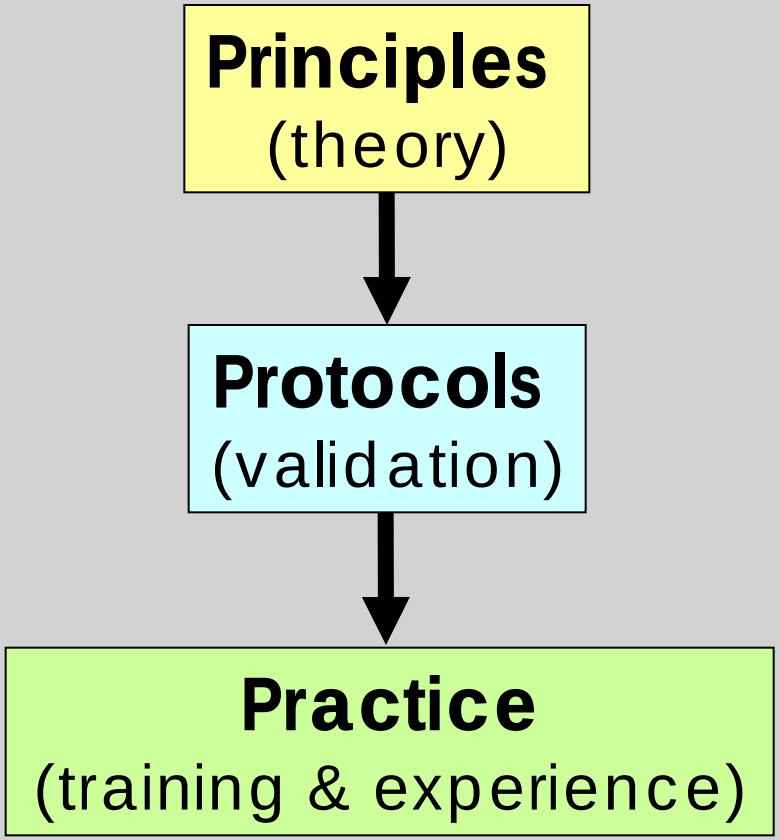
DNA Mixture Interpretation Workshop | *Michael D. Coble, PhD*

Design and Execution of Validation Studies for Establishing DNA Mixture Interpretation Procedures

Elements of DNA Mixture Interpretation



Consistency across analysts

ISFG Recommendations
SWGDAM Guidelines

Your Laboratory SOPs

Training within Your Laboratory

Periodic training will aid accuracy and efficiency within your laboratory.

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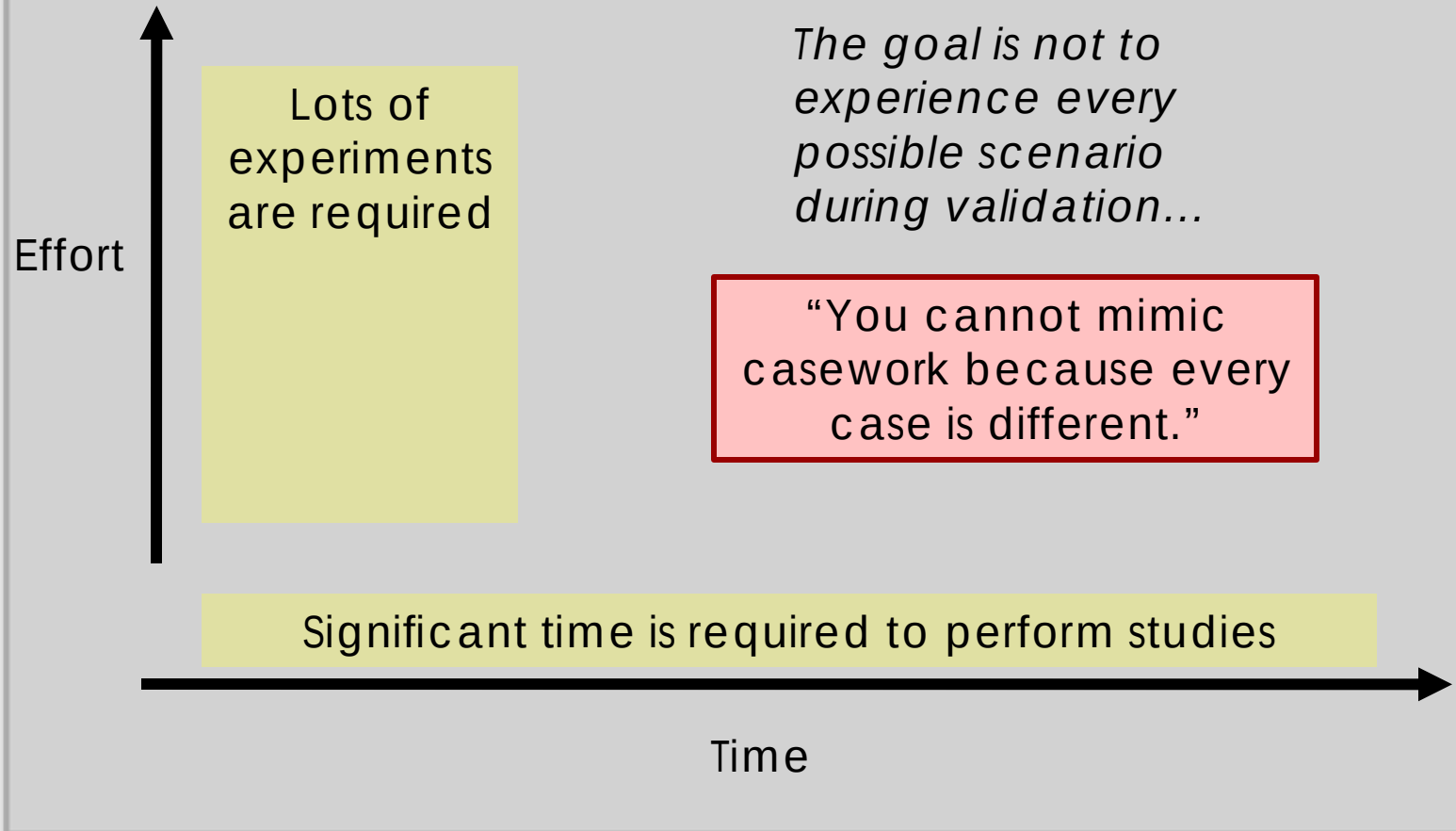
Common Perceptions of Validation



Validation Studies

- **Information from validation studies should be used to set laboratory-specific**
  - **Minimum Peak Heights (detection thresholds)**
  - **Stutter %**
  - **Peak Height Ratios**
  - **Relative balance across loci**

- **These values are all dependent on amount of input DNA**
  - **If low-level DNA is amplified, stutter % may be higher and peak height ratios may be lower**

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Setting Thresholds

- **Analytical (detection) threshold**
  - Dependent on ***instrument sensitivity***

  ~50 RFU
  - Impacted by instrument baseline noise

what is a peak?

- **Stochastic (drop-out) threshold**
  - Dependent on ***biological sensitivity***

  ~150-200 RFU
  - Impacted by assay and injection parameters

what is reliable PCR data?

Validation studies should be performed in each laboratory

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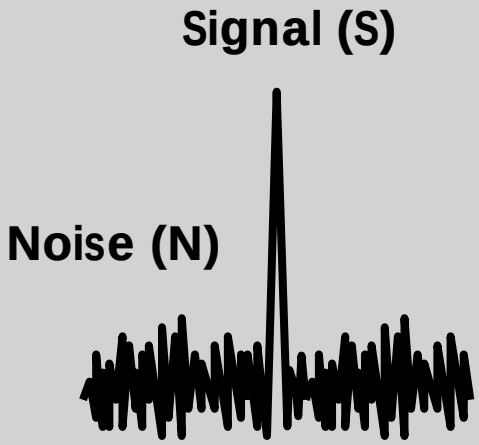
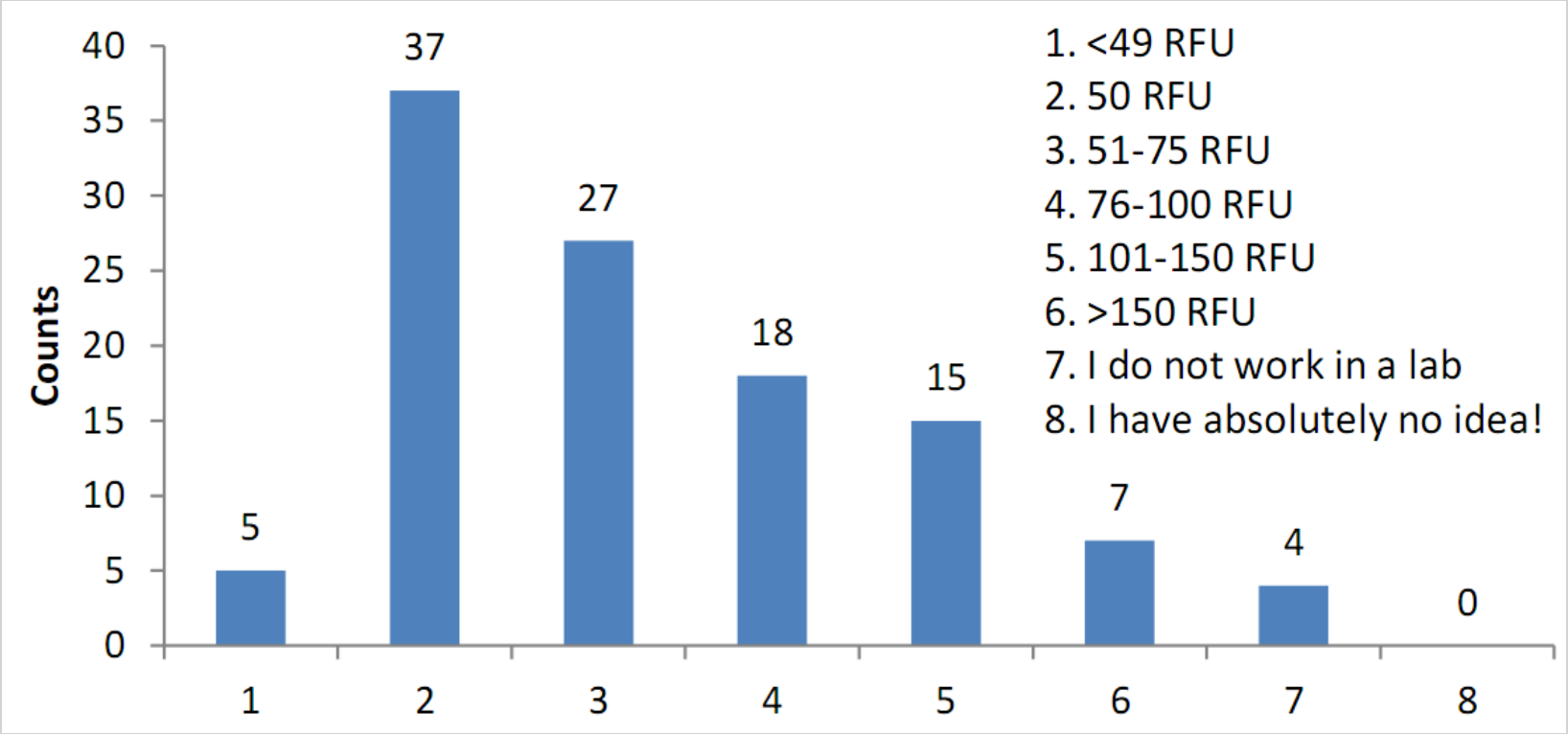
1.1. Analytical threshold

- **The Laboratory should establish an analytical threshold based on signal-to-noise analyses of internally derived empirical data.**



What is your AT? Question at the Promega meeting 10/2010 (n=113)



Slide Courtesy of Robin Cotton

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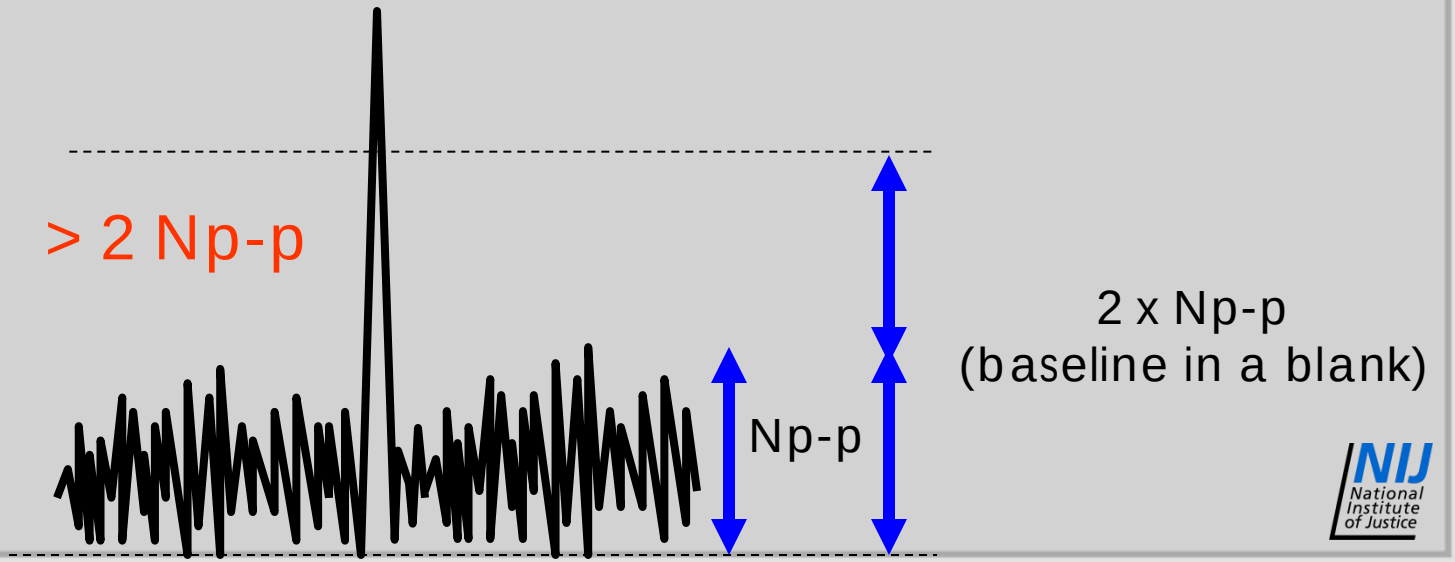
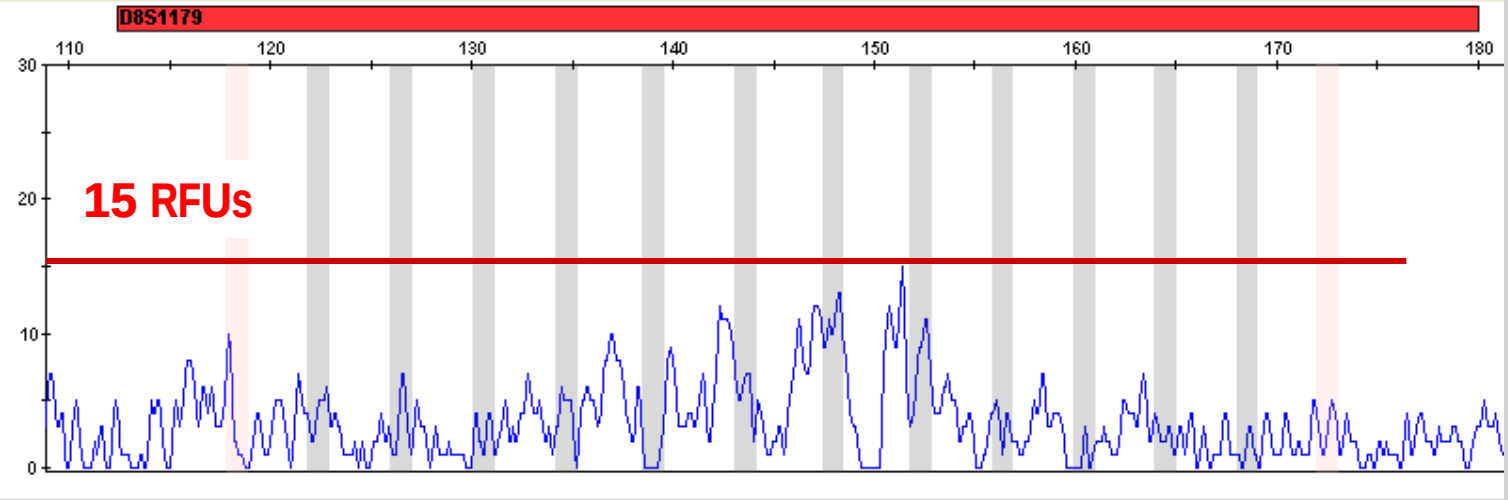
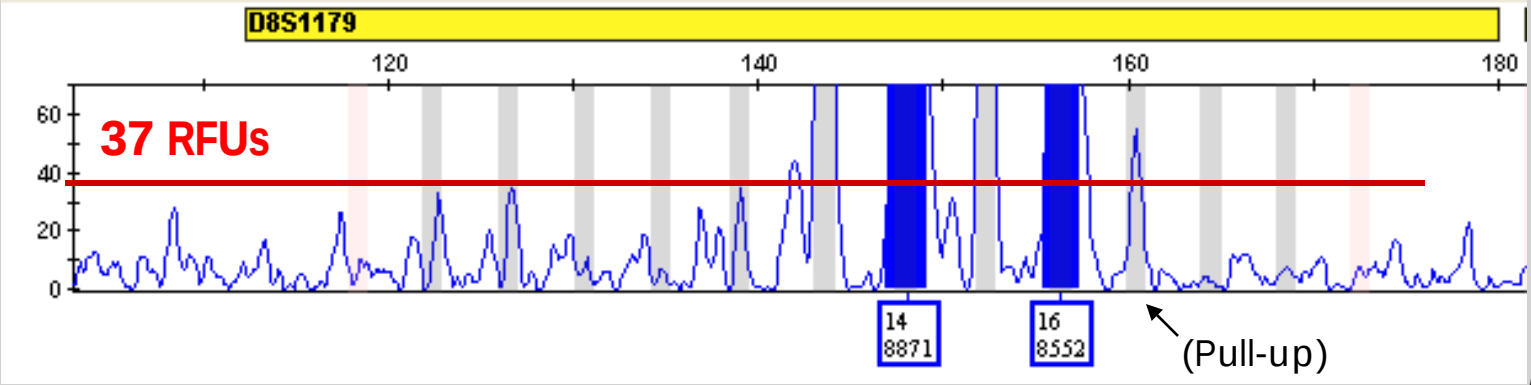
1.1. Analytical threshold

