

ISHI 2010 Mixture Interpretation Workshop:
Principles, Protocols, and Practice
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Mixture Ratios

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Outline for Mixture Ratios

- **GUIDELINES**
 - SWGDAM Guideline 3.5.3
- **PRINCIPLES**
 - Distinguishable vs. indistinguishable mixture
 - Relationship between PHR, stutter, and mixture ratios
- **PROTOCOLS**
 - Approaches and benefits to calculating mixture ratios
 - Variation across loci in a profile
 - Some validation mixture ratio experiments
- **PRACTICE**
 - Use in mixture deconvolution
 - A worked example (Case 2 later today)

GUIDELINES

Mixture Ratio

SWGDAM Guidelines glossary:

- Mixture ratio: **the relative ratio of the DNA contributions of multiple individuals** to a mixed DNA typing result, **as determined by the use of quantitative peak height information**; may also be expressed as a percentage

PRINCIPLES

What Exists in Sample vs. What is Estimated

Mixture Ratio(s)
(what actually exists in the sample)

vs.

Deduced Mixture Proportion(s)
(what is determined from the data by relative peak heights)

Other terms sometimes used
Mixture proportion (M_x), proportion (p) or mass ratio

GUIDELINES

Mixture ratios can help deduce contributor profiles

SWGDAM Guideline 3.5.3:

- A laboratory may define other quantitative characteristics of mixtures (e.g., **mixture ratios**) to aid in further refining the contributors.

Have you used mixture proportions or donor ratios to deduce mixtures?

1. Yes
2. No

PRINCIPLES

German Mixture Classification Scheme

Schneider et al. (2009) *Int. J. Legal Med.* 123: 1-5

(German Stain Commission, 2006):

- Type A:** no obvious major contributor, no evidence of stochastic effects
- Type B:** clearly distinguishable major and minor contributors; consistent peak height ratios of **approximately 4:1** (major to minor component) for all heterozygous systems, no stochastic effects
- Type C:** mixtures without major contributor(s), evidence for stochastic effects

SWGDAM

Type A "Indistinguishable"

Type B "Distinguishable"

Type C "Uninterpretable"

PROTOCOLS

Forensic Science International
91 (1998) 55-70

Analysis and interpretation of mixed forensic stains using DNA STR profiling

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Received 13 May 1997; received in revised form 9 October 1997; accepted 27 October 1997

This approach is supported by ISFG (2006); see Recommendation #4

Gill et al. (2006) DNA Commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Sci. Int.* 160: 90-101

PROTOCOLS

Steps in the interpretation of mixtures

(Clayton et al. *Forensic Sci. Int.* 1998; 91:55-70)

- Step #1 Identify the Presence of a Mixture
- Step #2 Designate Allele Peaks
- Step #3 Identify the Number of Potential Contributors
- Step #4 Estimate the Relative Ratio of the Individuals Contributing to the Mixture
- Step #5 Consider All Possible Genotype Combinations
- Step #6 Compare Reference Samples

PROTOCOLS

Calculation of Major/Minor Ratio

With 2-person mixtures, examine loci with four alleles

$$\frac{PH_A + PH_C}{PH_B + PH_D}$$

Sum of major allele peak heights / Sum of minor allele peak heights

$$\frac{PH_A + PH_C}{PH_A + PH_C + PH_B + PH_D}$$

Sum of major allele peak heights / Sum of all allele peak heights at the locus

Major = A, C
Minor = B, D

Formation of possible genotypes depends on PHRs allowed and the mixture ratio

PRACTICE

Mixture Ratio Example

PowerPlex 16 (1 ng)

3 alleles, 2 alleles, 3 alleles, 4 alleles, 3 alleles, 3 alleles, 3 alleles, 3 alleles, 4 alleles, 3 alleles, 3 alleles, 3 alleles, 2 alleles, 3 alleles

