00%	11, 11
YS391			
151656	16, 17.3	94.13%	F, F
	17.3, 17.3	3.53%	
	16, 16	2.34%	
125391	16, 19	94.02%	F, F
	16, 16	3.02%	
	19, 19	2.96%	
E33	29.2, 31.2	97.16%	F, F
	29.2, 29.2	1.79%	
	31.2, 31.2	1.04%	
10S1248	12, 15	92.66%	12, F
	12, 12	6.74%	
	15, 15	0.60%	
22S1045	15, 16	94.05%	F,F
	16, 16	3.50%	
	15, 15	2.45%	
195433	14, 15	94.49%	F, F
	14, 14	3.13%	
	15, 15	2.38%	
BS1179	10, 11	94.59%	F, F
	10, 10	3.63%	
	11,11	1.78%	
251338	17, 22	91.56%	F, F
	22, 22	5.68%	
	17, 17	2.76%	
25441	10, 15	95.41%	F, F
	15, 15	3.27%	
	10, 10	1.32%	
18551	15, 17	93.89%	F, F
	17, 17	3.95%	
	15, 15	2.16%	
GA .	20, 26	92.08%	F, F
	26, 26	6.09%	
	20, 20	1.84%	
16S539	11, 11	100.00%	11, 11
SF1PO	12, 12	100.00%	12, 12
135317	9, 12	94.15%	F, F
	12, 12	3.58%	
	9,9	2.27%	
55818	12, 12	100.00%	12, 12
75820	10, 12	91.66%	F, F
	12, 12	5.02%	
	10, 10	3.32%	



Unintuitive Mixture Proportions and/or Genotype Weights Case Example 1

- Increased burn-in and post-burn in iterations
 - Default
 - Burn-in accepts per chain 100,000
 - Post burn-in accepts per chain 50,000
 - Increased
 - Burn-in accepts per chain 500,000
 - Post burn-in accepts per chain 500,000
- STRmix deconvolution
 - Mixture proportions C1 100% / C2 0%
 - Intuitive genotype weights in comparison to EPG

Questioned contributor			
LOCUS	GENOTYPE	WEIGHT	COMPONENT ≥ 99%
TH01	9.3, 9.3	100.00%	9.3, 9.3
D3S1358	15, 15	100.00%	15, 15
vWA	15, 17	100.00%	15, 17
D21S11	30, 31	100.00%	30, 31
	30, 30	0.00%	
TPOX	11, 11	100.00%	11, 11
DYS391			
D1S1656	16, 17.3	100.00%	16, 17.3
D12S391	16, 19	100.00%	16, 19
SE33	29.2, 31.2	100.00%	29.2, 31.2
D10S1248	12, 15	100.00%	12, 15
D22S1045	15, 16	100.00%	15, 16
D19S433	14, 15	100.00%	14, 15
D8S1179	10, 11	100.00%	10, 11
	10, 10	0.00%	
D2S1338	17, 22	100.00%	17, 22
	17, 17	0.00%	
D2S441	10, 15	100.00%	10, 15
D18S51	15, 17	100.00%	15, 17
FGA	20, 26	100.00%	20, 26
	20, 20	0.00%	
D16S539	11, 11	100.00%	11,11
CSF1PO	12, 12	100.00%	12, 12
D13S317	9, 12	100.00%	9,12
D5S818	12, 12	100.00%	12, 12
D7S820	10, 12	100.00%	10, 12
	10, 10	0.00%	



Reporting -

Results of the unintuitive deconvolution was not reported

- Case record addresses any deconvolutions that are not reported and the reason
 - "Genotype weightings were unintuitive data not reported. Re-run with extra burn-in and post burn-in iterations. This data reported"
- Comparison to POI supported exclusion in both runs



Case Example 2

Homicide

- 3 victims
- Firearm was recovered from suspect flight path
 - Q1: Swabs from rifle
 - Reference standards from S1 and S2
 - No known familial relationship between S1/S2

The partial DNA profile obtained from this item was interpreted as a mixture of four individuals with at least two male contributors.