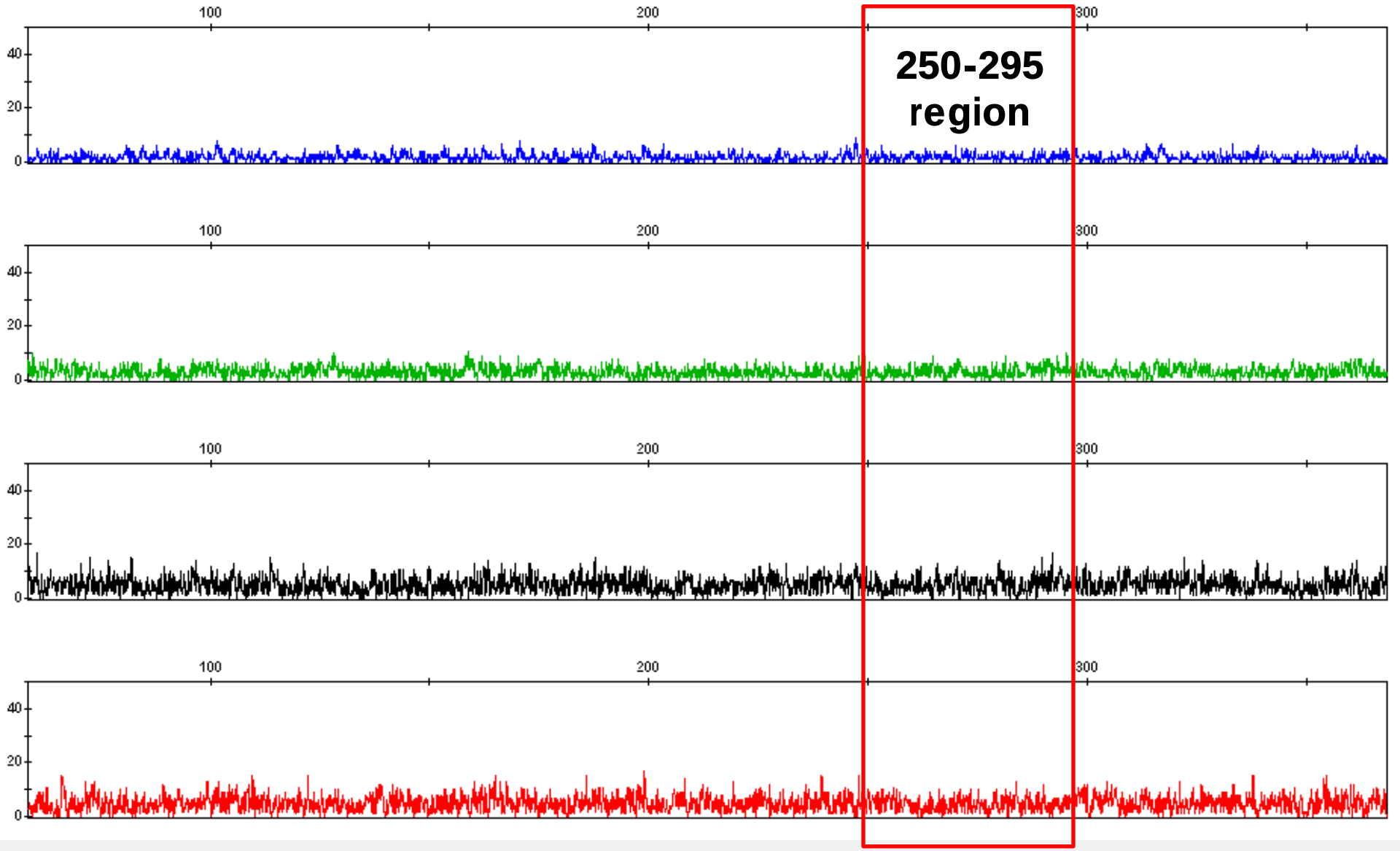
- **As an example, an analytical threshold may be based on two times the intensity difference between the highest peak and lowest trough within the instrumental noise data. *Other scientific methods may be used.***



Sample Source – Negatives? Positives?





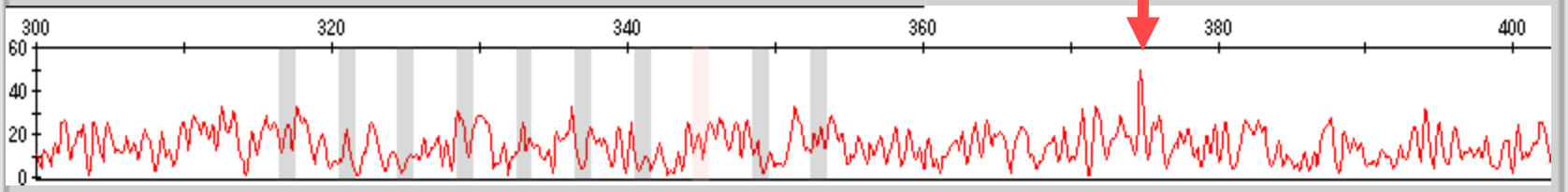


Analytical Thresholds can be determined for each dye channel

New Instruments, New Thresholds…

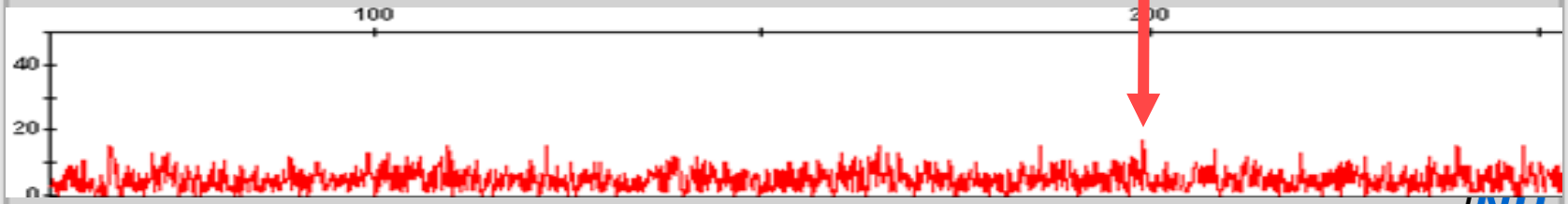
ABI 3500
1.2 kV, 15 sec (Default)

50 RFUs



ABI 3130
3.0 kV, 10 sec (Default)

16 RFUs



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How to set an analytical threshold (AT)? Some Examples…

SWGDAM: Two times the intensity difference between the highest peak and lowest trough (as an example).

"The Ballpark": Three times the highest peak.

Gilder et al. (2007): Determined LOD by examining Pos, Neg, RB from 150 cases.

$$LOD = \mu_b + 3\sigma_b$$

J Forensic Sci, January 2007, Vol. 52, No. 1
doi:10.1111/j.1556-4029.2006.00318.x
Available online at: www.blackwell-synergy.com

TECHNICAL NOTE

Jason R. Gilder,[1] M.S.; Travis E. Doom,[2] Ph.D.; Keith Inman,[3] M. Crim.; and Dan E. Krane,[4] Ph.D.

Run-Specific Limits of Detection and Quantitation for STR-based DNA Testing

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Gilder et al. (2007)

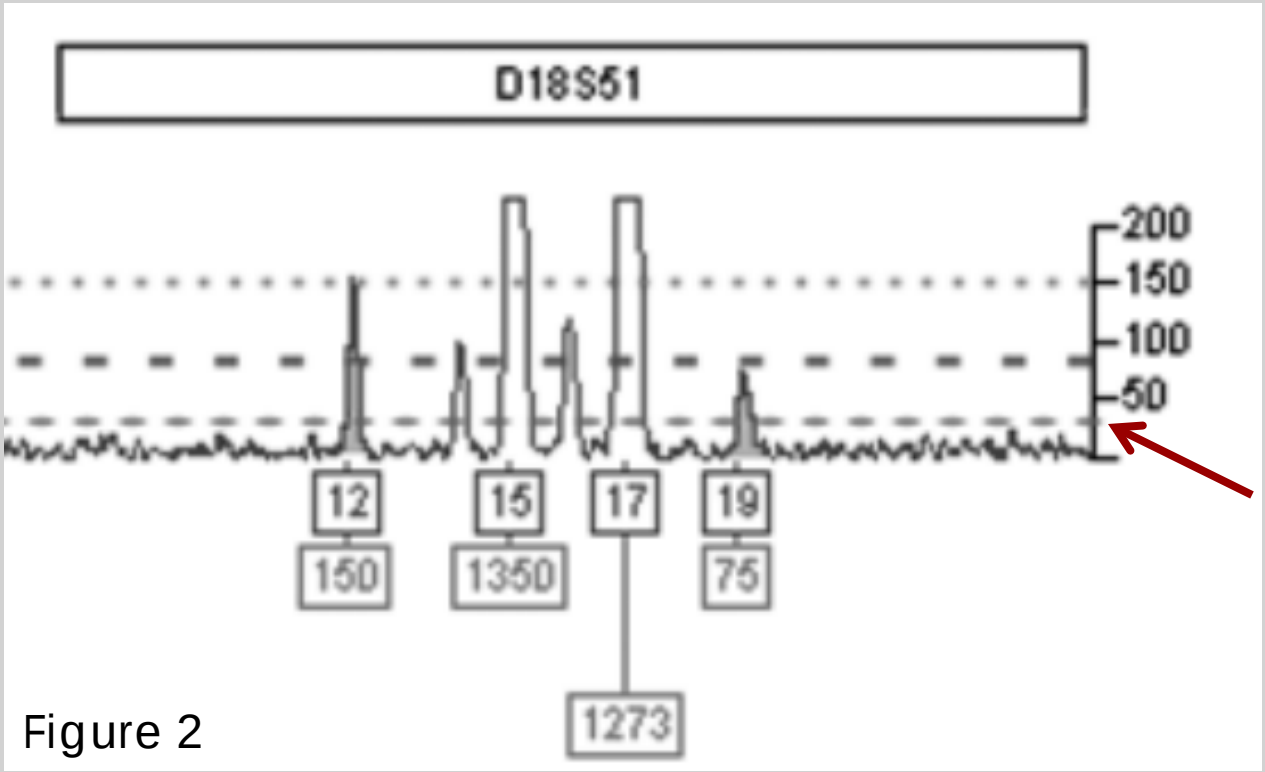

Figure 2

10:1 mixture

LOD = 29 RFUs

Gilder et al. (2007)

TABLE 1—*Maximum, minimum, and average baseline levels observed in the set of reagent blanks, negative controls, and positive controls (determined from controls in 50 different runs).*

	μ_b	σ_b	$\mu_b+3\sigma_b$	$\mu_b+10\sigma_b$
Positive Control				
Maximum	6.7	6.9	27.4	75.7
Average	5.0	3.7	16.1	42.0
Minimum	3.7	2.4	10.9	27.7
Negative Control				
Maximum	13.4	13.2	53.0	145.4
Average	5.4	3.9	17.1	44.4
Minimum	4.0	2.6	11.8	30.0
Reagent Blank				
Maximum	6.5	11.0	39.5	116.5
Average	5.3	4.0	17.3	45.3
Minimum	4.0	2.6	11.8	30.0
All three controls averaged				
Maximum	7.1	7.3	29.0	80.1
Average	5.2	3.9	16.9	44.2
Minimum	3.9	2.5	11.4	28.9

All values are in RFUs.

How to set an analytical threshold (AT)? Some Examples…

SWGDAM: Two times the intensity difference between the highest peak and lowest trough (as an example).

"The Ballpark": Three times the highest peak.

Gilder et al. (2007): Determined LOD by examining Pos, Neg, RB from 150 cases.

Catherine Grgicak (Boston U.) presentation at the 2010 ISHI (Promega) mixture workshop. (http://www.cstl.nist.gov/biotech/strbase/mixture.htm)

Multiple methods for determining AT

- **Method 1.**
  - **Kaiser (IUPAC 1976)**
    - **Winefordner 1983 and Krane 2007**
- **Method 2.**
  - **Currie (IUPAC 1995)**
    - **Winefordner 1983**
- **Method 3.**
  - **Example in SWGDAM Guidelines**

Negative Controls (at least 20)

- **Method 4.**
  - **Miller & Miller. *Statistics for Analytical Chemistry (Ellis Horwood & Prentice Hall)***
    - **IUPAC 1997 ElectroAnalytical Committee**

DNA Dilution Series

- **Method 5.**
  - **1997 IUPAC ElectroAnalytical Committee Recommendations**

Courtesy of Catherine Grgicak

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Multiple methods for determining AT

$$AT_{M1} = \bar{Y}_{bl} + ks_{bl}$$

$$AT_{M3} = 2(Y_{\max} - Y_{\min})$$

$$AT_{M2} = \bar{Y}_{bl} + t_{1-\alpha,v}\frac{s_{bl}}{\sqrt{n}}$$

Negative Controls
(at least 20)

$$AT_{M4} = b + 3S_y$$

$$AT_{M5} = b + t_{n-1,\alpha}S_y$$

(http://www.cstl.nist.gov/biotech/strbase/mixture.htm)

Courtesy of Catherine Grgicak

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Multiple methods for determining AT

Method	Origin	Analytical Threshold for green 5s injection example
1	Negatives	7
2	Negatives	4
3	Negatives	20
4	DNA Series	31
5	DNA Series	39

Courtesy of Catherine Grgicak

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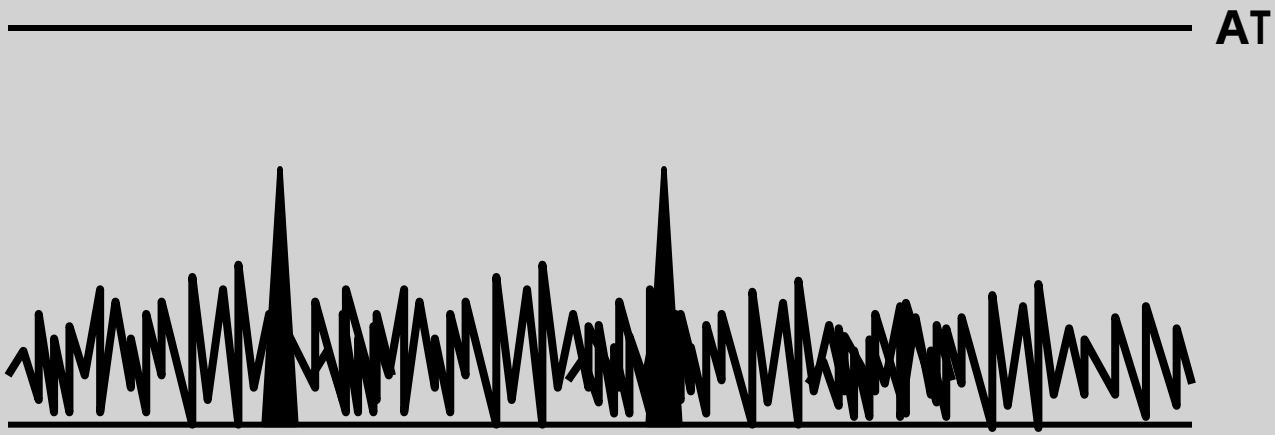
What about peaks below AT?

