21st International Symposium on Human Identification

Mixture Interpretation Workshop:

Principles, Protocols, and Practice

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Stutter

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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

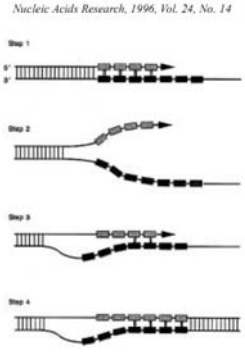
3.1. Non-Allelic Peaks

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

Stutter Products

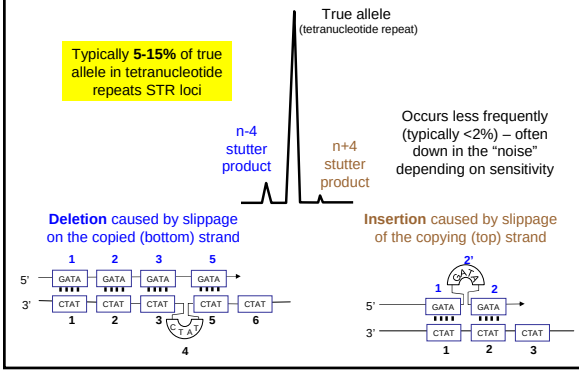
- Peaks that show up primarily one repeat less than the true allele as a result of strand slippage during DNA synthesis

Strand Slippage Model



Walsh, P.S., et al. (1996). Sequence analysis and characterization of stutter products at the tetranucleotide repeat locus vWA. *Nucleic Acids Research, 24*, 2807-2812.

Stutter Products



PRINCIPLES

Review of the Literature

Study	Kit	Measured	TH01	vWA	D18S51
Greenspoon *et al.* (2004)	PP16 BIO	mean + 3SD	5	14	13
Krenke *et al.* (2002)	PP16	mean + 1SD	3	10	9
Moretti *et al.* (2001)	Pro+/CoFiler	mean + 3SD	15.9	11.7	13.9
Mulero *et al.* (2008)	MiniFiler	max %	-	-	**17.3**
Hill et al. (2010)	PP ESX	mean + 3SD	4.2	14.6	14.6
User Manual	Identifiler	max%	5.1	12.6	17
User Manual	IDfiler Direct	mean + 3SD	4.7	11.9	12.8
User Manual	IDfiler Plus	mean + 3SD	4	12.4	13.6

Many labs just use a flat 15%

PRINCIPLES

Types of STR Repeat Units

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

- **Di**nucleotide (CA)(CA)(CA)(CA)
- **Tri**nucleotide (GCC)(GCC)(GCC)
- **Tetra**nucleotide (AATG)(AATG)(AATG)
- **Penta**nucleotide (AGAAA)(AGAAA)
- **Hexa**nucleotide (AGTACA)(AGTACA)

GUIDELINES

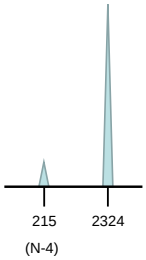
Interpretation of DNA Typing Results

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.***

PROTOCOLS

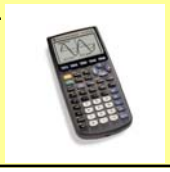
Calculating Stutter

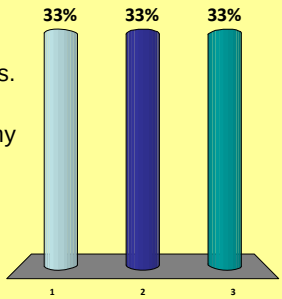


$$\text{Stutter \%} = \frac{\text{N-4 peak}}{\text{allele peak}} = \frac{215}{2324} = 9.25\%$$

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 %

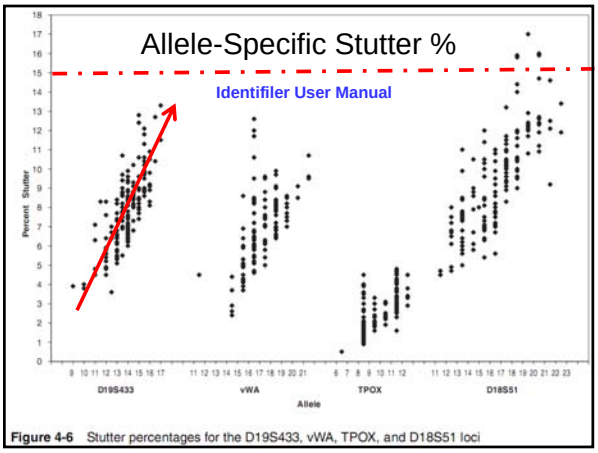


Figure 4-6 Stutter percentages for the D19S433, vWA, TPOX, and D18S51 loci

PROTOCOLS

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

STR_StutterFreq!

