Topics and Techniques for Forensic DNA Analysis

Continuing Education Seminar

Data Interpretation & Statistical Analysis

NYC OCME
Dept of Forensic Biology

New York City, NY

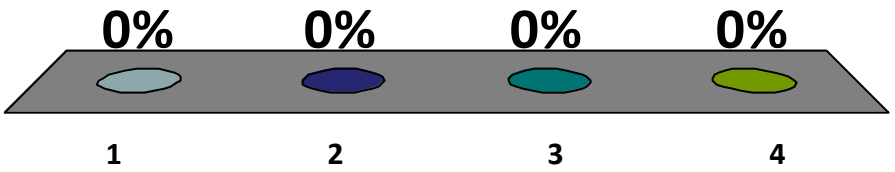
April 18, 2012

Dr. John M. Butler
National Institute of Standards and Technology

john.butler@nist.gov

What topic are you most interested in learning about today? (select only one)

1. SWGDAM Guidelines
2. Problems with CPI statistics & mixtures
3. John's new book on interpretation
4. How to set thresholds



Planned Presentation Outline

- Overview/thoughts on interpretation & statistics
- SWGDAM 2010 interpretation guidelines
- Thoughts on setting thresholds
- Problems with CPI/CPE statistics
- Plan for my new *Interpretation* book

Quality Assurance Standard Requirement for Literature Review

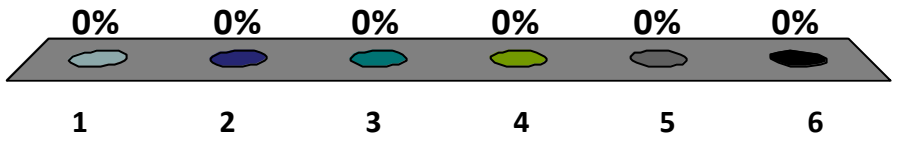
Quality Assurance Standards for Forensic DNA Testing Laboratories
(effective September 1, 2011)

5.1.3.2. The laboratory shall have a program approved by the technical leader for the **annual review of scientific literature** that documents **the analysts' ongoing reading of scientific literature**. **The laboratory shall maintain or have physical or electronic access to a collection of current books, reviewed journals, or other literature applicable to DNA analysis**.

http://www.fbi.gov/about-us/lab/codis/qas-standards-for-forensic-dna-testing-laboratories-effective-9-1-2011

How long has it been since you read a DNA-related journal article?

1. Last week
2. Last month
3. Six months ago
4. Over 12 months
5. None, I only read the abstracts
6. I don’t have time to read!



President John F. Kennedy

Yale University commencement address (June 11, 1962)

"For the greatest enemy of truth is very often not the lie – deliberate, contrived and dishonest – but the myth – persistent, persuasive, and unrealistic. Too often we hold fast to the clichés of our forebears. We subject all facts to a prefabricated set of interpretations. **We enjoy the comfort of opinion without the discomfort of thought**."

http://www.jfklibrary.org/Research/Ready-Reference/Kennedy-Library-Miscellaneous-Information/Yale-University-Commencement-Address.aspx

Written summary of a recent interview…

The CAC News • 1st Quarter 2012 pp. 8-11

norah rudin & keith inman • the proceedings of lunch

www.forensicdna.com • norah@forensicdna.com • kinman@ix.netcom.com

The Discomfort of Thought
—a discussion with John Butler

Several years ago, we began to keep a list of topics that we thought would either be worthwhile topics for a Proceedings (POL in our vernacular, for Proceedings of Lunch, capitalization optional), or just fun to talk or write about. Recently we added *discussion with John Butler* to the list. Although one of us (NR) has had sporadic conversations with John over the years, we've never actually had the opportunity to share a meal. Fortuitously, all three of us attended the recent CAC meeting in Sacramento (We don't think we provided Mr. Houde with any photo ops, but there were reliable witnesses), and were able to huddle around the salad and other lunch offerings to at least begin this session. John has indicated that he routinely reads the *CACNews*, including this column. And he expressed some fascination with the process of how these Proceedings actually come about. What better way to find out than to participate in one? We agreed to present him with a list of questions to

What, we wonder, was the impetus for the SWGDAM 2010 Autosomal STR Interpretation Guidelines? What was wrong with the previous SWGDAM guidelines? Or what needed updating? John responds by saying that the Quality Assurance Standards (QAS) were, after a decade hiatus, revised in 2009. It was felt that the SWGDAM STR Interpretation Guidelines should also be updated to include more information and specifically to aid with mixture interpretation. The previous SWGDAM STR Interpretation Guidelines were released in 2000 and were very general. The 2010 guidelines expanded the text from 4 pages (1066 words) to 28 pages (9862 words) but followed the same general format. More information was needed on mixture interpretation and statistical approaches as the 2000 guidelines only had a few sentences on these topics without any real detail.