- **The Analytical Threshold is the "floor" of the EPG. Apparent peaks below the AT are not to be trusted!**



"The Sixth Peak"

Setting the Stochastic Threshold

Determining the Dropout (Stochastic) Threshold

Gill *et al.* (2008) *FSI Genetics* 2(1): 76–82

- **The dropout threshold can be determined experimentally for a given analytical technique from a series of pre-PCR dilutions of extracts of known genotype technique (it will probably vary between analytical methods). These samples can be used to determine the point where allelic dropout of a heterozygote is observed relative to the size of the survivor companion allele. The threshold is the maximum size of the companion allele observed. This is also the point where Pr(D) approaches zero (Fig. 4).**

Dropout threshold will change depending on instrument and assay conditions (e.g., longer CE injection will raise dropout threshold)

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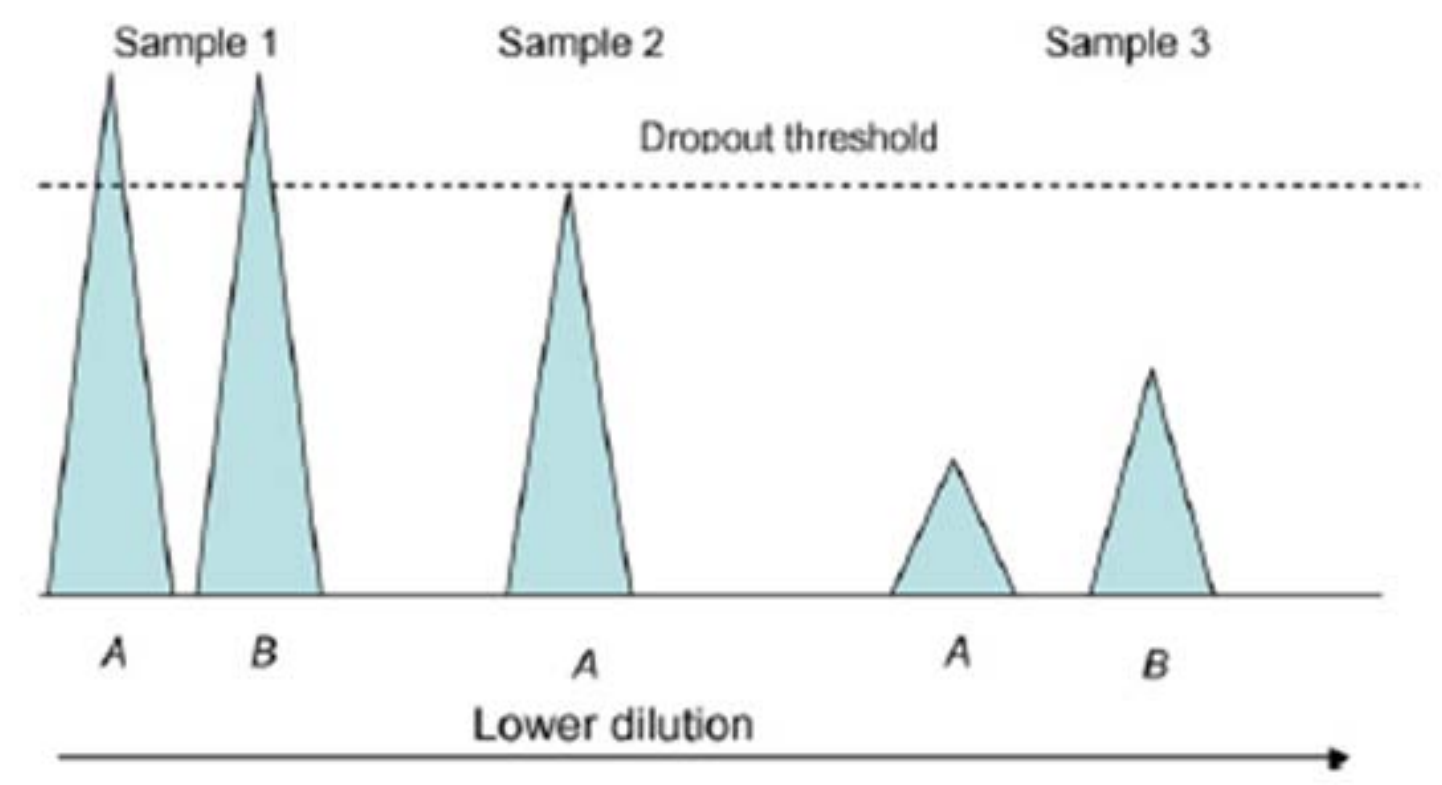
Hypothetical Examples

Gill *et al.* (2008) *FSI Genetics* 2(1): 76–82



Forensic Science International: Genetics 3 (2009) 104–111

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

The *low-template-DNA* (stochastic) threshold—Its determination relative to risk analysis for national DNA databases

Peter Gill [a,b,*], Roberto Puch-Solis [c], James Curran [d]

[a] *University of Strathclyde, Royal College, 204 George Street, Glasgow G1 1XW, UK*
[b] *Institute of Forensic Medicine, University of Oslo, 0027 Oslo, Norway*
[c] *Forensic Science Service, Trident Court, Solihull B37 7YN, UK*
[d] *Department of Statistics, University of Auckland, Private Bag 92019, New Zealand*

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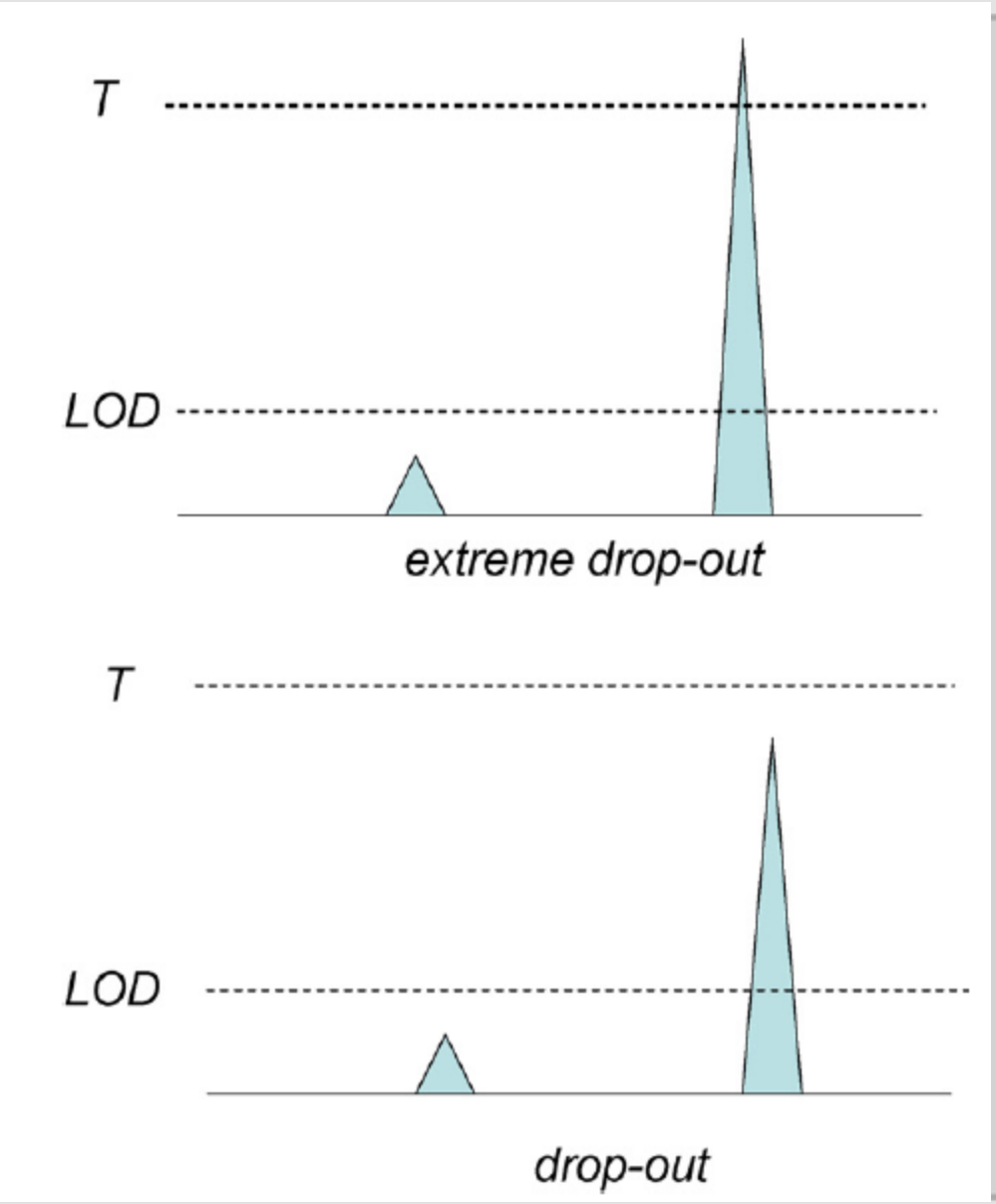
Gill *et al.* (2009) *FSI-G* 3: 104-111



Gill *et al.* (2009) *FSI-G* 3: 104-111

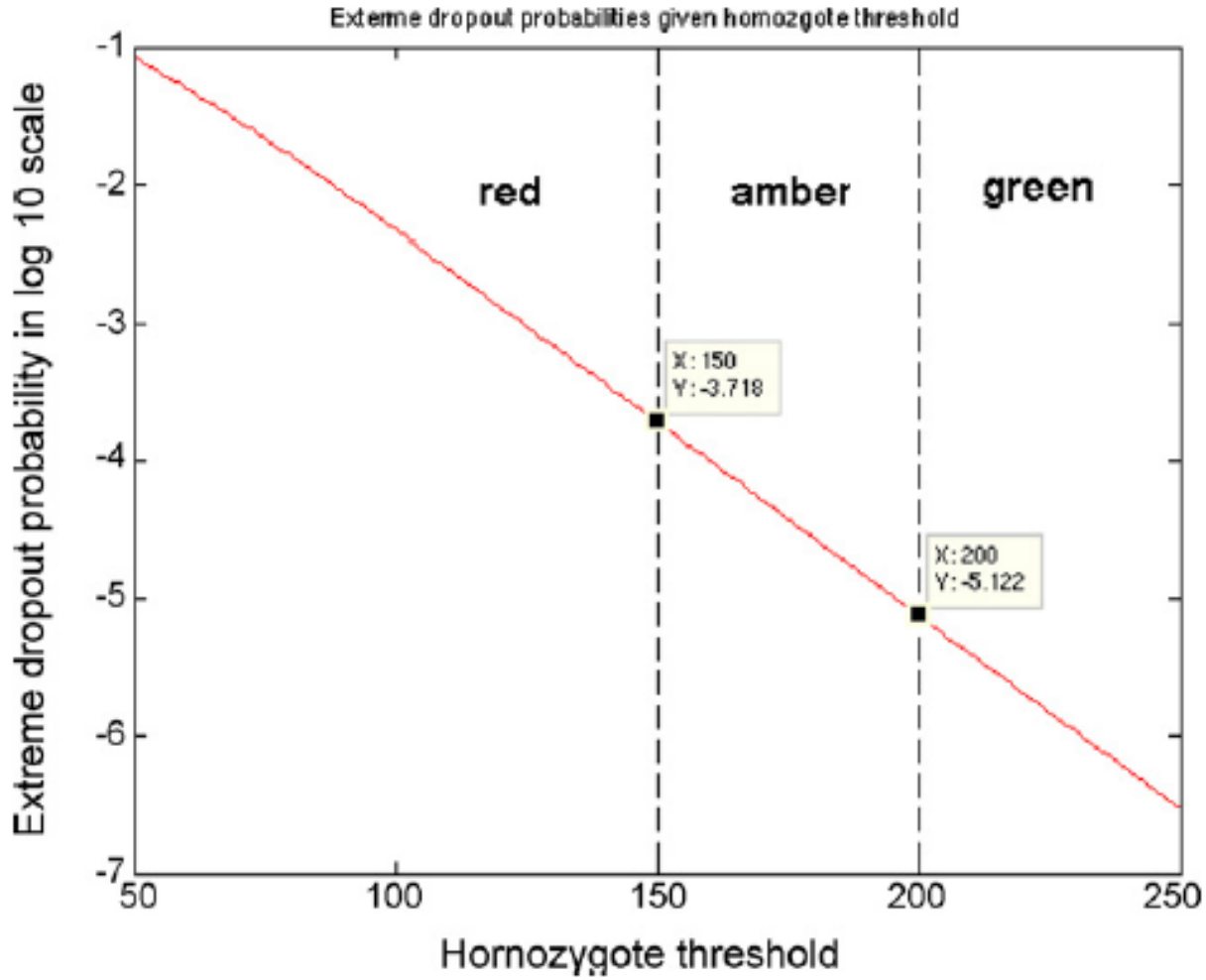


Fig. 3. A diagrammatic representation of the three profile categories and their associated risks relative to the low-level-DNA (homozygote) threshold.

Forensic Science International: Genetics 3 (2009) 222–226

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Estimating the probability of allelic drop-out of STR alleles in forensic genetics

Torben Tvedebrink [a,*], Poul Svante Eriksen [a,1], Helle Smidt Mogensen [b,2], Niels Morling [b,3]

[a] Department of Mathematical Sciences, Aalborg University, Fredrik Bajers Vej 7G, DK-9220 Aalborg East, Denmark
[b] Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health Sciences, University of Copenhagen, Fredrik V's Vej 11, DK-2100 Copenhagen East, Denmark

Table 3
Mean peak heights (rfu) for various drop-out probabilities for 10 STR loci.