Welcome to STR_StutterFreq!
Version <04-Jan-10>

STR_StutterFreq is a specialty analysis tool for characterizing stutter frequency…
Development of STR_StutterFreq was funded in part by the National Institute of Justice.

- Program developed by Dave Duewer (NIST) to rapidly calculate stutter frequencies.
- Manuscript in progress, to be placed in the STRBase software section.

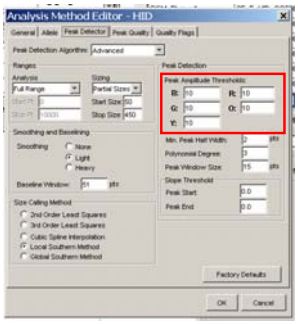
PROTOCOLS

STR_StutterFreq!

Set stutter filter to zero

PROTOCOLS

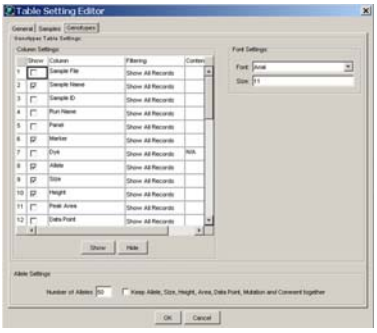
STR_StutterFreq!



Set peak detection to 10 RFUs

PROTOCOLS

STR_StutterFreq!

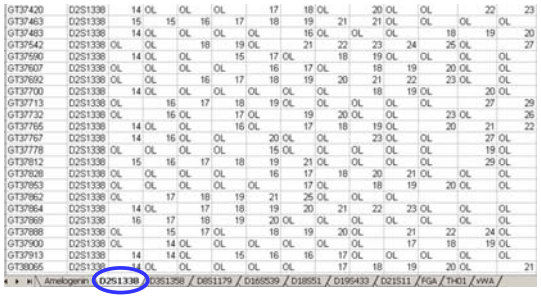


Export 50 alleles for each marker (separately)

Info
Allele
Size
Height

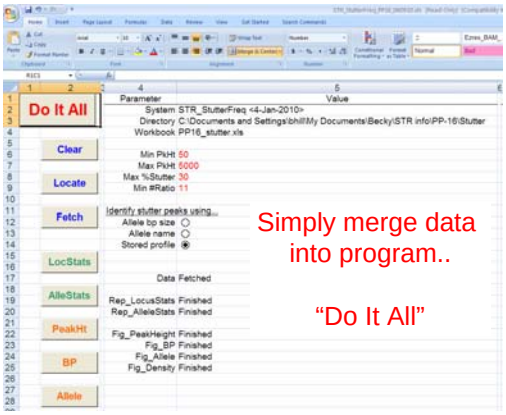
PROTOCOLS

STR_StutterFreq!



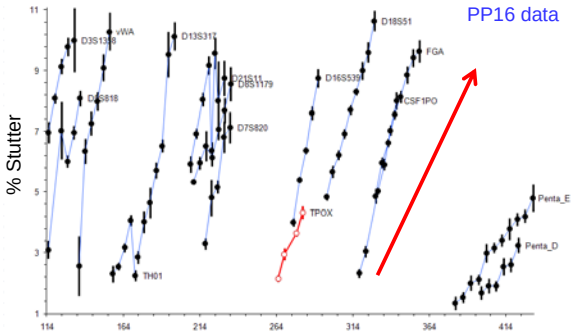
Size and Height

PROTOCOLS



PROTOCOLS NEW SLIDE

Allele-Specific Stutter



PROTOCOLS

TPOX – $[AATG]_N$

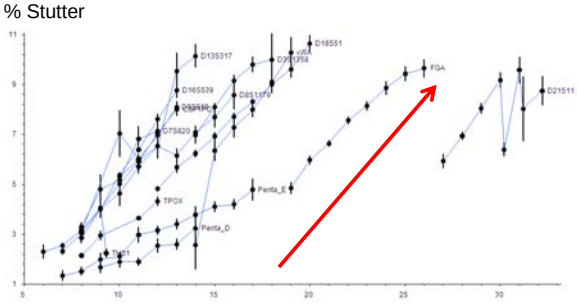
Locus	Allele	Size	Stutter #	Stutter Median	Stutter MADe
TPOX	8	265.2	86	2.1	0.5
	9	269.2	21	2.9	0.4
	11	277.2	75	3.6	0.4
	12	281.2	14	4.3	0.4
		Avg	196	3.3	0.4
		SD		0.9	