S1 AND S2 BOTH ASSIGNED CONTRIBUTOR 1

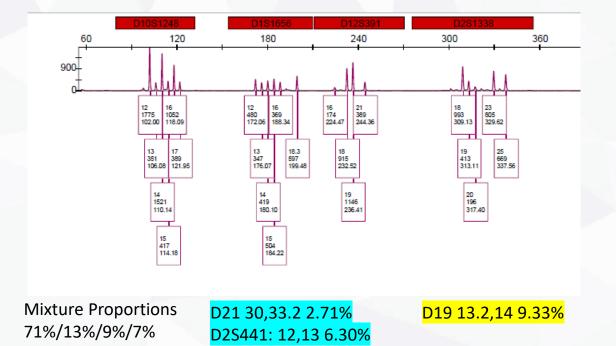
UMMARY OF CONTRIBUTORS				
CONTRIBUTORS	1	2	3	4
Template (rfu)	1600	297	206	147
Mixture Proportion	71%	13%	9%	7%
Degradation starts at 84bp (rfu/bp)	6,555	1.007	0,506	0.331

Obtaining this mixture profile is approximately 1.17 quintillion times more likely if the DNA originated from S1 and three unknown, unrelated individuals than if the DNA originated from four unknown, unrelated individuals.

Obtaining this mixture profile is approximately 1.13 trillion times more likely if the DNA originated from S2 and three unknown, unrelated individuals than if the DNA originated from four unknown, unrelated individuals.

Case Example 2

- Concerns for Mixture proportions
- Red flags for contributor assignment
- Complex mixture + Degradation



D3S1358	16, 17	85.94%	F, F
	17, 17	11.25%	·
	16, 16	1.44%	
	15, 17	0.70%	
	15, 16	0.66%	
D21S11	30, 31	54.14%	F,F
	31, 31	31.74%	
	31, 33.2	4.82%	
	29, 31	2.98%	
	30, 33.2	2.71%	
	29, 30	1.64%	
	30, 30	1.36%	
	29, 33.2	0.34%	
	Q, 31	0.12%	
	Q, 30	0.08%	
	Q, 33.2	0.03%	
	Q, 29	0.02%	
	33.2, 33.2	0.01%	
D2S441	11, 12	60.64%	F,F
	11, 14	12.61%	
	12, 14	9.57%	
	11, 13	8.56%	
	12, 13	6.30%	
	13, 14	2.26%	
	11,11	0.04%	
	12, 12	0.03%	
	14, 14	0.00%	
	13, 13	0.00%	
D19S433	14, 14	90.58%	14, F
	13.2, 14	9.33%	
	14, 15	0.07%	
	13, 14	0.01%	
	13.2, 15	0.01%	
	13.2, 13.2	0.00%	
D10S1248	12,14	72.70%	F,F
	12, 16	13.66%	
	14, 16	12.87%	
	12, 12	0.59%	
	14, 14	0.07%	
	12, 17	0.04%	
	14, 17	0.04%	
	16, 17	0.01%	
	12, 15	0.01%	
	14, 15	0.00%	
	15, 16	0.00%	
	16, 16	0.00%	



Reporting

The partial DNA profile obtained from this item was interpreted as a mixture of three individuals with at least one male contributor.

Obtaining this mixture profile is approximately 1.17 quintillion times more likely if the DNA originated from S1 and three unknown, unrelated individuals than if the DNA originated from four unknown, unrelated individuals. The statistical calculation for S1 may be overestimated based on the contributor designation assigned by STRmix.

Obtaining this mixture profile is approximately 1.13 trillion times more likely if the DNA originated from S2 and three unknown, unrelated individuals than if the DNA originated from four unknown, unrelated individuals. The statistical calculation for S2 may be overestimated based on the contributor designation assigned by STRmix.



Explaining the Reporting Statement

The statistical calculation for [Name] may be overestimated based on the contributor designation assigned by STRmix. Please contact this laboratory as needed for additional information.

