Stutter

Michael D. Coble

Outline for Stutter

• GUIDELINES
  – SWGDAM Guideline 3.1 – Non-Allelic Peaks
  – Related SWGDAM Guidelines 3.1.1.1.; 3.1.1.3; 3.5.4.2; 3.5.8; 3.5.8.1; 3.5.8.2; 3.5.8.3; 5.2.2.2

• PRINCIPLES
  – Creation of stutter

• PROTOCOLS
  – Data collection and calculating stutter

• PRACTICE
  – Stutter peaks in mixture interpretation

Interpretation of DNA Typing Results

3.1. Non-Allelic Peaks

• Generally, non-allelic data such as stutter, non-template dependent nucleotide addition, disassociated dye, and incomplete spectral separation are reproducible;

Guidelines

Stutter Products

• Peaks that show up primarily one repeat less than the true allele as a result of strand slippage during DNA synthesis

Stutter Products

Typically 5-15% of true alleles in tetranucleotide repeats STR loci

Strand Slippage Model

**Review of the Literature**

Many labs just use a flat 15%

**Types of STR Repeat Units**

Requires size based DNA separation to resolve different alleles from one another

- **Dinucleotide** (CA)(CA)(CA)(CA)
- **Trinucleotide** (GCC)(GCC)(GCC)
- **Tetranucleotide** (AATG)(AATG)(AATG)
- **Pentanucleotide** (AGAAA)(AGAAA)
- **Hexanucleotide** (AGTACA)(AGTACA)

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**Interpretation of DNA Typing Results**

3.1.1.1.

In general, the empirical criteria are based on qualitative and/or quantitative characteristics of peaks. As an example, dye artifacts and spikes may be distinguished from allelic peaks based on morphology and/or reproducibility. Stutter and non-template dependent nucleotide addition peaks may be characterized based on size relative to an allelic peak and amplitude.

**Calculating Stutter**

Stutter % = \( \frac{N-4 \text{ peak}}{\text{allele peak}} \)

\( \frac{215}{2324} = 9.25\% \)

**How did you determine your stutter percentages?**

1. Internal validation with multiple samples.
2. Used the kit manufacturer guidelines.
3. Used the random number generator on my TI-83.

**Allele-Specific Stutter %**

![Image of Allele-Specific Stutter %](https://via.placeholder.com/150)
Developing Stutter Filter Values

- **Samples** – Ideally at least 5 observations of each stutter product per locus from relevant populations (e.g., longer repeats in FGA alleles are observed mostly among African Americans).
- Use typical DNA input quantities (0.5 – 2.0ng), but may want to assess stutter at lower levels (e.g., <150pg). Excessive DNA (5-10ng) can skew your average percentages.
- **Now what??**
**PROTOCOLS**

Simply merge data into program...

“Do It All”

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**Allele-Specific Stutter**

% Stutter

Allele Size, bp

PP16 data

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**TPOX – [AATG]_n**

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<th>Allele</th>
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MADe – Median Absolute Deviation

Mutation Rate: 0.01%

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**Allele-Specific Stutter**

% Stutter

Number of Repeats

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**D3S1358 – TCTA[TCTG]_n[TCTA]_n**

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PRINCIPLES

Stutter Trends

- Tetra
- Tetra w/ LTDNA
- Penta

Repeat Length

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Becky
**1. Highest value observed per locus (+3 SD).**

**2. An average across all loci is determined and used across the board.**

**3. ABI made me use their default stutter percentages.**
Interpretation of Potential Stutter Peaks in a Mixed Sample

• 3.5.8.1. For mixtures in which minor contributors are determined to be present, a peak in stutter position (generally n-4) may be determined to be 1) a stutter peak, 2) an allelic peak, or 3) indistinguishable as being either an allelic or stutter peak.

Consideration of Peaks in Stutter Positions

- Generally, when the height of a peak in the stutter position exceeds the laboratory’s stutter expectation for a given locus, that peak is consistent with being of allelic origin and should be designated as an allele.

• 3.5.8.2. If a peak is at or below this expectation, it is generally designated as a stutter peak. However, it should also be considered as a possible allelic peak, particularly if the peak height of the potential stutter peak(s) is consistent with (or greater than) the heights observed for any allelic peaks that are conclusively attributed (i.e., peaks in non-stutter positions) to the minor contributor(s).

Do you ever include stutter as a potential minor allele? (Section 3.5.8.3)

1. Yes, only if it matches the suspect.
2. Yes, and we make that determination prior to looking at the suspect(s) profile.
3. No, we do not do this.
Summary

• Stutter can vary across profiles, loci, or alleles.

• Stutter becomes especially problematic for mixtures when samples are at low [DNA] levels.

• Labs should decide when is it appropriate to turn off stutter filters, especially when the minor component alleles are nearly the same height as stutter peaks.

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michael.coble@nist.gov
301-975-4330