MADe – Median Absolute Deviation

Mutation Rate: 0.01%

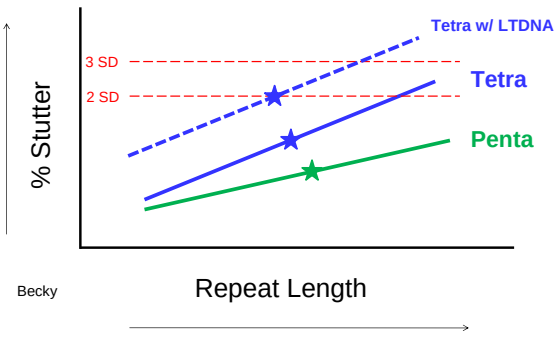
PROTOCOLS NEW SLIDE

Allele-Specific Stutter



PRINCIPLES

Stutter Trends



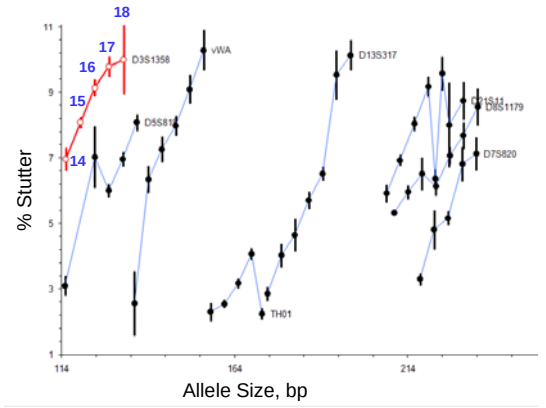
Becky

PROTOCOLS

D3S1358 – $TCTA[TCTG]_N[TCTA]_N$

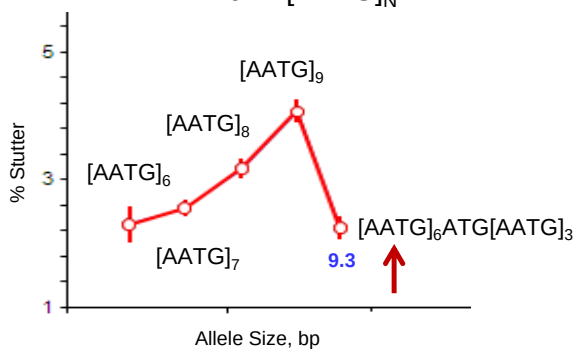
Locus	Allele	Size	Stutter #	Stutter Median	Stutter MADe
D3S1358	14	115.2	26	7.0	0.9
	15	119.4	66	8.1	0.7
	16	123.5	47	9.1	0.9
	17	127.7	47	9.8	1.1
	18	131.9	41	10.0	3.4
		Avg	227	8.8	1.7
		SD		1.3	

PROTOCOLS



PROTOCOLS NEW SLIDE

TH01 - $[AATG]_N$



PROTOCOLS

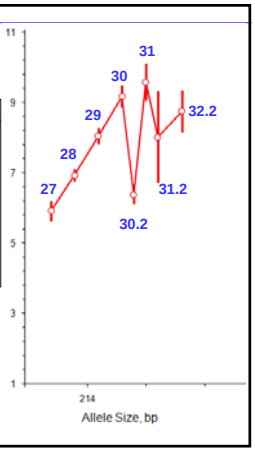
D21S11 – a complex repeat

$[TCTA]_N$ $[TCTG]_N$ $[TCTA]_N$ TA $[TCTA]_N$ TCA $[TCTA]_N$ TCCATA $[TCTA]_N$

			Stutter		
Locus	Allele	Size	#	Median	MADe
D21S11	27	207.8	20	5.9	0.6
	28	211.8	69	6.9	0.7
	29	215.8	59	8.0	0.8
	30	219.9	66	9.2	1.2
→	30.2	221.9	11	6.4	0.5
	31	223.9	21	9.6	1.2
	31.2	226.0	28	8.0	3.4
	32.2	230.0	33	8.7	1.7
		Avg	307	7.8	1.6
		SD		1.3	