- "STRmix works in two parts. First, it determines the most likely profiles for contributors in the sample. Then, in comparison to a known sample, it assigns the known sample to a best fit contributor and a statistic is generated. The STRmix results are always evaluated to make sure the results are intuitive."
- "Based on an evaluation of this mixed DNA profile, I believe the statistic for the sample could
 potentially be falsely elevated. When I evaluated the results in comparison to John Doe there
 were indications that they may be assigned to the wrong contributor for this sample, which is
 one of the factors the statistic is based off."
- Case by case basis:
 - Issue could be inherent to the complexity of the profile
 - Request more information regarding familial relationship
 - Request reference standards from first degree relatives if relevant to the case



Case Example 3

Homicide involving three victims

- V1/V2 were pistol whipped with a gun, V3 (deceased) was shot
- V1 and V2 are mother and daughter
- Gun was recovered from the alleged suspect
 - Q1: Swabs from grips, trigger, slide, magazine release
 - Reference standards from V1, V2, V3 and S1

The partial DNA profile obtained from this item was interpreted as a mixture of three

individuals with at least one male contributor.

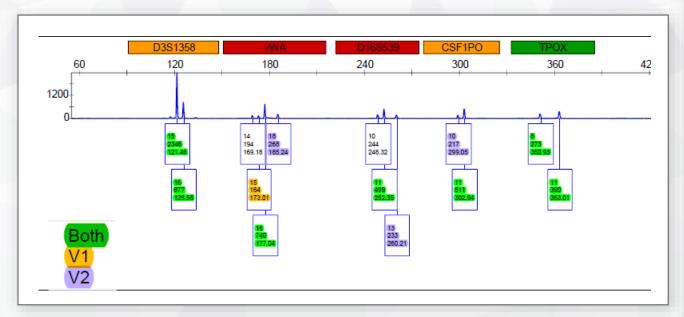
UMMARY OF CONTRIBUTORS			
CONTRIBUTORS	1	2	3
Template (rfu)	1650	528	209
Mixture Proportion	69%	22%	9%
Degradation starts at 85bp (rfu/bp)	6.703	1.918	0.579

MOTHER (V1) AND DAUGHTER (V2) BOTH ASSIGNED CONTRIBUTOR 1

Obtaining this mixture profile is approximately 6.83 quadrillion times more likely if the DNA originated from V1 and two unknown, unrelated individuals than if the DNA originated from three unknown, unrelated individuals.

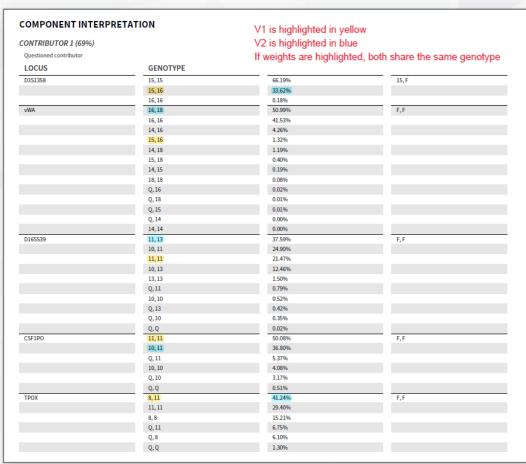
Obtaining this mixture profile is approximately 8.52 trillion times more likely if the DNA originated from V2 and two unknown, unrelated individuals than if the DNA originated from three unknown, unrelated individuals