$P(D\|\hat{H})$	D3	vWA	D16	D2	D8	D21	D18	D19	TH0	FGA	Overall
0.0001	556	577	622	562	558	461	531	722	723	692	648
0.0005	384	399	430	388	385	318	367	499	499	478	439
0.0010	327	340	366	331	328	271	313	425	426	407	371
0.0050	226	235	253	228	226	187	216	293	294	281	251
0.0100	192	200	215	194	193	159	184	250	250	239	212
0.0500	132	137	147	133	132	109	126	171	171	164	142
0.1000	111	115	124	112	111	92	106	144	144	138	119
0.2000	92	95	103	93	92	76	88	119	120	114	98
0.3000	81	84	91	82	81	67	78	105	106	101	86
0.4000	73	76	82	74	74	61	70	95	95	91	77
0.5000	67	69	75	68	67	55	64	87	87	83	70
0.6000	61	63	68	62	61	50	58	79	79	76	63
0.7000	55	57	62	56	55	46	53	71	71	68	57
0.8000	49	50	54	49	49	40	46	63	63	60	50
0.9000	40	42	45	41	40	33	39	52	52	50	41
0.9500	34	35	38	34	34	28	32	44	44	42	34
0.9900	23	24	26	23	23	19	22	30	30	29	23

3.2. Application of Peak Height Thresholds to Allelic Peaks

- **It is noted that a stochastic threshold may be established by assessing peak height ratios across multiple loci in dilution series of DNA amplified in replicate. The RFU value above which it is reasonable to assume that, at a given locus, allelic dropout of a sister allele has not occurred constitutes a stochastic threshold.**

3.2. Application of Peak Height Thresholds to Allelic Peaks

- **3.2.1. The laboratory establishes a stochastic threshold based on empirical data derived within the laboratory and specific to the quantitation and amplification systems (e.g., kits) and the detection instrumentation used.**

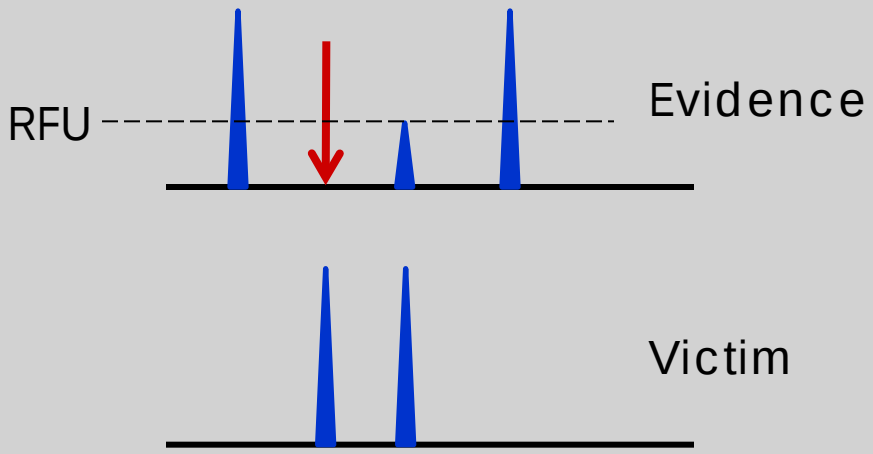
Setting Stochastic Thresholds (some examples)

Mass. State Police Approach-Identifiler (Courtesy of Joanne Sgueglia)

- **Titration sets of at least two sources (have used 5 sources for some validations).**
- **Target DNA using two fold dilution series from 5 ng, 2.5 ng, 1.25 ng …….39 pg.**
- **Establish AT prior to ST and use as a guide to have ST satisfy two criteria:**
  - **1. Three fold the AT**
  - **2. Obtain a partial profile at 150pg. This ensures no data is interpreted with statistical weight in the stochastic or low copy number region (gray zone).**

Using "Real World" Data (Courtesy of Dr. Robin Cotton)

- **Examine sexual assault casework data from known heterozygous loci using:**
  - **two person mixtures**
  - **one component is consistent with a known victim**
  - **loci with 4 alleles**



Setting Stochastic Thresholds (NIST)

- **Multiple samples, replicates, and concentrations are ideal to get a feel for how the system is working**
  - **We used 3 fully heterozygous samples with 10 replicates at 2 ng, 1 ng, 800 pg, 500 pg, 400 pg, 300 pg, 200 pg, 100 pg, 30 pg, & 10 pg**

Sample Selection

Description	CSF1PO	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D21S11	FGA	TH01	TPOX	vWA	Penta D	Penta E	D2	D19
Genomic 8	10, 12	15, 18	12, 13	9, 10	12,14	9, 13	9, 11	15,18	30,31	24, 28	7, 8	8,12	15,17	8, 9	5,10	22,22	12.2,15
9947A	10, 12	14, 15	11, 11	10, 11	13,13	11, 11	11, 12	15,19	30,30	23, 24	8, 9.3	8,8	17,18	12,12	12,13	19,23	14,15

9947A – 5/13 loci are homozygous

Setting Stochastic Thresholds (NIST)

- **Multiple samples, replicates, and concentrations are ideal to get a feel for how the system is working**
  - **We used 3 fully heterozygous samples with 10 replicates at 2 ng, 1 ng, 800 pg, 500 pg, 400 pg, 300 pg, 200 pg, 100 pg, 30 pg, & 10 pg**
- **Stochastic thresholds are not perfect or "cut and dry"**
  - **Can vary between loci and dye channels**

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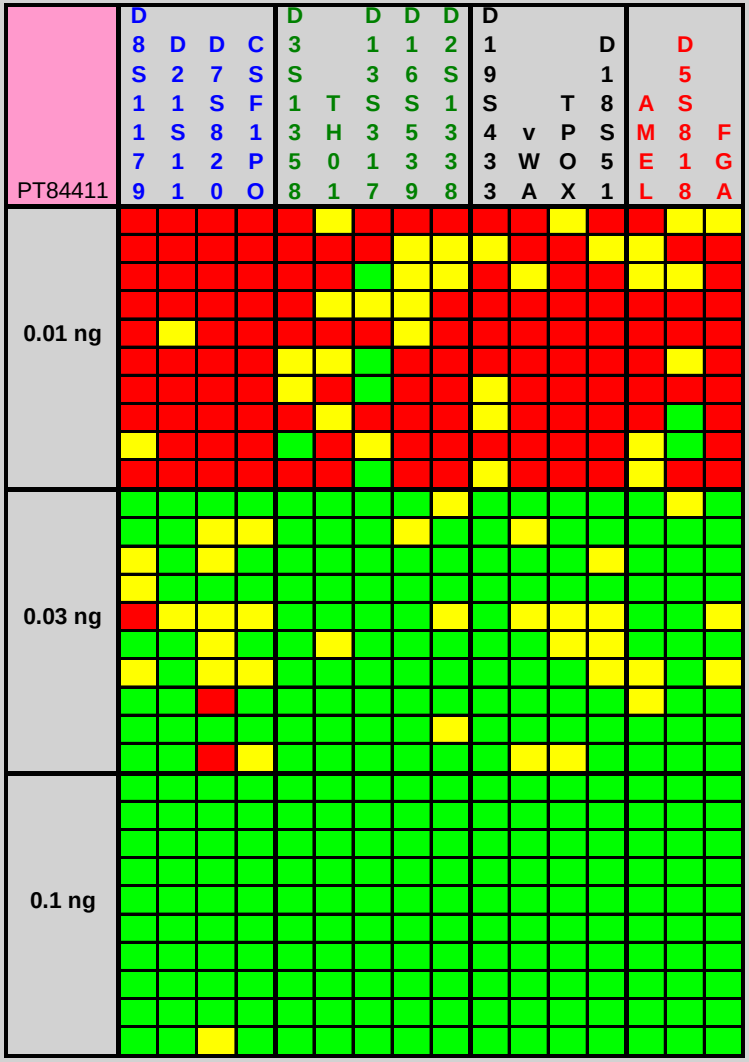
Setting Stochastic Thresholds



Identifiler, 28 cycles

3130*xl*, 10 sec @ 3kV inj

Slide courtesy of Becky Hill (NIST)

Setting Stochastic Thresholds

Identifiler, 28 cycles

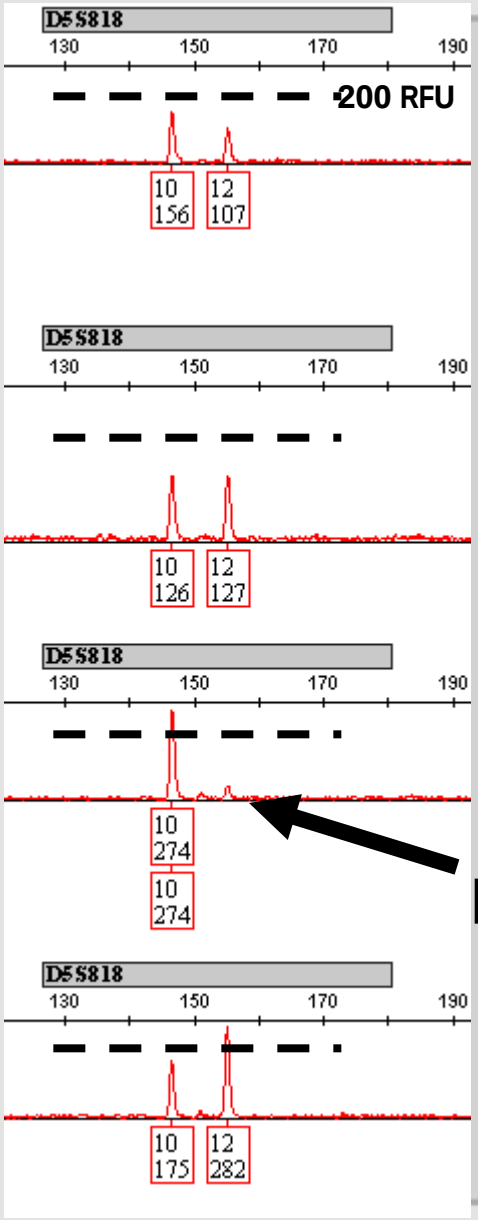
3130*xl*, 10 sec @ 3kV inj

0.03 ng									57						274	
			114	119				242			142					
	149		122										185			
	152															
		92	52	76					166		76	75	88			95
			89			140						73	133			
	61		71	51									84	91		74
														82		
									156							
				51							95	114				

Highest peak height of "false homozygote" = 274 RFU

Allelic drop-out is prevalent at 30 – 50 pg DNA

Slide courtesy of Becky Hill (NIST)



Setting Stochastic Thresholds

- **Stochastic threshold – point at which data is considered reliable**
- **"Level of risk": the higher you go, the less risk you have of calling a false homozygote - but you start to lose more data for statistics**

Drop-out

*False homozygote because it is above the 200 RFU stochastic threshold

Slide courtesy of Becky Hill (NIST)

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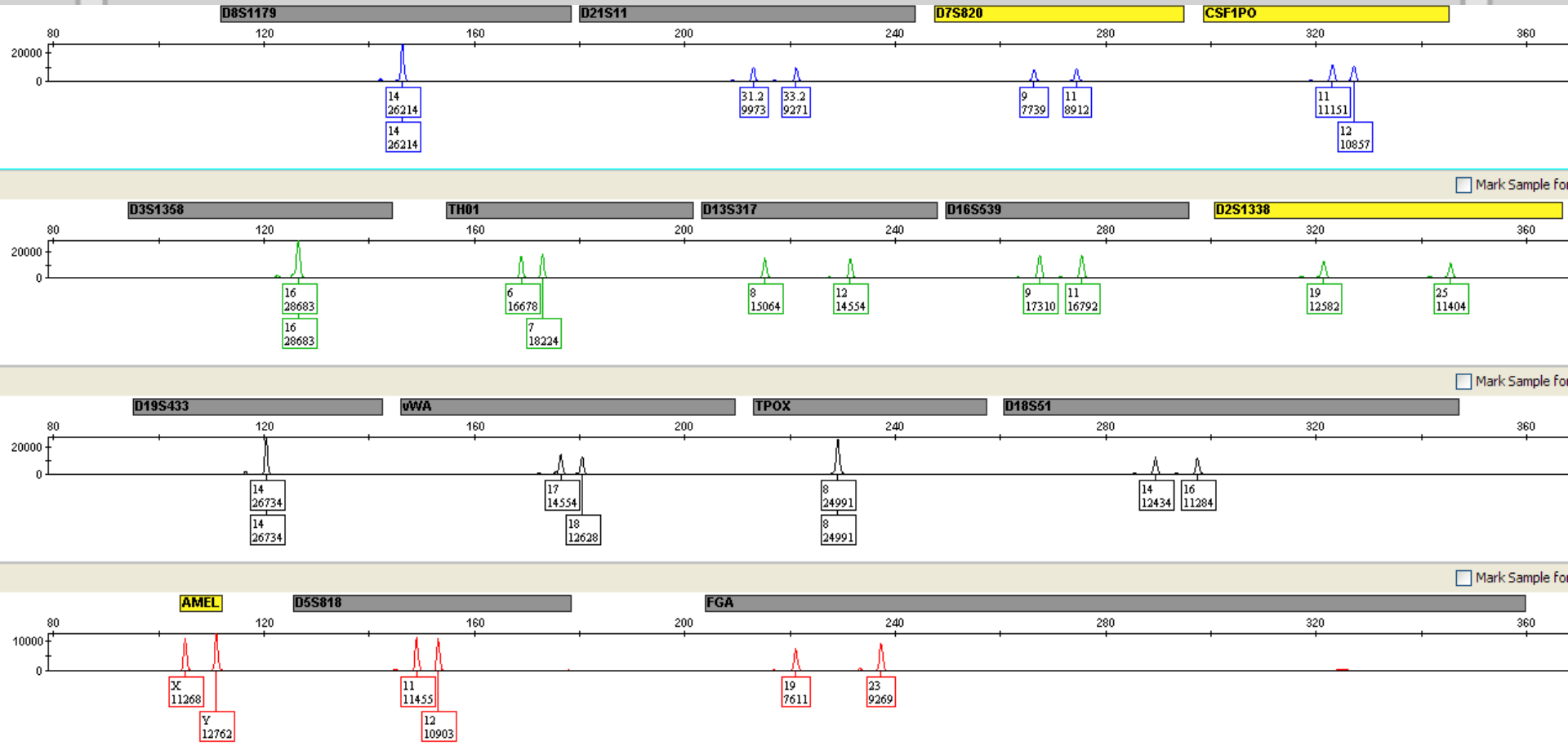
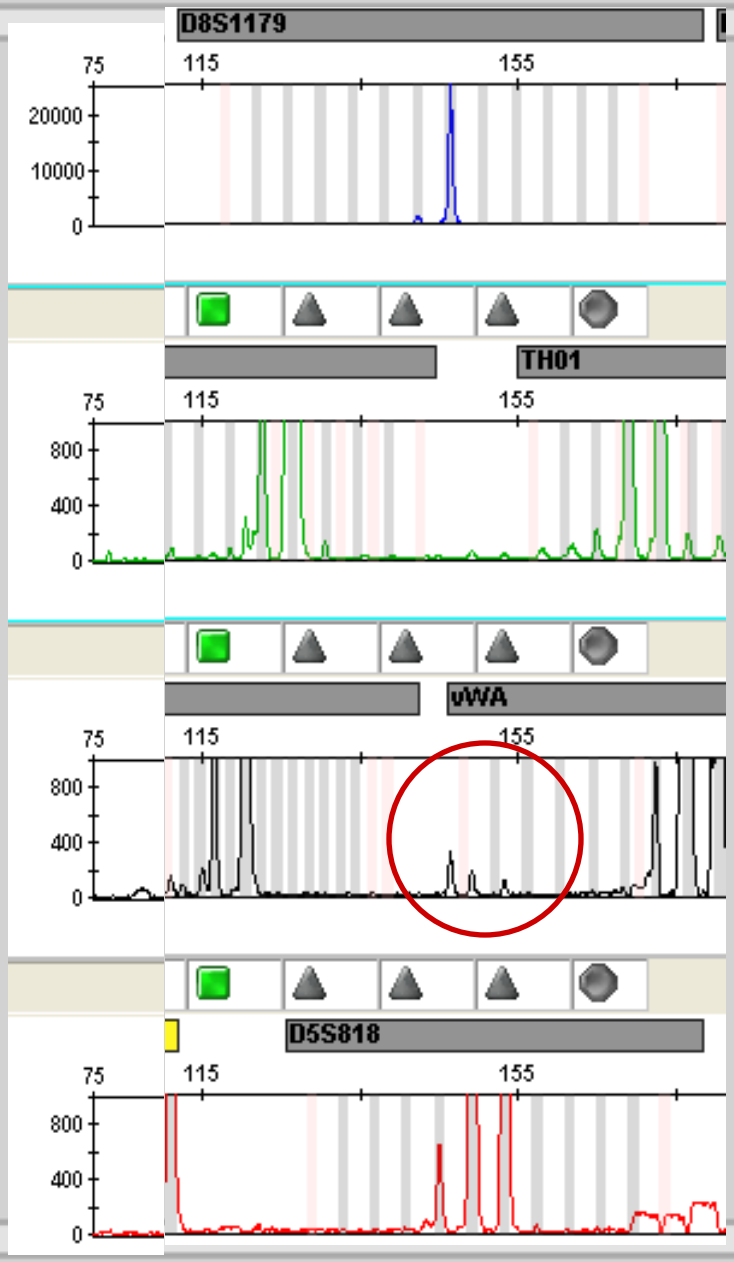
New Instruments, New Thresholds…