"For the greatest enemy of truth is very often not the lie – deliberate, contrived and dishonest – but the myth – persistent, persuasive, and unrealistic. Too often we hold fast to the clichés of our forebears. We subject all facts to a prefabricated set of interpretations. We enjoy the comfort of opinion without the discomfort of thought."

—John F. Kennedy

Available at **http://www.cacnews.org/news/1stq12.pdf**

"…**we should spend as much time developing our interpretation skills as we do our methodological skills.** Technological progress (more sensitivity in detecting DNA, for example), can be a double–edged sword; without equivalent progress in interpretation skill, we are just as likely to cut ourselves as we are the target."

"Your interpretation and statistical methods should have consistent assumptions and go together for each assumption being made (e.g., you may interpret a mixture under alternative sets of assumptions)…"

Results Depend on Assumptions

- "Although courts expect one simple answer, statisticians know that **the result depends on how questions are framed and on assumptions tucked into the analysis**."
  - Mark Buchanan, Conviction by numbers. *Nature* (18 Jan 2007) 445: 254-255

Uncertainty and Probability

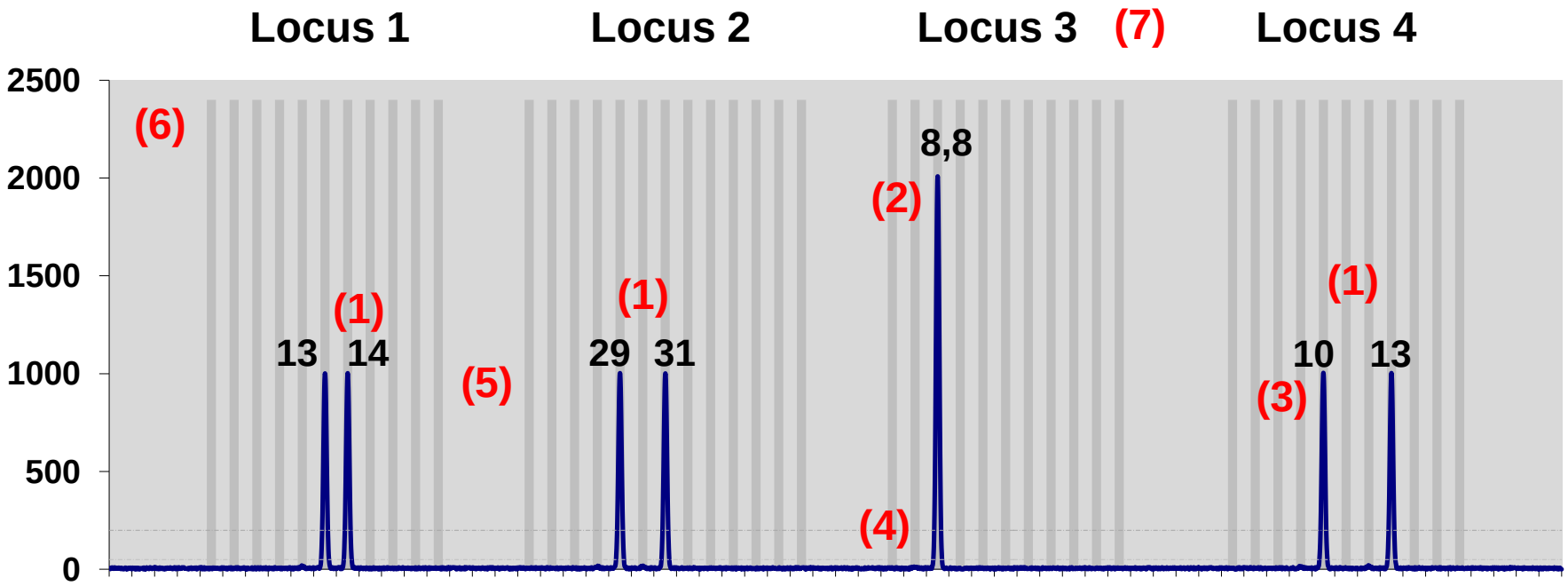
- "Contrary to what many people think, **uncertainty is present throughout any scientific procedure**."
  - Dennis V. Lindley, in his foreword to Aitken & Taroni (2004) *Statistics and the Evaluation of Evidence for Forensic Scientists, Second Edition*