PROTOCOLS

			Stutter		
Locus	Allele	Size	#	Median	MADe
D21S11	27	207.8	20	5.9	0.6
	28	211.8	69	6.9	0.7
	29	215.8	59	8.0	0.8
	30	219.9	66	9.2	1.2
	30.2	221.9	11	6.4	0.5
	31	223.9	21	9.6	1.2
	31.2	226.0	28	8.0	3.4
	32.2	230.0	33	8.7	1.7
		Avg	307	7.8	1.6
		SD		1.3	



PROTOCOLS

			Stutter		
Locus	Allele	Size	#	Median	MADe
D18S51	12	296.9	43	4.8	0.4
	13	300.7	27	5.7	0.5
	14	304.6	35	6.2	0.5
	15	308.5	55	6.9	0.6
	16	312.4	46	7.7	0.5
	17	316.2	47	8.3	0.4
	18	320.2	38	9.0	0.9
	19	324.0	30	9.6	0.9
	20	328.0	24	10.6	0.8
		Avg	345	7.7	0.6
		SD		1.9	

$\overline{X}$ + 3 SD

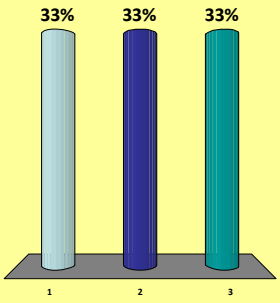
= 7.7 + 3(1.9)

= 13.4%

Max Stutter % = 13.4%

What stutter percentages do you use?

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



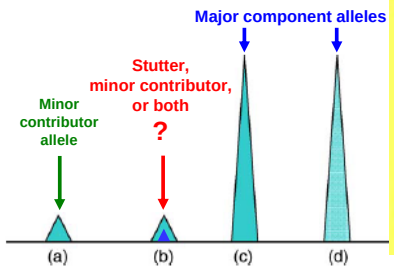
GUIDELINES

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.

PRACTICE

Consideration of Peaks in Stutter Positions



Possibilities for Minor
a,a
a,b
a,c
a,d

Probability of Inclusion = $(f_a + f_b + f_c + f_d)^2$

If peak height of peak a is within established PHR of peak b peak height

f_a = freq. of allele a

Fig. 4. *c* and *d* are unambiguous alleles, *b* is a minor allele in a stutter position and *a* is an unambiguous minor allele.

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

GUIDELINES

Interpretation of Potential Stutter Peaks in a Mixed Sample

- 3.5.8.2. 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.

GUIDELINES

Interpretation of Potential Stutter Peaks in a Mixed Sample

- 3.5.8.3. 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).

PRACTICE

ISFG Recommendation #6 Example

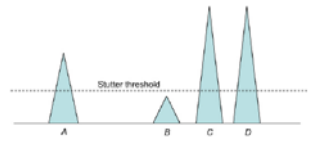


Fig. 2. A two person mixture with major peaks *C*, *D* and minor peaks *A*. There is an additional peak present in a stutter position (*B*).

Likely a AA

(homozygote)

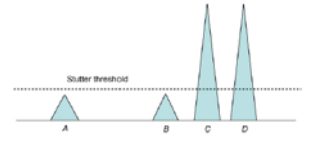


Fig. 3. A two person mixture with major peaks *C*, *D* and minor peaks *A*, *B*, where *B* is in a stutter position.

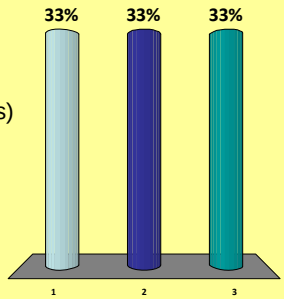
Possibly AB

(heterozygote)

Could also be AC, AD, AA, or A,? (dropout)

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.

Acknowledgments

- NIJ Funding to our NIST Group through NIST OLES interagency agreement 2008-DN-R-121
- Dave Duewer and Becky Hill for stutter data.

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