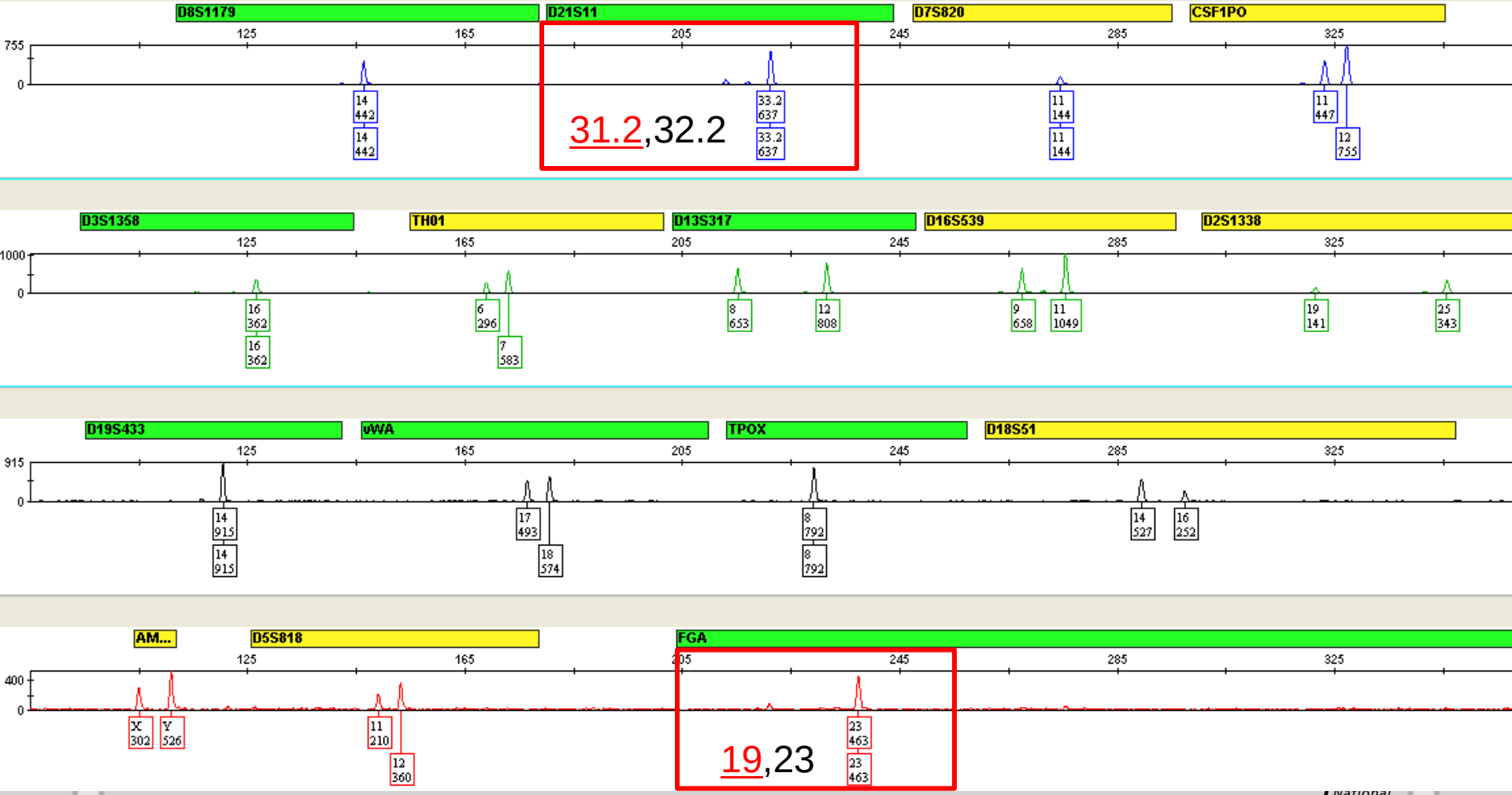
Identifiler Plus 1.0ng input DNA



26214 RFUs

1000 RFU scale

New Instruments, New Thresholds…



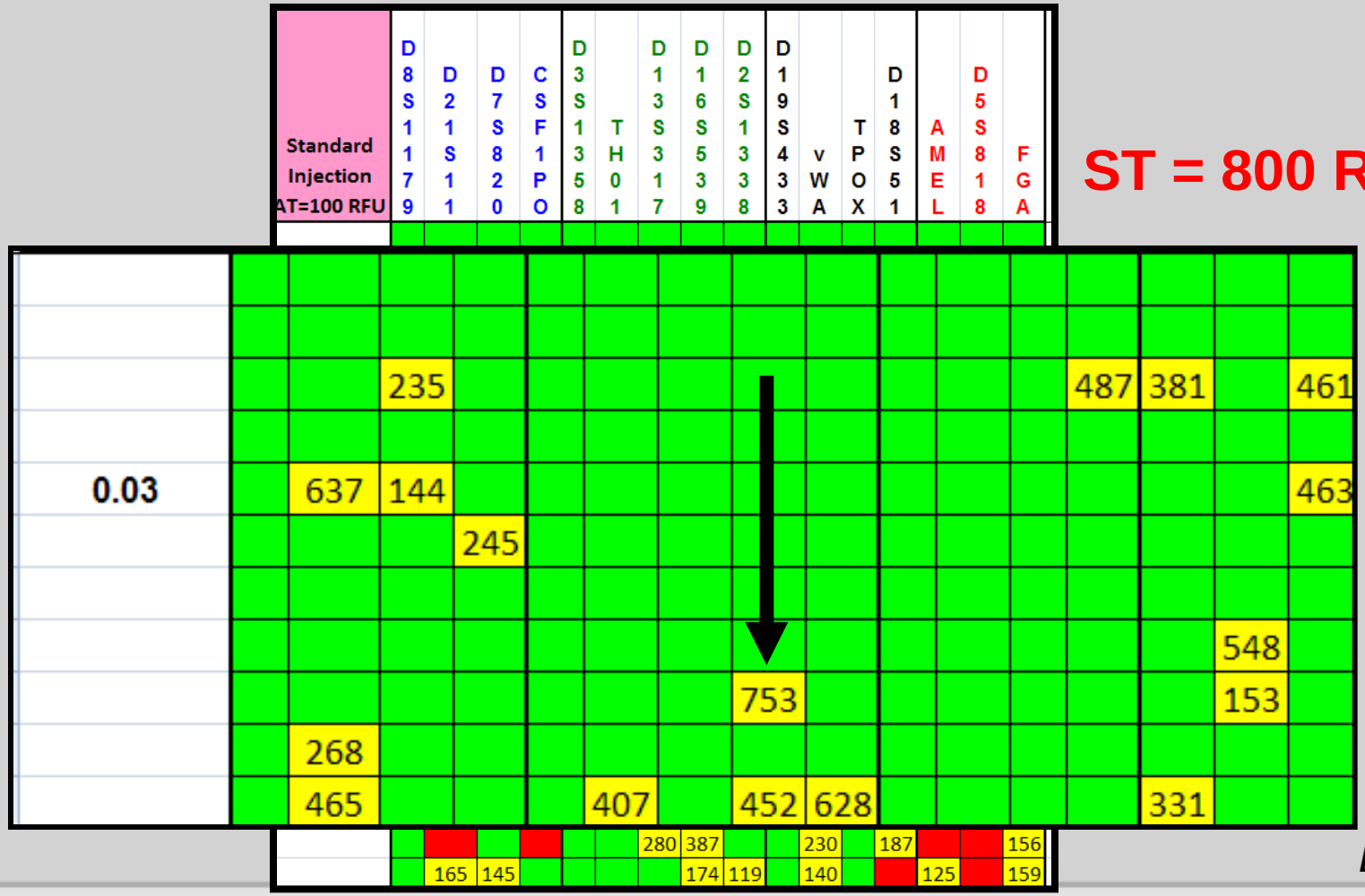
Identifiler Plus 0.03ng input DNA

Identifiler Plus, 28 cycles
3500, 15 sec @ 1.2 kV inj

Standard Injection AT=100 RFU	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	AMEL	D5S818	FGA
			235										487	381		461
0.03		637	144													463
				245												
															548	
								753							153	
		268														
		465				407		452	628					331		
		401	116	436				261								
				184		353					103					366
		231	131			429			222		162					
				118				201					129		114	165
0.01				183		240			232							110
		176				172		541	173		155					
		180		262										156		172
			252			239		256	181					108		
			167	166				340	105					154		108
							280	387			230		187			156
		165	145					174	119		140			125		159

Identifiler Plus, 28 cycles
3500, 15 sec @ 1.2 kV inj

Highest peak height of "false homozygote" = 753 RFU



Forensic Science International: Genetics 4 (2010) 111–114

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journal homepage: www.elsevier.com/locate/fsig

Examination of the variability in mixed DNA profile parameters for the Identifiler™ multiplex

Jo-Anne Bright, Jnana Turkington, John Buckleton *

ESR, 120 Mt Albert Road, PB 92021, Auckland, New Zealand

"The use of bounds applied to data that show continuous variation is common in forensic science and is often a pragmatic decision. However it should be borne in mind that applying such bounds has arbitrary elements to it and that there will be cases where the data lie outside these bounds."

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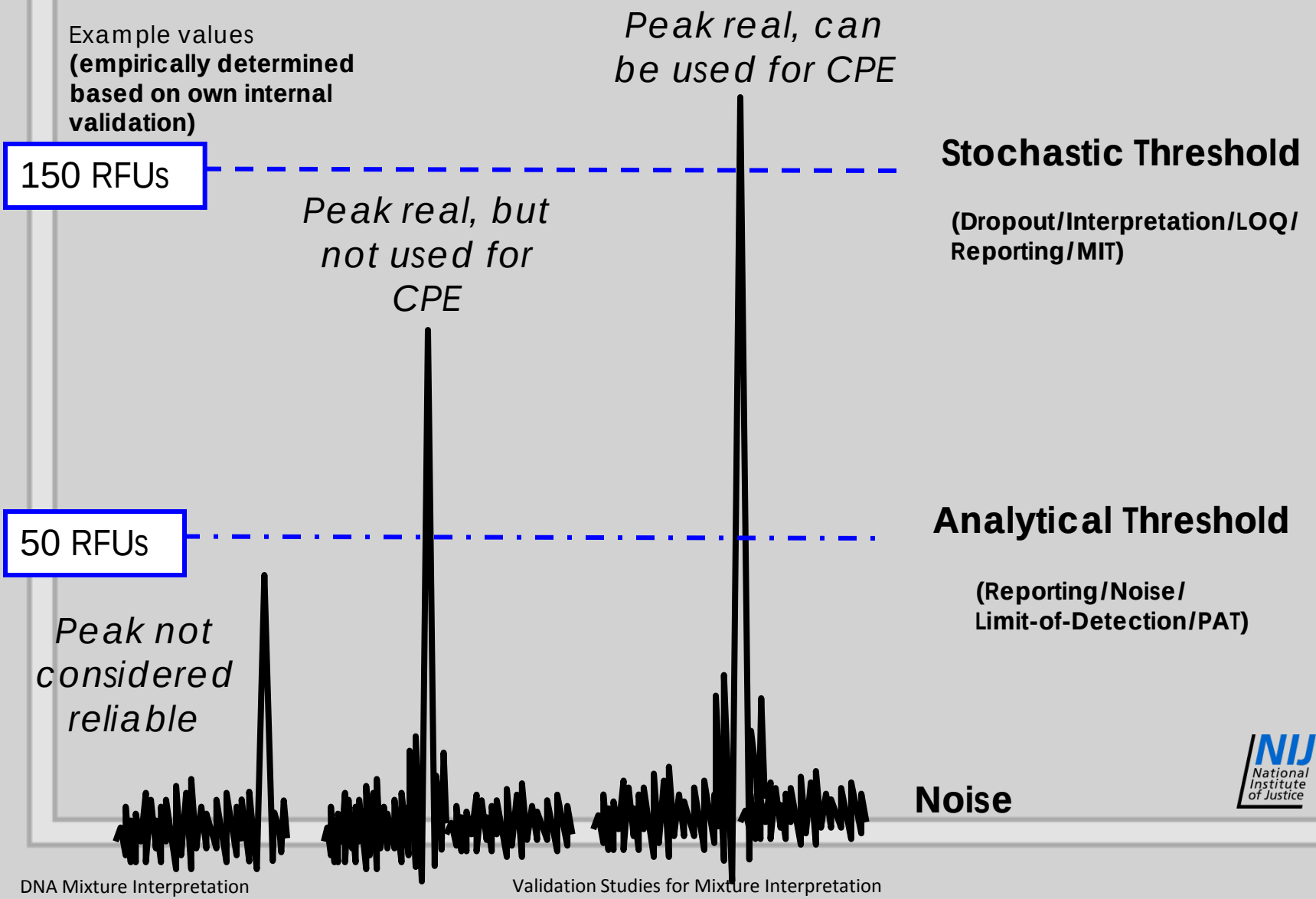
Different Thresholds



Stutter Thresholds

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%

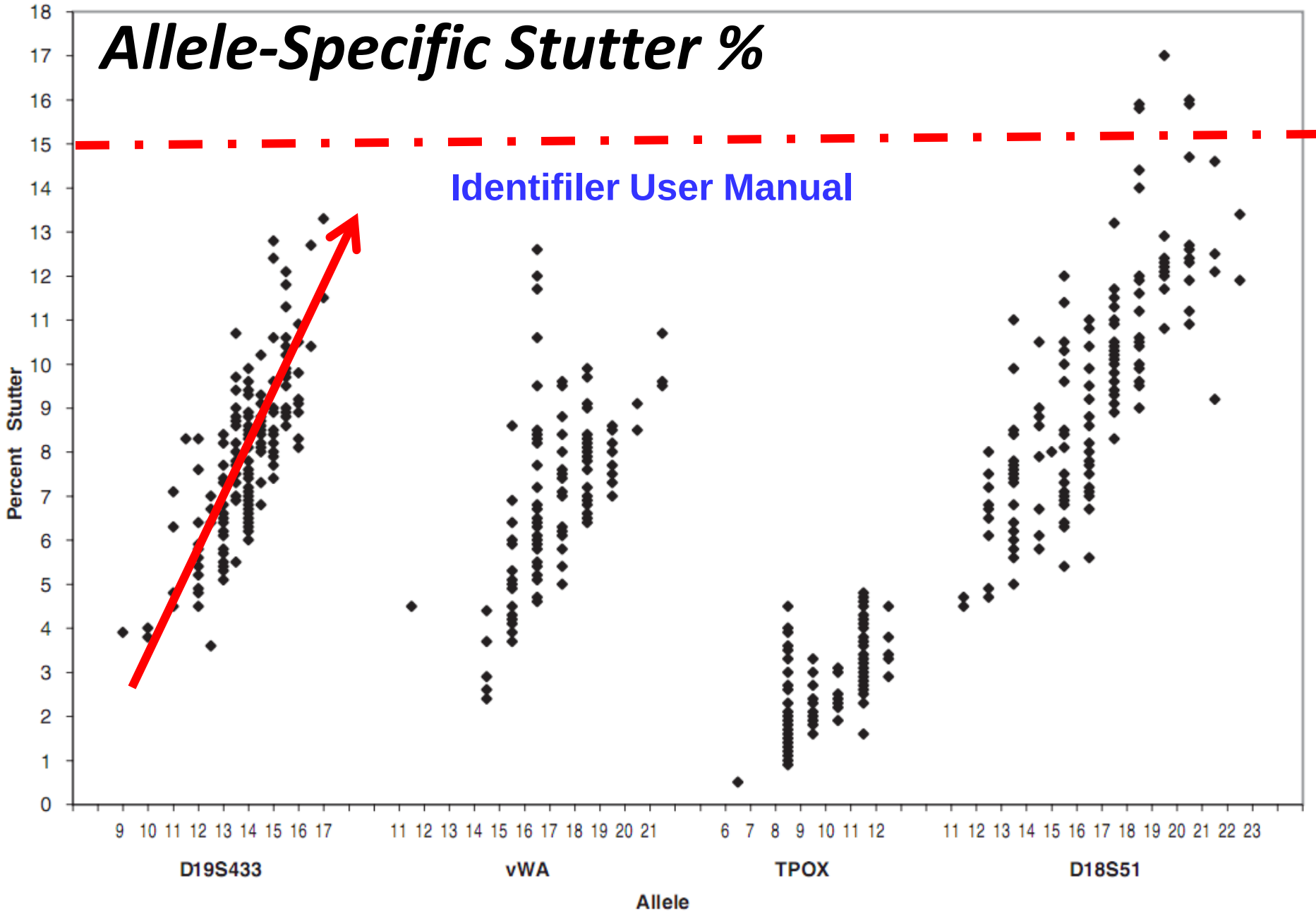
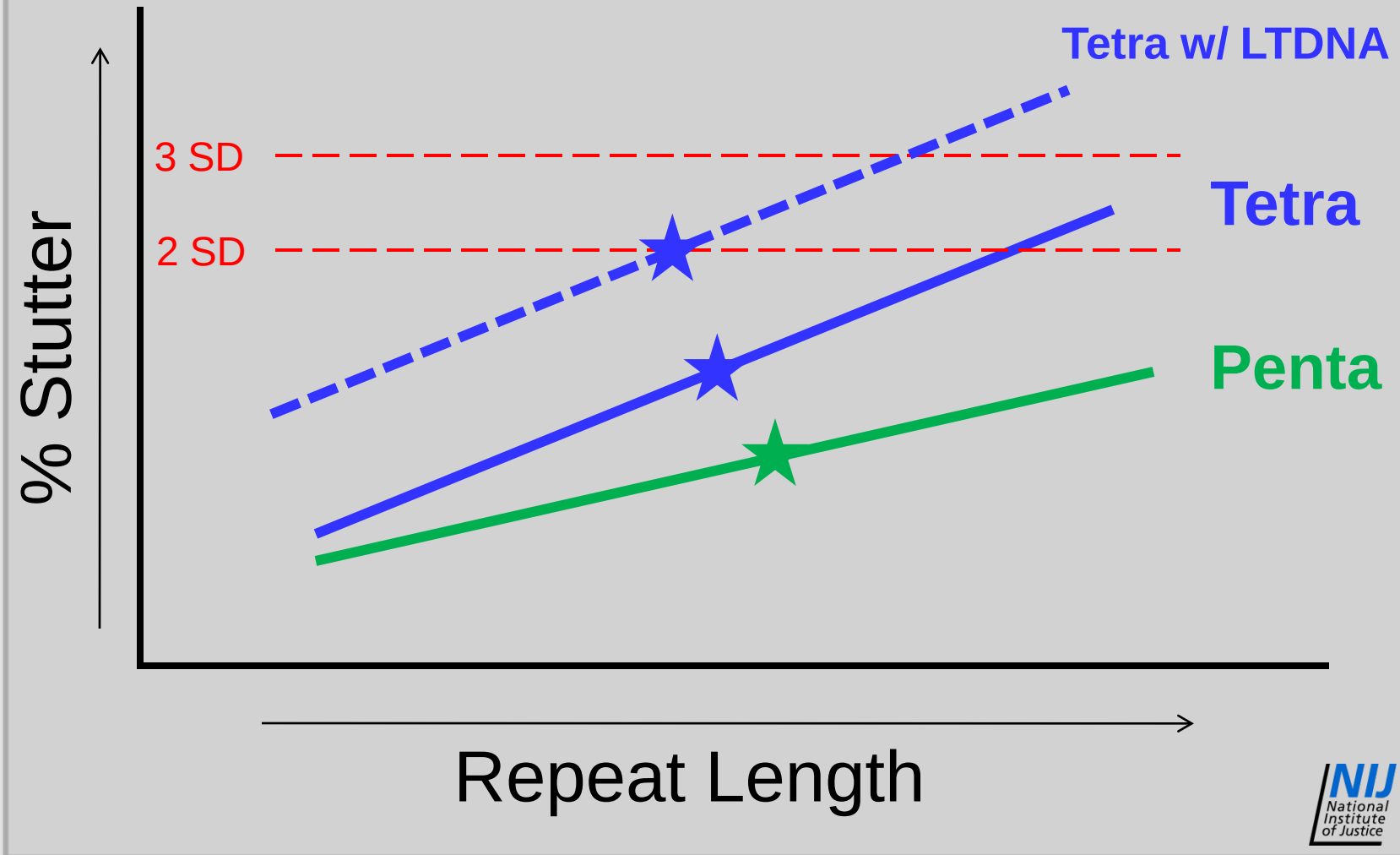