Balanced X:Y

PRACTICE UPDATED SLIDE

Analysis of the 4-Allele Loci

Variability exists between loci

D18S51

14: 3384 rfu
20: 3007 rfu
16: 988 rfu
18: 519 rfu

$$\frac{(3384 + 3007)}{(988 + 519)} = 4.24$$

PHR = 52.5%

Penta D

8: 3287 rfu
12: 3281 rfu
2.2: 612 rfu
10: 774 rfu

$$\frac{(3287 + 3281)}{(612 + 774)} = 4.74$$

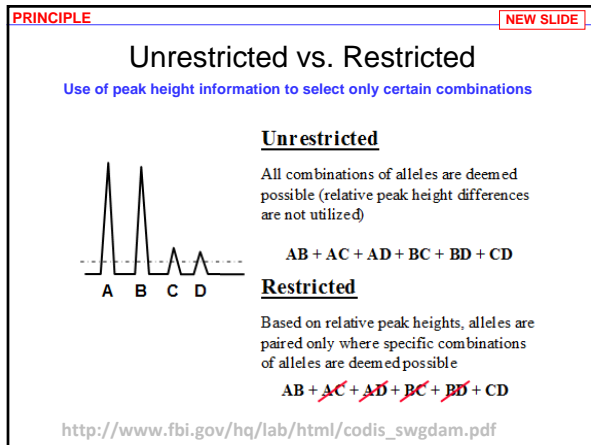
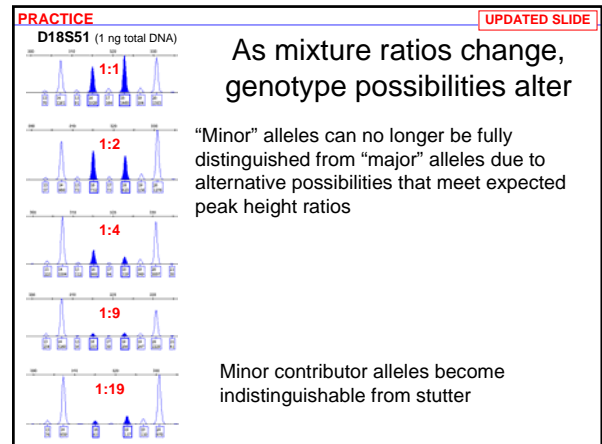
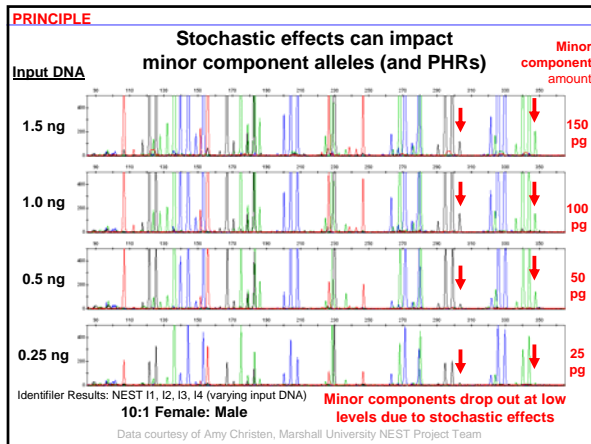
PHR = 79.1%

D8S1179

13: 2698 rfu
16: 3086 rfu
14: 841 rfu
15: 1072 rfu

$$\frac{(2698 + 3086)}{(841 + 1072)} = 3.02$$

PHR = 78.5% (stutter -875)



GUIDELINES

Recognition that degradation can impact reliable mixture ratios across a DNA profile

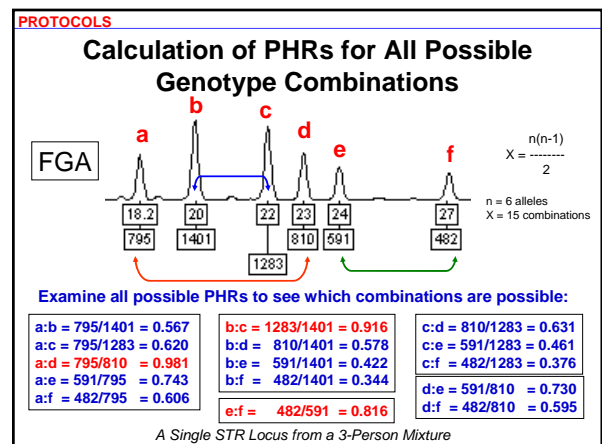
- 3.5.3.1. Differential degradation of the contributors to a mixture may impact the **mixture ratio** across the entire profile.

GUIDELINES

Evaluation of all pair-wise comparisons

SWGAM Guideline 3.5.1:

- The laboratory should establish guidelines based on peak height ratio assessments for evaluating potential sharing of allelic peaks among contributors and for determining whether contributors to a mixed DNA typing result are distinguishable. **When assessing peak height ratios, pair-wise comparison of all potential genotypic combinations should be evaluated.**



PRINCIPLES

All possible 3-contributor combinations

23 "families" of possibilities

150 total combinations

3 allele pattern has 8 "families"

This "family" has 30 possibilities

Adapted slide from Tim Kalafut, USACIL, (AAFS 2008 workshop)

PROTOCOLS

Software tools can help perform calculations

Prof Amp Tolerance: 90%
Mixing Proportion Tolerance: 10%
Heterozygote: H

Weight Minimum: 20% 4.1
Weight Maximum: 20% 4.1

Locus	Allele	Area	Contributor 1	Contributor 2	Contributor 1	Contributor 2	Mix Est	BC	Contributor 1	Contributor 2
D3S1358	15	4405	10	10	15	15	-	Y	-	Y
	15	2430	15	15	15	15	89%	Y	100%	Y
	-	-	15	15	15	15	89%	Y	89%	Y
	-	-	15	15	15	15	100%	Y	142%	Y
	-	-	16	16	16	16	-	Y	89%	1.1
	-	-	18	18	18	18	100%	Y	89%	1.1
	-	-	18	18	18	18	100%	Y	119%	4.1
	-	-	18	18	18	18	142%	Y	100%	Y
	-	-	15	10	10	10	142%	Y	100%	Y
	Database Consolidation for D3S1358									
16 F F F										

Database Consolidation for FGA

19	7227	21	22	19	20	147%	Y	237%	N	55% 1.1	N	-	-	-	-	
20	3051	20	22	19	21	88%	Y	142%	Y	65% 1.2	N	-	-	-	-	
21	5101	20	21	19	22	80%	Y	208%	N	87% 1.1	N	-	-	-	-	
22	3470	19	22	20	21	205%	N	60%	Y	43% 2.1	Y	-	-	-	-	
-	-	19	21	20	22	142%	Y	88%	Y	35% 2.1	Y	Include	19	21	20	22
-	-	19	20	21	22	237%	N	147%	Y	45% 1.1	Y	-	-	-	-	
Database Consolidation for FGA																
19 21 20 22																

FSS-i3 ISTRM Mixture Interpretation Tool

Does your lab use quantitative peak heights in mixture interpretation?

1. Yes
2. No
3. Why should we?
4. None of the above

Summary

- Mixture ratios may be used to help deduct major and minor components of mixtures
- With 2-person mixtures, loci possessing 4 alleles can be used to help establish the contributor ratios
- This mixture ratio may then be used to eliminate possible contributor genotype combinations for loci possessing only two or three alleles
- Software tools are helpful to performing these calculations