Case Example 3 - Blue Channel



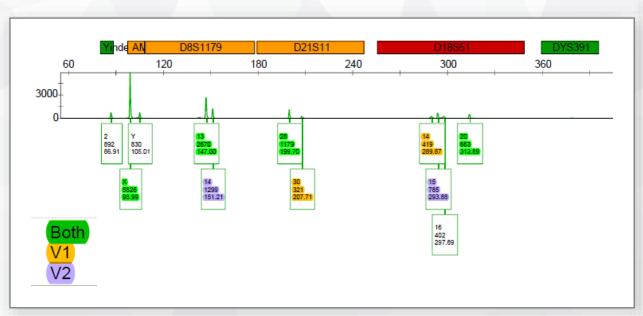
Mixture Proportions 69%/22%/9%

vWA: V1 15,16 1.32%



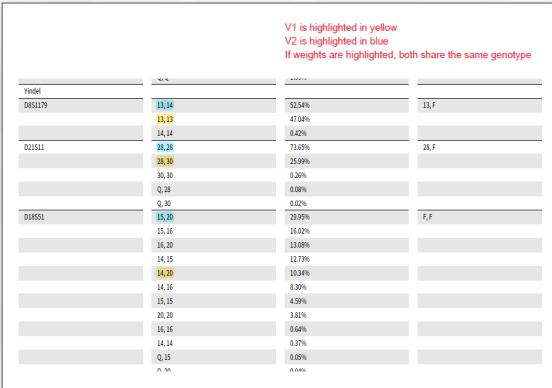


Case Example 3 - Green Channel



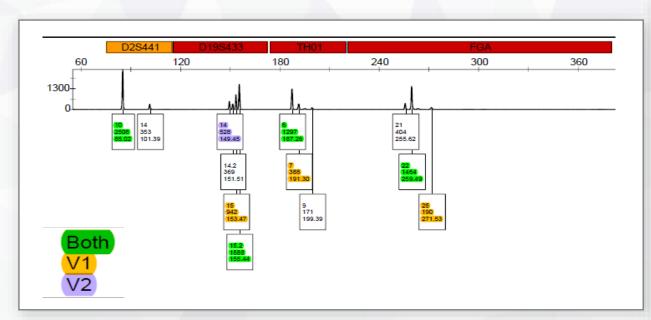
Mixture Proportions 69%/22%/9%

D18: V1 14,20 10.34%





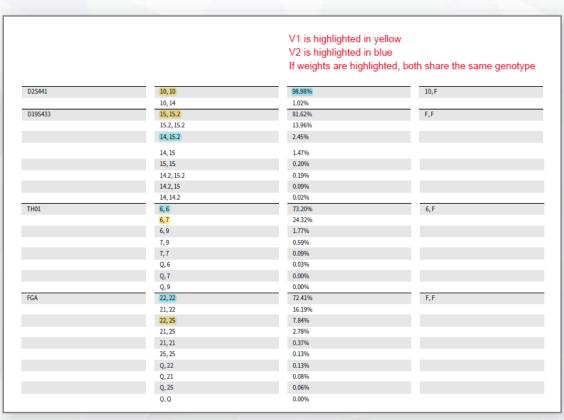
Case Example 3 - Yellow Channel



Mixture Proportions 69%/22%/9%

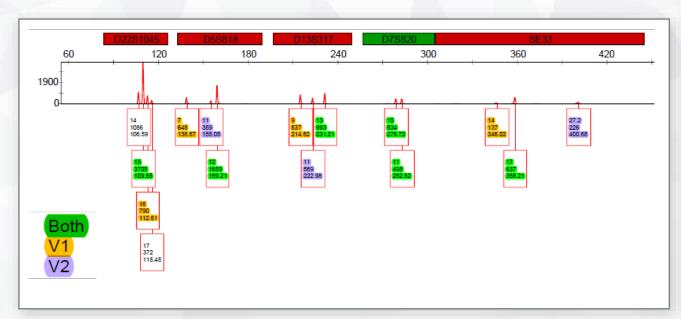
D19: V2 14,15.2 2.45%

FGA: V1 22,25 7.84%





Case Example 3 - Red Channel



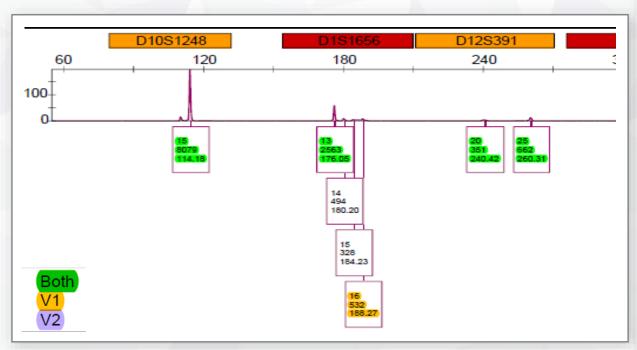
Mixture Proportions 69%/22%/9%

D22: V1 15,16 2.20% D5: V2 11,12 0.94%

		V1 is highlighted in	
		V2 is highlighted in	blue
		If weights are highli	ghted, both share the same genotype
		Holgino al o iligini	ginea, bear enare are came generype
D22S1045	15, 15	95.07%	15, F
	14, 15	2.38%	
	15, 16	2.20%	
	14, 16	0.17%	
	15, 17	0.15%	
	14, 17	0.02%	
	16, 17	0.01%	
	16, 16	0.00%	
D5S818	12, 12	79.11%	12, F
	7, 12	19.51%	
	11, 12	0.94%	
	7,11	0.38%	
	7,7	0.06%	
D13S317	9,13	46.99%	F,F
	11, 13	23.43%	
	9,11	17.98%	
	13, 13	9.35%	
	9,9	1.89%	
	11, 11	0.34%	
	Q, 13	0.01%	
	Q, 11	0.01%	
	Q, 9	0.01%	
D7S820	10, 11	54.82%	F, F
	11, 11	21.64%	
	10, 10	20.82%	
	Q, 11	1.34%	
	Q, 10	1.31%	
	Q, Q	0.08%	
SE33	17, 27.2	30.01%	F, F
	17, 17	25.48%	
	14, 17	16.28%	
	14, 27.2	12.47%	
	27.2, 27.2	7.80%	
	Q, 17	2.60%	
	Q, 27.2	2.08%	
	Q, 14	1.82%	
	14, 14	1.19%	
	Q, Q	0.27%	



Case Example 3 - Purple Channel



Mixture Proportions 69%/22%/9%

D1: V1 13,16 6.54%

		V2 is highlighted i	V1 is highlighted in yellow V2 is highlighted in blue If weights are highlighted, both share the same genotyp		
D10S1248	15, 15	100.00%	15,15		
D1S1656	13, 13	89.58%	13,F		
	13, 16	6.54%			
	13, 14	2.96%			
	13, 15	0.40%			
	14, 16	0.40%			
	15, 16	0.09%			
	14, 15	0.04%			
D12S391	25, 25	52.96%	F,F		
	20, 25	42.87%			
	20, 20	2.85%			
	Q, 25	0.75%			
	Q, 20	0.53%			
	Q, Q	0.03%			
D2S1338	23,23	32.98%	F,F		
	19, 23	23.99%			
	17,23	22.56%			
	17, 19	14.35%			
	19, 19	2.14%			
	17,17	1.59%			
	Q, 23	0.93%			
	Q, 17	0.76%			
	Q, 19	0.65%			
	0.0	0.05%			



Run	Most Conservative Total LR	Contributor 1	Contributor 2	Contributor 3
3p (V1)	8.52 trillion	69%	22%	9%
3p (V2)	6.83 quadrillion	69%	22%	9%
3p (V1 assuming V2)	8.42 billion	62% (V2)	31% (V1)	7%
3p (V2 assuming V1)	1.05 trillion	59% (V1)	33% (V2)	8%