Figure 4-6 Stutter percentages for the D19S433, vWA, TPOX, and D18S51 loci

Developing Stutter Filter Values

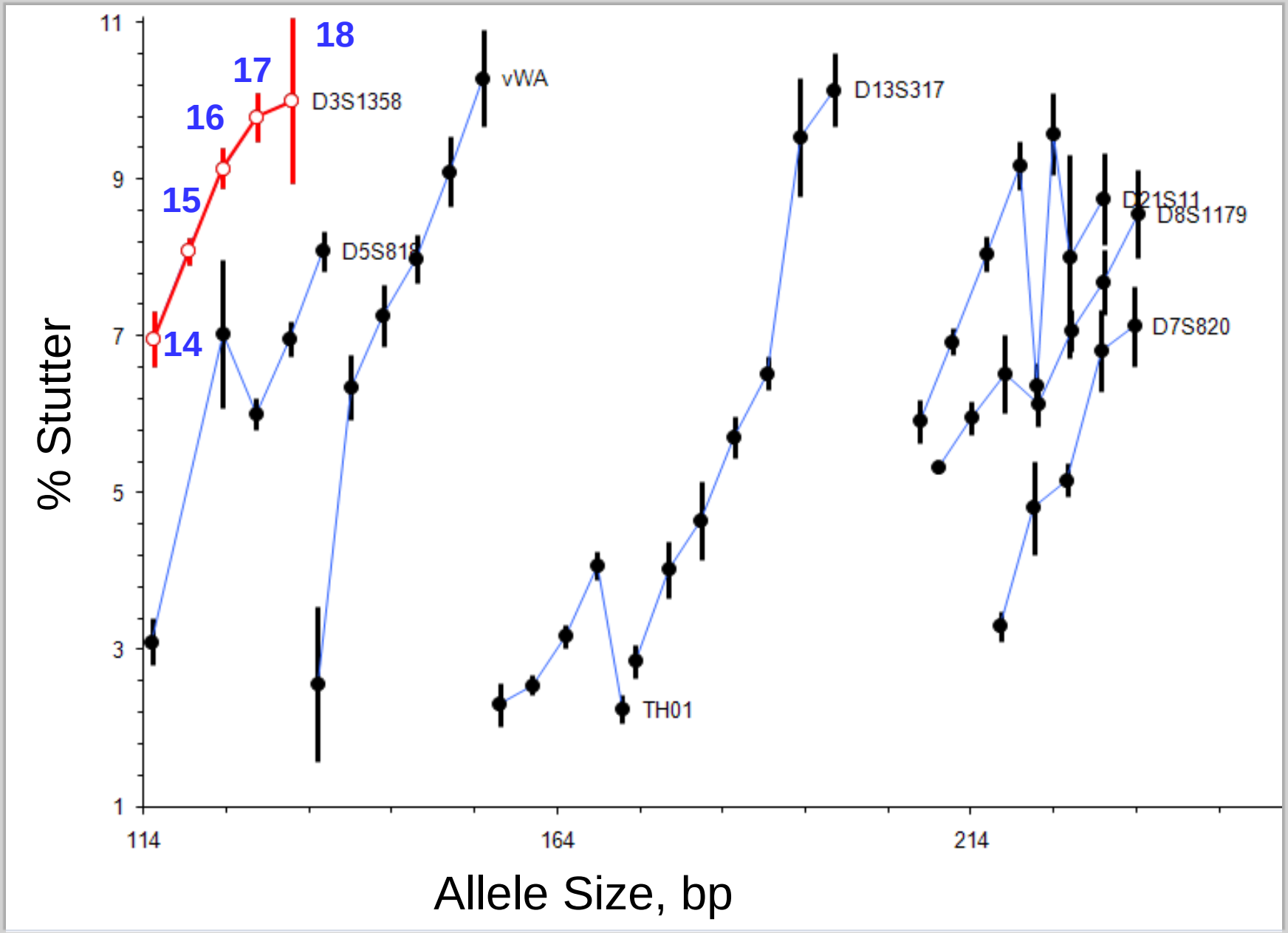
- **Samples – Ideally at least 5 observations of each stutter product per locus from relevant populations (e.g. longer repeats in FGA alleles are observed mostly among African Americans).**
- **Use typical DNA input quantities (0.5 – 2.0ng), but may want to assess stutter at lower levels (e.g. <150pg).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

Stutter Trends



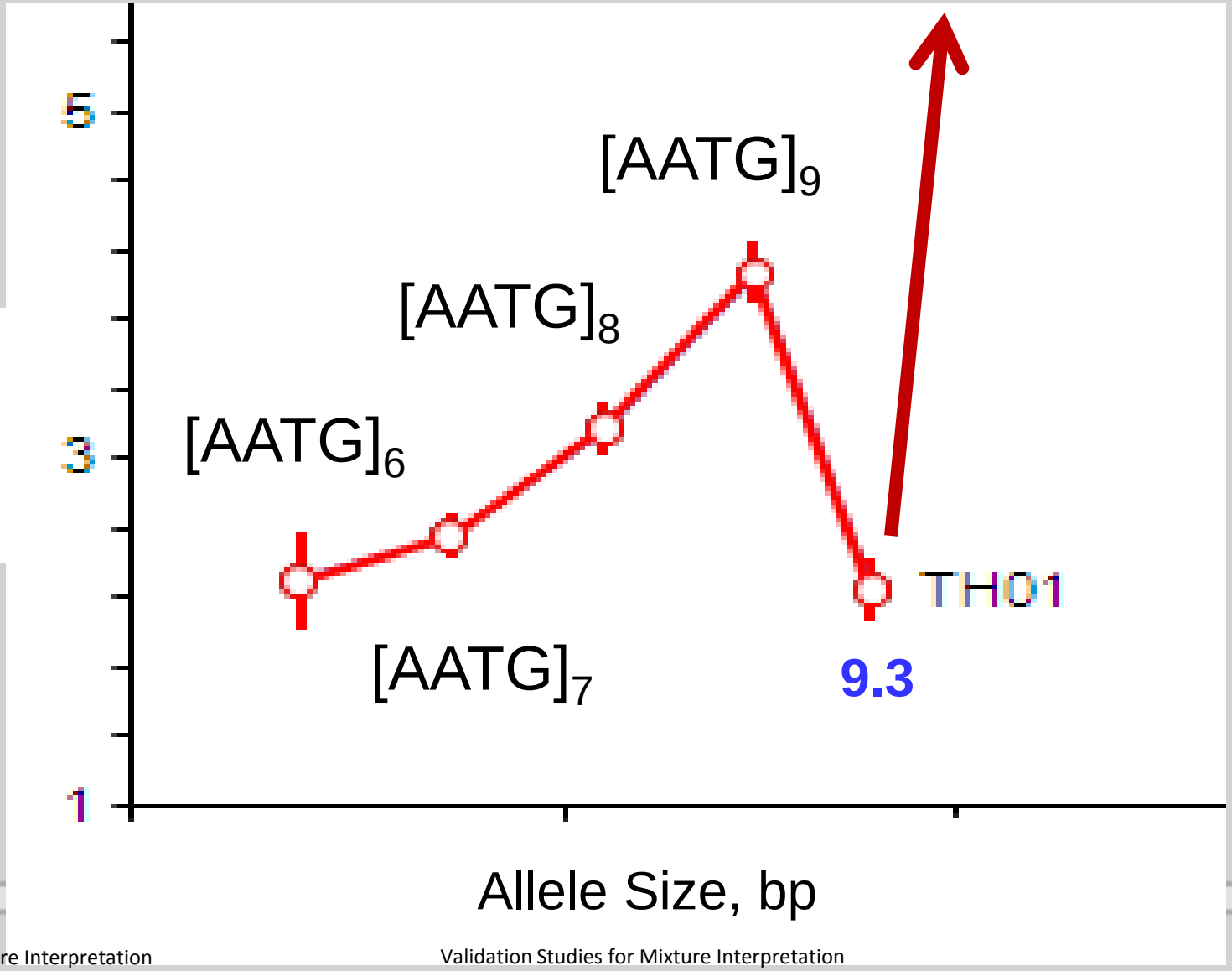
D3S1358 – $TCTA[TCTG]_N[TCTA]_N$

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	


% Stutter
Allele Size, bp
11
9
7
5
3
1
114
164
214
18
17
16
15
14
D3S1358
vWA
D5S818
D13S317
TH01
D21S11
D8S1179
D7S820

TH01 - [AATG]$_N$

$[AATG]_6ATG[AATG]_3$



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.**

ISFG Recommendation #6 Example

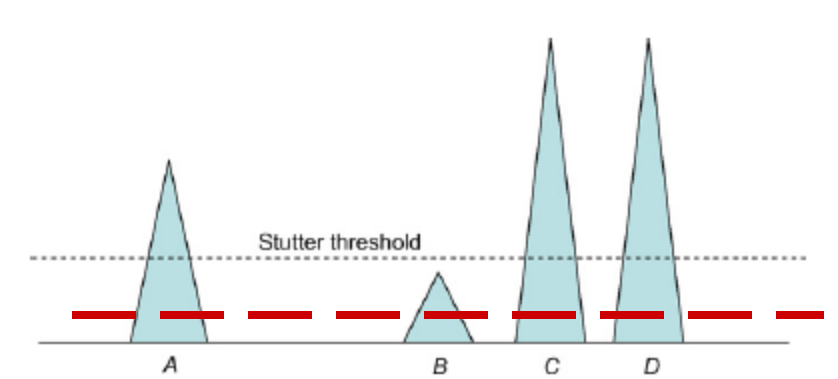


Fig. 2. A two person mixture with major peaks *C*, *D* and minor peaks *A*. There is an additional peak present in a stutter position (*B*).

Likely a AA

(homozygote)

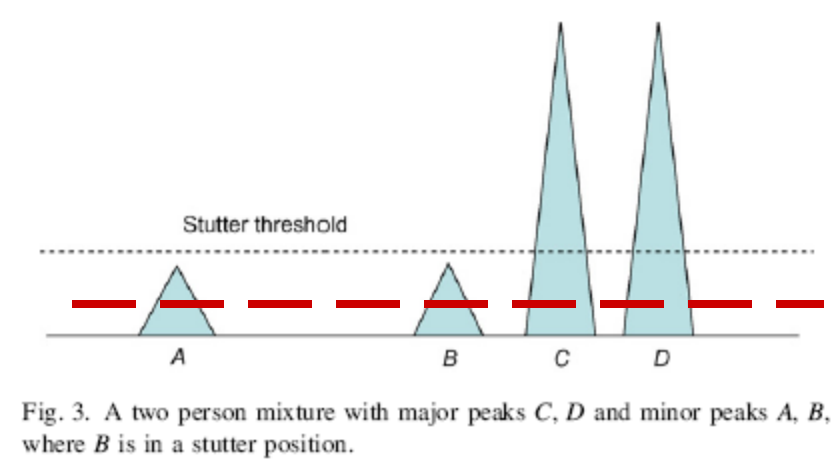


Fig. 3. A two person mixture with major peaks *C*, *D* and minor peaks *A*, *B*, where *B* is in a stutter position.

