Module 8: Calculating & Using Mixture Ratios

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)

Mixture Ratios

John M. Butler

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), 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

http://www.cstl.nist.gov/biotech/strbase/training.htm
German Mixture Classification Scheme

(Schneider et al., 2009) 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

<table>
<thead>
<tr>
<th>Type A</th>
<th>Type B</th>
<th>Type C</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Indistinguishable&quot;</td>
<td>&quot;Distinguishable&quot;</td>
<td>&quot;Uninterpretable&quot;</td>
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</table>

**Steps in the interpretation of mixtures**

- Identify the Presence of a Mixture
- Designate Allele Peaks
- Identify the Number of Potential Contributors
- Estimate the Relative Ratio of the Individuals Contributing to the Mixture
- Consider All Possible Genotype Combinations
- Compare Reference Samples

**Calculation of Major/Minor Ratio**

*With 2-person mixtures, examine loci with four alleles*

\[
\begin{align*}
PH_A + PH_C &= \text{Sum of major allele peak heights} \\
PH_B + PH_D &= \text{Sum of minor allele peak heights}
\end{align*}
\]

\[
\begin{align*}
\text{PHR}_{\text{A}} &= \frac{PH_A + PH_C}{PH_B + PH_D} \\
\text{PHR}_{\text{B}} &= \frac{PH_B + PH_D}{PH_A + PH_C} \\
\text{PHR}_{\text{C}} &= \frac{PH_A + PH_C}{PH_B + PH_D}
\end{align*}
\]

**Mixture Ratio Example**

**PowerPlex 16 (1 ng)**

**Analysis of the 4-Allele Loci**

Variability exists between loci

<table>
<thead>
<tr>
<th>Locus</th>
<th>PowerPlex 16 (1 ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D18S51</td>
<td>Variability exists between loci</td>
</tr>
<tr>
<td></td>
<td>(3344 + 3027) / (565 + 539) = 4.24</td>
</tr>
<tr>
<td></td>
<td>PHR = 52.5%</td>
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<tr>
<td>Penta D</td>
<td>Variability exists between loci</td>
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<td></td>
<td>(3287 + 3281) / (612 + 774) = 4.74</td>
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<tr>
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<td>PHR = 76.1%</td>
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<tr>
<td>D8S1179</td>
<td>Variability exists between loci</td>
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<td></td>
<td>(3208 + 3006) / (841 + 1072) = 3.02</td>
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<td>PHR = 78.3%</td>
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http://www.cstl.nist.gov/biotech/strbase/training.htm
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### PRINCIPLE

**Stochastic effects can impact minor component alleles (and PHRs)**

<table>
<thead>
<tr>
<th>Input DNA</th>
<th>Minor component amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5 ng</td>
<td>150 pg</td>
</tr>
<tr>
<td>1.0 ng</td>
<td>100 pg</td>
</tr>
<tr>
<td>0.5 ng</td>
<td>50 pg</td>
</tr>
<tr>
<td>0.25 ng</td>
<td>25 pg</td>
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</table>

Data courtesy of Amy Christen, Marshall University NEST Project Team

**Minor components drop out at low levels due to stochastic effects**

### PRACTICE

**As mixture ratios change, genotype possibilities alter**

- "Minor" alleles can no longer be fully distinguished from "major" alleles due to alternative possibilities that meet expected peak height ratios

- Minor contributor alleles become indistinguishable from stutter

### GUIDELINES

**Unrestricted vs. Restricted**

*Use of peak height information to select only certain combinations*

**Unrestricted**

- All combinations of alleles are deemed possible (relative peak height differences are not utilized)

**Restricted**

- Based on relative peak heights, alleles are paired only where specific combinations of alleles are deemed possible

### PROTOCOLS

**Calculation of PHRs for All Possible Genotype Combinations**

Examine all possible PHRs to see which combinations are possible:

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<td>0.567</td>
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### EVALUATION

**Evaluation of all pair-wise comparisons**

**SWGDAM 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.

### 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.

### PROTOCOLS

**Calculation of PHRs for All Possible Genotype Combinations**

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### PRINCIPLES

**All possible 3-contributor combinations**

- 23 "families" of possibilities
- 150 total combinations
- This "family" has 30 possibilities
- 3 allele pattern has 8 "families"

### PROTOCOLS

**Software tools can help perform calculations**

FSS-i3 iSTReam Mixture Interpretation Tool

### 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

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