Due to the known familial relationship, additional propositions were considered in order to explore the contributor assignment

By assuming one and running LR for the other, genotype weights for C2 assignment were more intuitive



Reporting

The partial DNA profile obtained from this item was interpreted as a mixture of three individuals with at least one male contributor.

Obtaining this mixture profile is approximately 6.83 quadrillion times more likely if the DNA originated from V1 and two unknown, unrelated individuals than if the DNA originated from three unknown, unrelated individuals. The statistical calculation for V1 may be overestimated based on the contributor designation assigned by STRmix.

Obtaining this mixture profile is approximately 8.52 trillion times more likely if the DNA originated from V2 and two unknown, unrelated individuals than if the DNA originated from three unknown, unrelated individuals. The statistical calculation for V2 may be overestimated based on the contributor designation assigned by STRmix.



Reporting

The partial DNA profile obtained from this item was also interpreted as a mixture of three individuals with at least one male contributor and with V1 as an assumed contributor. Obtaining this mixture profile is approximately 8.42 billion times more likely if the DNA originated from V1, V2, and one unknown, unrelated individual than if the DNA originated from V1 and two unknown, unrelated individuals.

The partial DNA profile obtained from this item was also interpreted as a mixture of three individuals with at least one male contributor and with V2 as an assumed contributor. Obtaining this mixture profile is approximately 1.05 trillion times more likely if the DNA originated from V2, V1, and one unknown, unrelated individual than if the DNA originated from V2 and two unknown, unrelated individuals.



Final Thoughts

Evaluate all information as a whole when considering whether results are unintuitive.

Low genotype weights can be observed in:

- Complex Mixtures
- High molecular weight loci in degraded samples
- Equal contributors to a mixture

Caution: May be inherent to the profile rather than a red flag for contributor assignment

Propositions explored for likelihood ratios should take into consideration case information



QUESTIONS?

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