Possibly AB

(heterozygote)

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

NIJ National Institute of Justice

Stutter effects

- **In case of doubt a suspicious peak *in the position of a stutter band* has to be considered as a true allele and part of the DNA profile, and should be included into the biostatistical interpretation.**

Slide from Peter Schneider
(presented at EDNAP meeting in Krakow in April 2007)

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.**

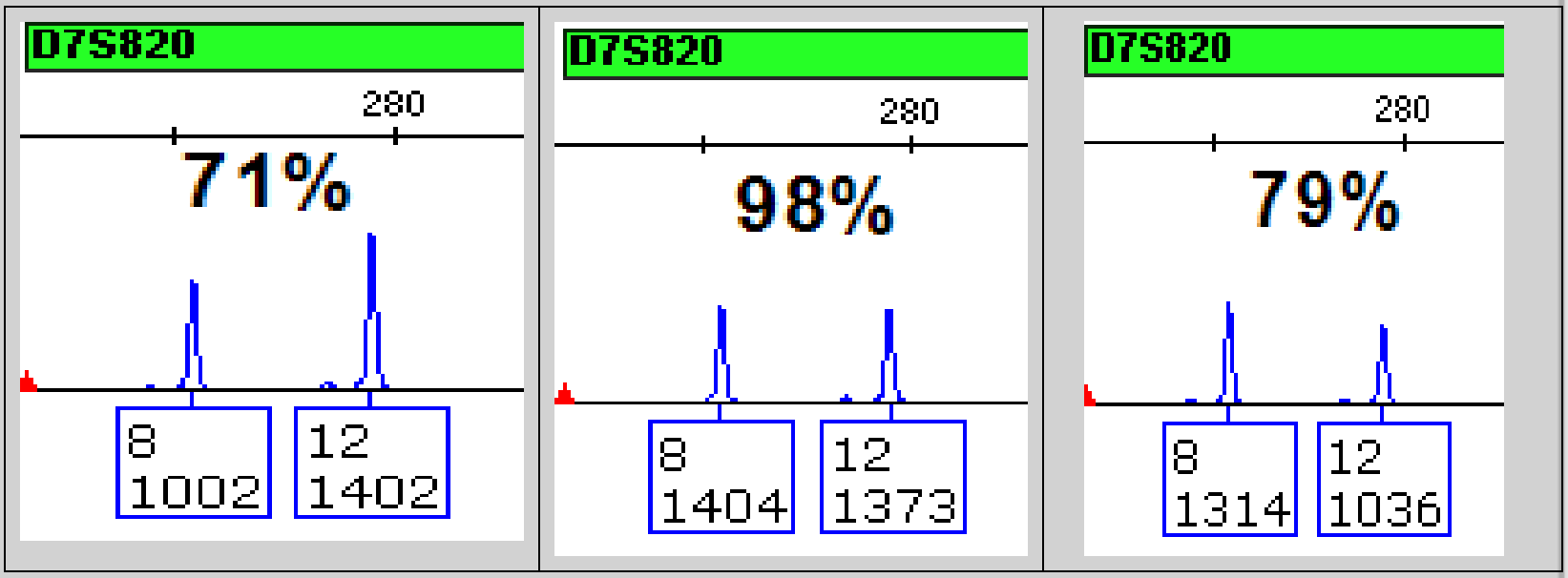
Peak Height Thresholds

3. Interpretation of DNA Typing Results

- **3.2. Application of Peak Height Thresholds to Allelic Peaks**

- **Amplification of low-level DNA samples may be subject to stochastic effects, where two alleles at a heterozygous locus exhibit considerably different peak heights (i.e., peak height ratio generally <60%) or an allele fails to amplify to a detectable level (i.e., allelic dropout).**

Peak Height Ratio Variation

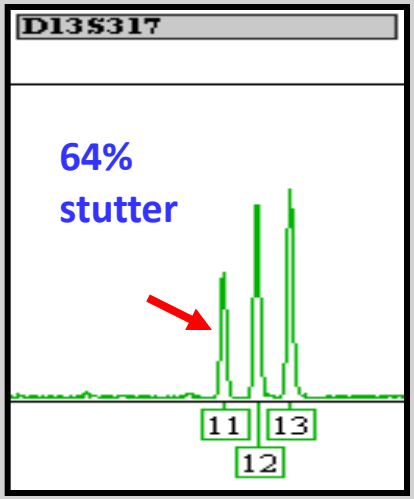


Peak heights will vary from sample-to-sample, even for the same DNA sample amplified in parallel

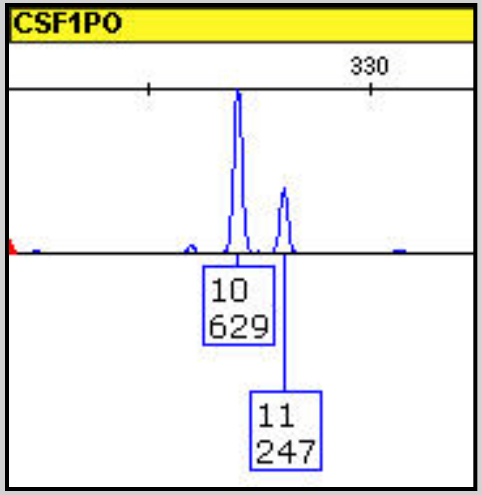
Causes of Peak Height Imbalance

Single Source Samples

- **LT DNA and stochastic effects**
  - **Elevated Stutter – artifact, not true allele**
  - **Unequal sampling of true alleles – the two alleles are not sampled and amplified equally**



12,13



0.25 ng

How to calculate Peak Height Ratios?

From Validation Studies

- **Sensitivity Series at different amounts of DNA**
- **Non-probative single-source samples with good quality profiles amplified with different amounts of DNA (or at least with different peak height ranges)**
- **Perform for each kit validated as PHRs may vary for the same locus amplified with different kits**

Courtesy of Charlotte Word
(http://www.cstl.nist.gov/biotech/strbase/mixture.htm)

How to calculate Peak Height Ratios?

From Casework and Training samples

- **Known standards and single-source samples with good quality profiles amplified with different amounts of DNA (or at least with different peak height ranges)**
- **Database samples (as long as same procedures being used for casework)**

Courtesy of Charlotte Word
(http://www.cstl.nist.gov/biotech/strbase/mixture.htm)

How to calculate Peak Height Ratios?

- **Use a sufficient number and variety of samples to get representative data from each locus, especially for loci with a wide range of alleles and HMW markers (e.g., FGA, D18).**

Courtesy of Charlotte Word
(http://www.cstl.nist.gov/biotech/strbase/mixture.htm)

Peak Height Ratio Data (Dave Duewer)

PowerPlex 16

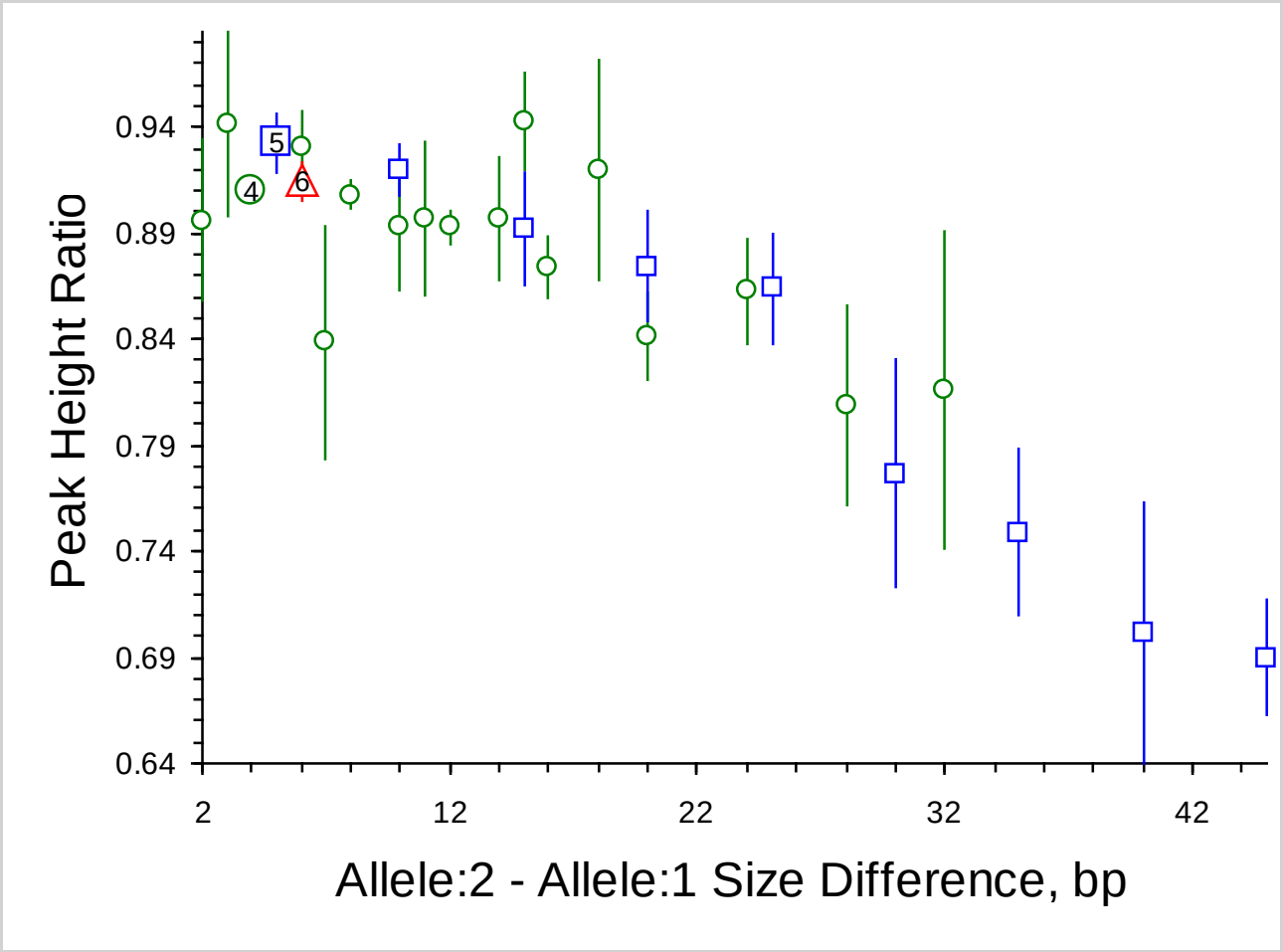
			Mean		Median		Percentiles	
Locus	Δbp	#	X	s(X)	X	s(X)	Min	Max
D13S317	4	103	0.913	0.082	0.930	0.079	0.637	1.000
	8	49	0.879	0.083	0.900	0.091	0.652	0.998
	12	24	0.867	0.079	0.874	0.084	0.639	0.979
	16	20	0.855	0.080	0.847	0.070	0.696	0.997
	20	11	0.828	0.069	0.822	0.067	0.742	0.959
D18S51	4	63	0.878	0.097	0.900	0.100	0.554	0.998
	8	49	0.894	0.100	0.905	0.112	0.704	0.998
	12	44	0.866	0.104	0.876	0.116	0.583	0.997
	16	27	0.872	0.107	0.895	0.119	0.574	0.995
	20	22	0.807	0.100	0.796	0.112	0.644	0.963
	28	10	0.795	0.115	0.785	0.138	0.641	0.936
D8S1179	4	105	0.884	0.082	0.886	0.079	0.683	0.997
	8	61	0.895	0.090	0.908	0.085	0.714	0.990
	12	26	0.857	0.105	0.898	0.099	0.485	1.000
	16	14	0.886	0.088	0.891	0.094	0.620	0.999

Peak Height Ratio
Observations

1. **Range of PHRs is observed within a locus**
   - **Minimum vs. Maximum %**
   - **Mean and Median**
   - **Alleles further apart tend to have lower PHR**

2. **Ranges of PHRs vary across loci**

Peak Height Ratio Data

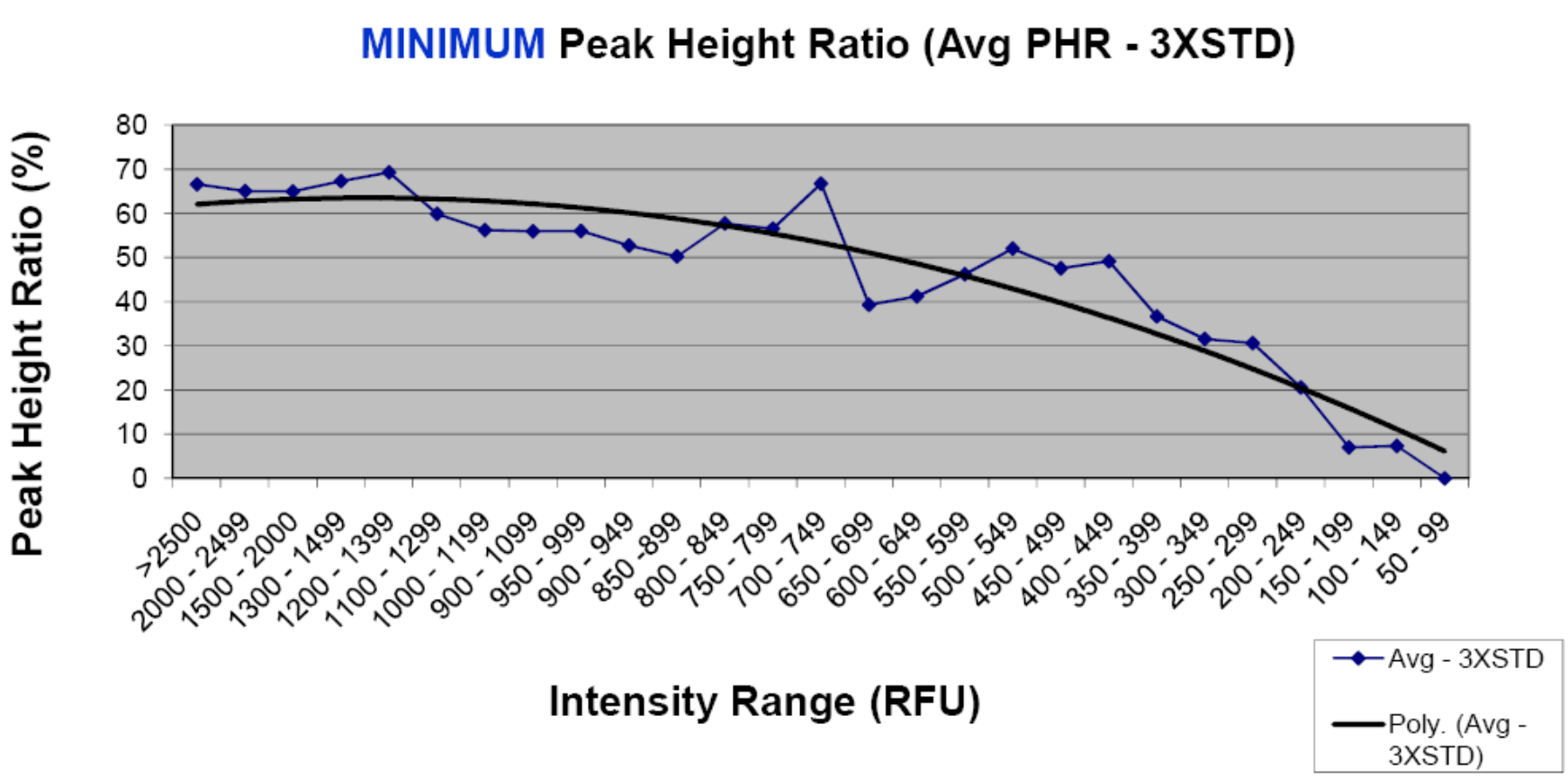


>8000 alleles

PHR Requirements

SWGDAM Interpretation Guideline 3.3.1

The laboratory should establish PHR requirements based on empirical data for interpretation of DNA typing results from single-source samples. Different PHR expectations can be applied to individual loci (e.g., 70% for D3S1358, 65% for vWA, etc.); alternatively, a single PHR expectation can be applied to multiple loci (e.g., 60%).



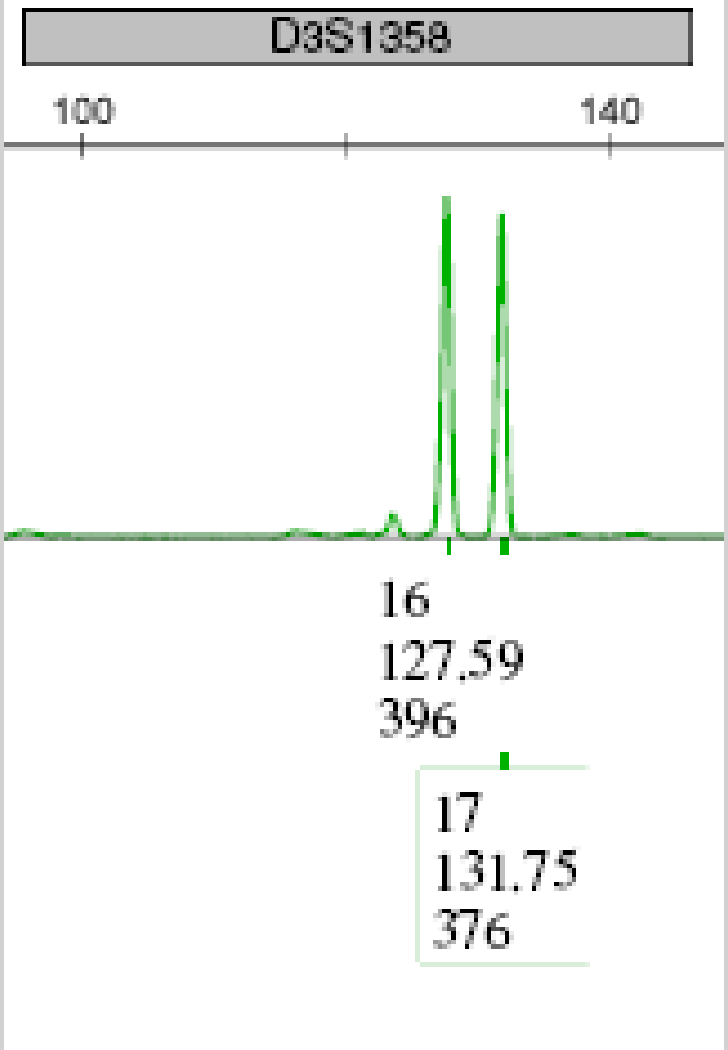
Slide courtesy of Todd Bille (ATF)

An example using the MEPHR

2 person mixture

2:1 ratio

150 ST

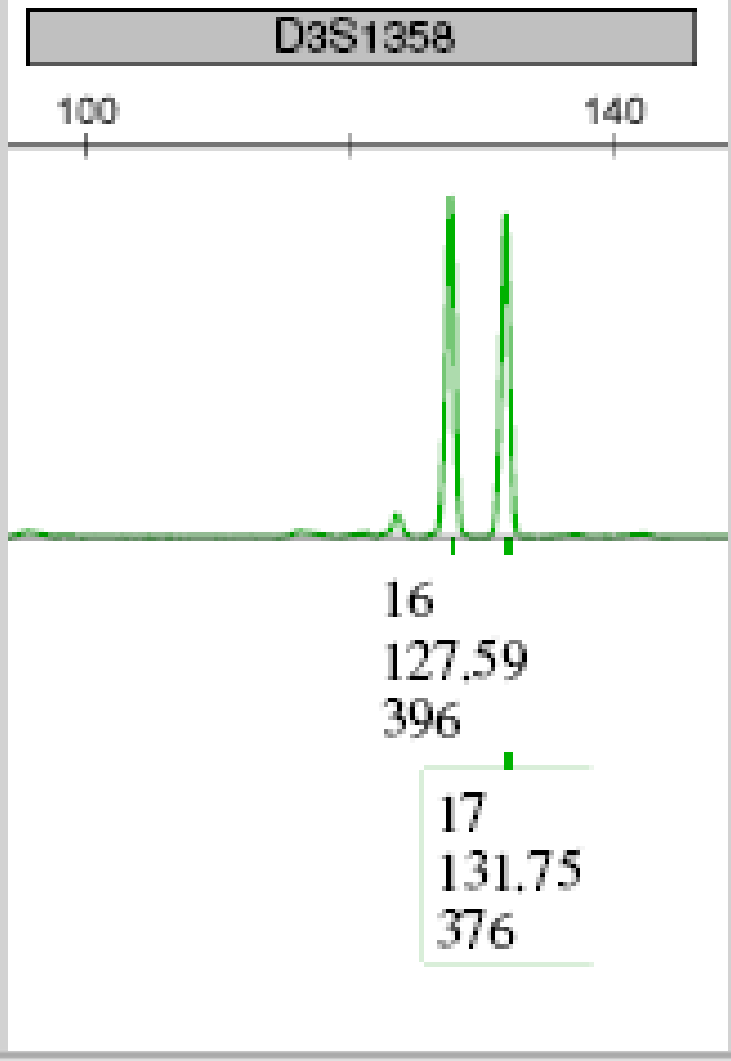


An example using the MEPHR

Is it reasonable to believe all alleles are represented ?

16,17 and 16, --

16,17 and 17, --

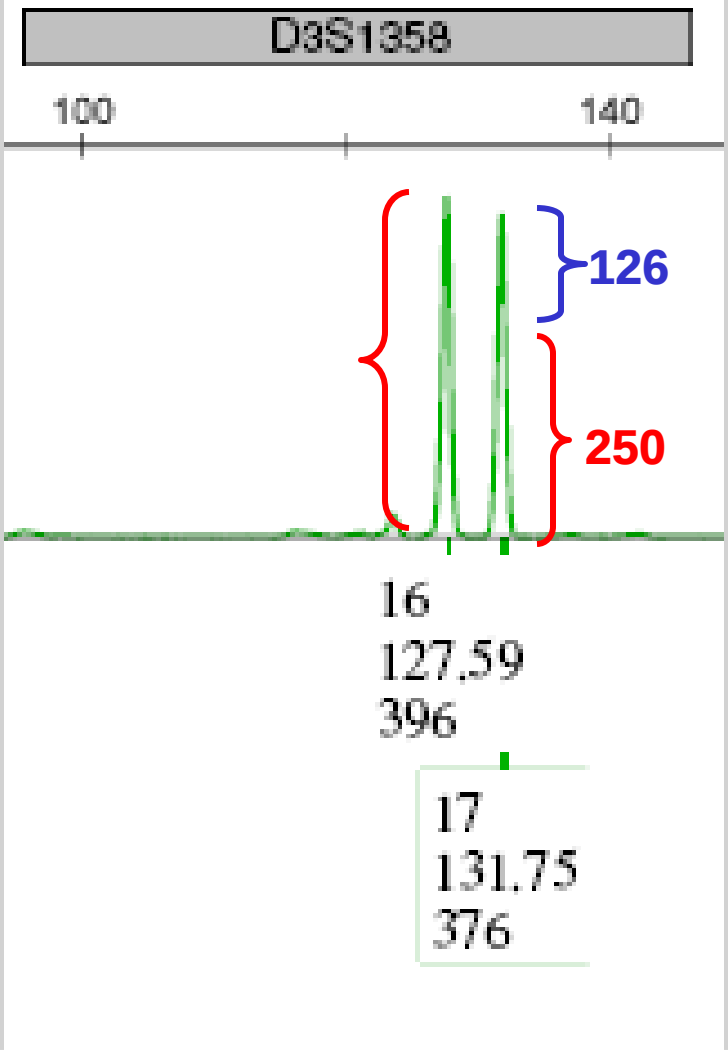


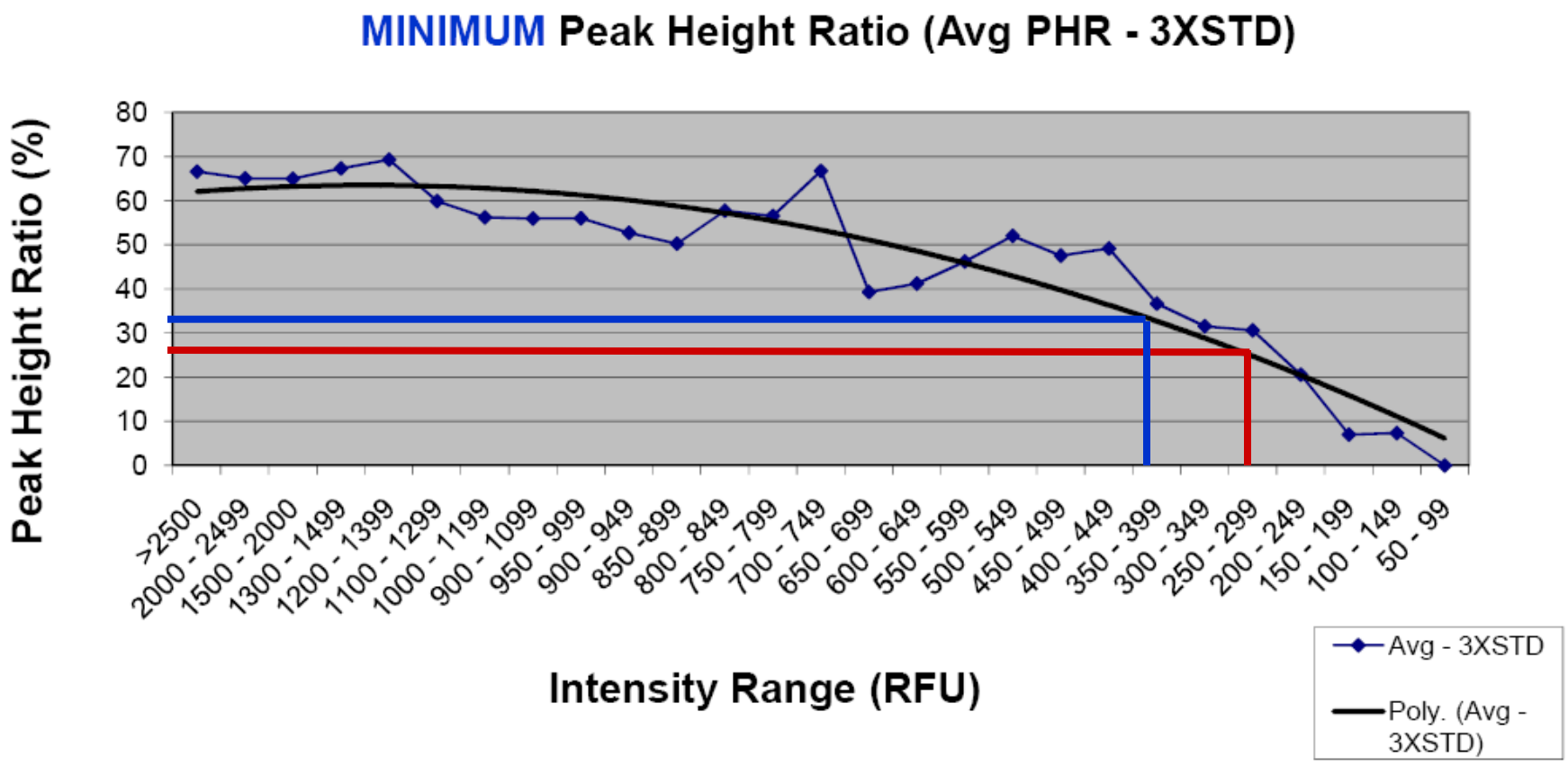
An example using the MEPHR

16,17 and 17, --

~250 RFUs of the 17 allele can be attributed to the 16,17 genotype

(2:1 ratio)
250/396 = 63%





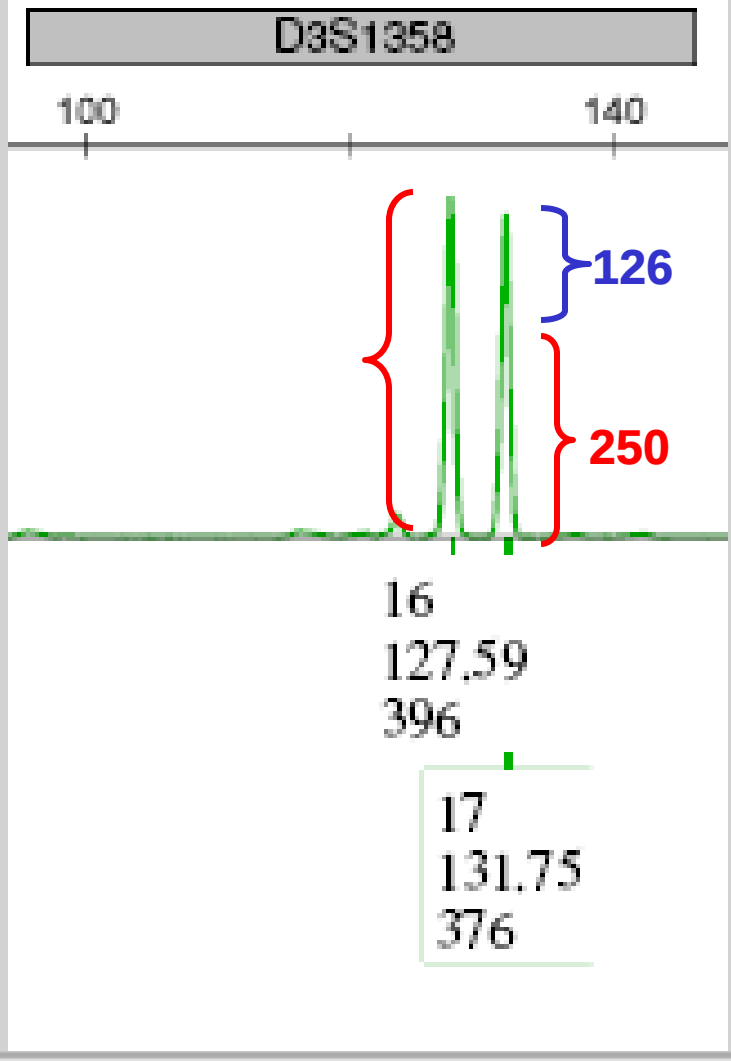
Slide courtesy of Todd Bille (ATF)

An example using the MEPHR

16,17 and 17, --

396 and 250 RFU fall within MEPHR for the16,17.

The remaining 126 RFU for the 17,-- is below the ST.

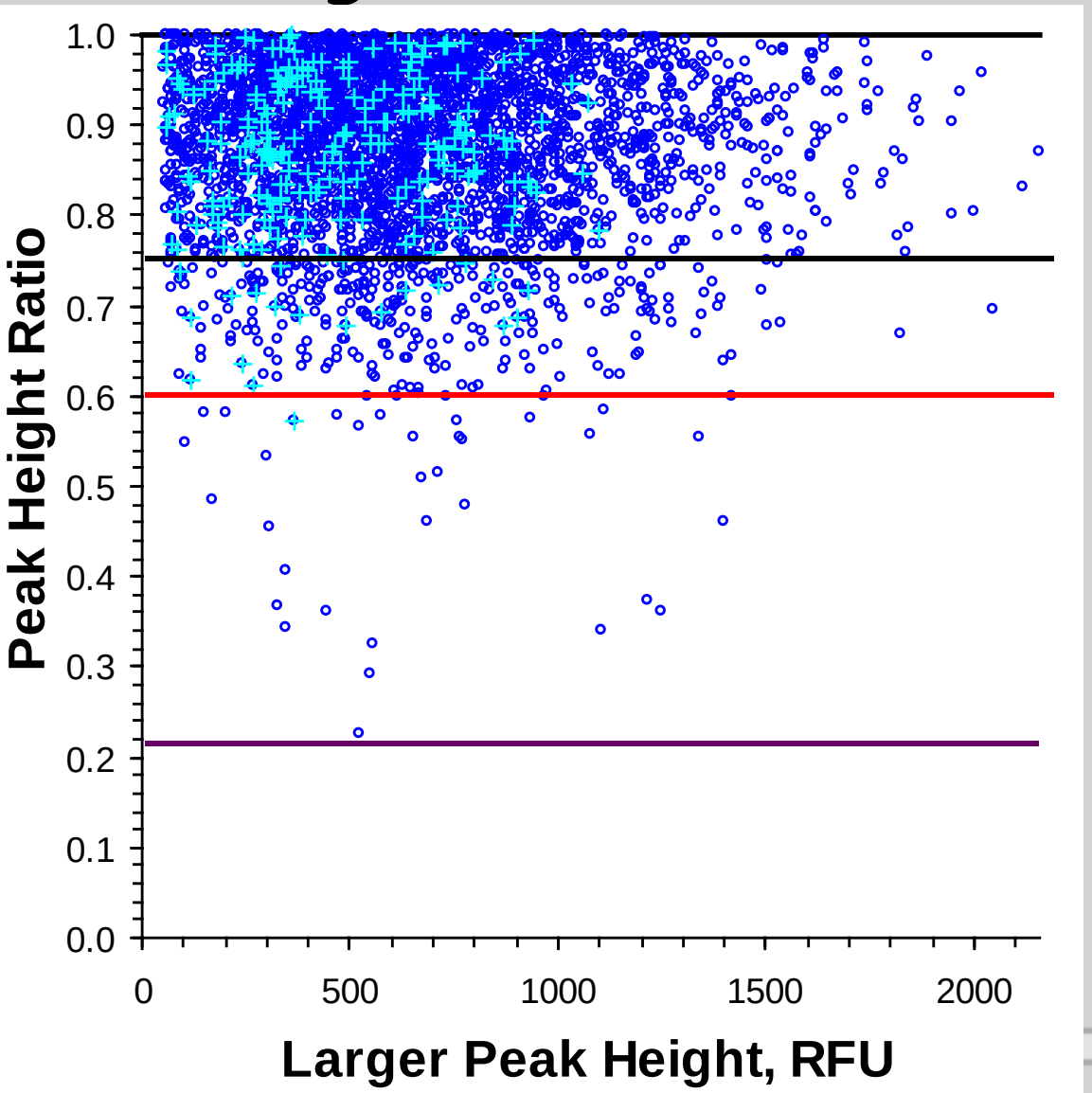


Peak Height Ratio Expectations

SWGDAM Interpretation Guideline 3.3.1.1

It is noted that different PHR expectations at different peak height ranges may be established.

Peak Height Ratio Data



Most peaks >1500 RFU

Most peaks

All peaks

Peak Height Ratio

SWGDAM Interpretation Guideline 3.3.2

PHR requirements are only applicable to allelic peaks that meet or exceed the stochastic threshold.

Summary

- Validation studies are necessary to establish thresholds for mixture interpretation.
- In addition to testing only single source samples, mixtures should also be a part of the validation study.

Questions?

Acknowledgements

- Becky Hill, Erica Butts, Peter Vallone, Dave Duewer and John Butler (NIST)
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NIJ National Institute of Justice