- "It is now recognized that **the only tool for handling uncertainty is probability**."
  - Dennis V. Lindley, in his foreword to Aitken & Taroni (2004) *Statistics and the Evaluation of Evidence for Forensic Scientists, Second Edition*

D.N.A. Approach to Understanding

- **D**octrine or Dogma (why?)
  - A fundamental law of genetics, physics, or chemistry
    - Offspring receive one allele from each parent
    - Stochastic variation leads to uneven selection of alleles during PCR amplification from low amounts of DNA templates
    - Signal from fluorescent dyes is based on …
- **N**otable Principles (what?)
  - The amount of signal from heterozygous alleles should be similar
- **A**pplications (how?)
  - Peak height ratio measurements

Using **Ideal Data** to Discuss Principles

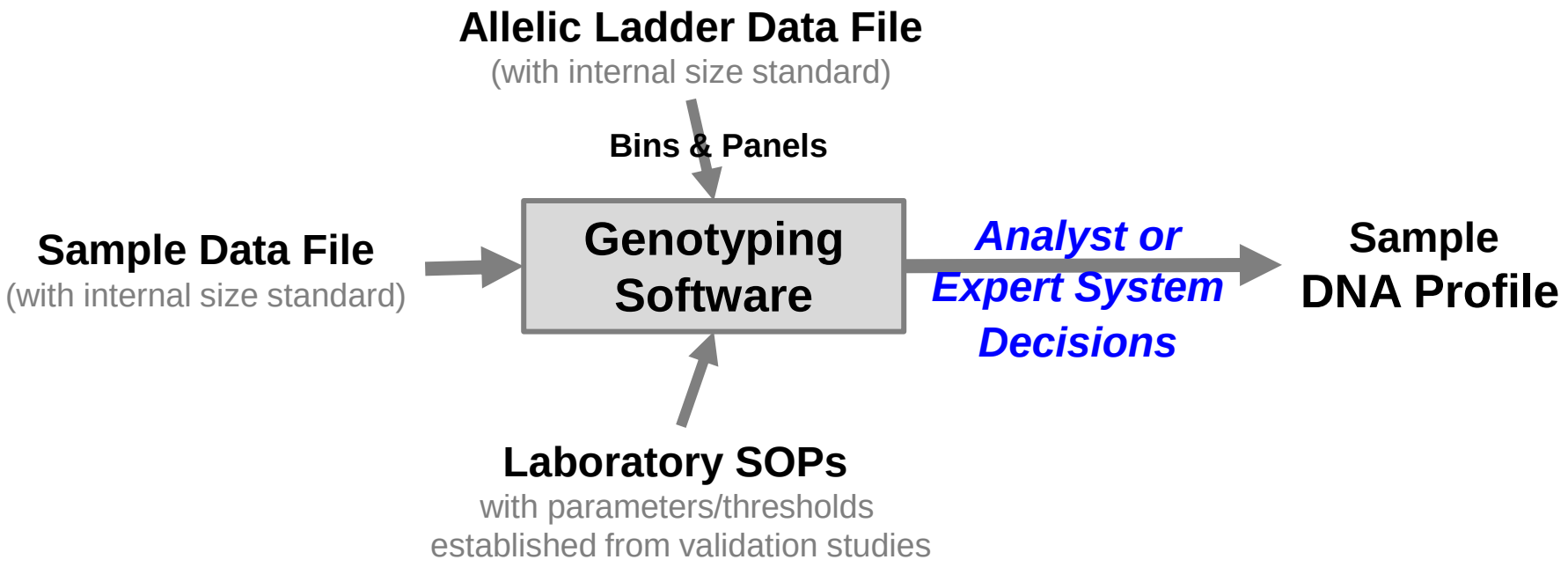


(1) 100% PHR between heterozygous alleles
(2) Homozygotes are exactly twice heterozygotes due to allele sharing
(3) No peak height differences exist due to size spread in alleles (any combination of resolvable alleles produces 100% PHR)
(4) No stutter artifacts enabling mixture detection at low contributor amounts
(5) Perfect inter-locus balance
(6) Completely repeatable peak heights from injection to injection on the same or other CE instruments in the lab or other labs
(7) Genetic markers that are so polymorphic all profiles are fully heterozygous with distinguishable alleles enabling better mixture detection and interpretation

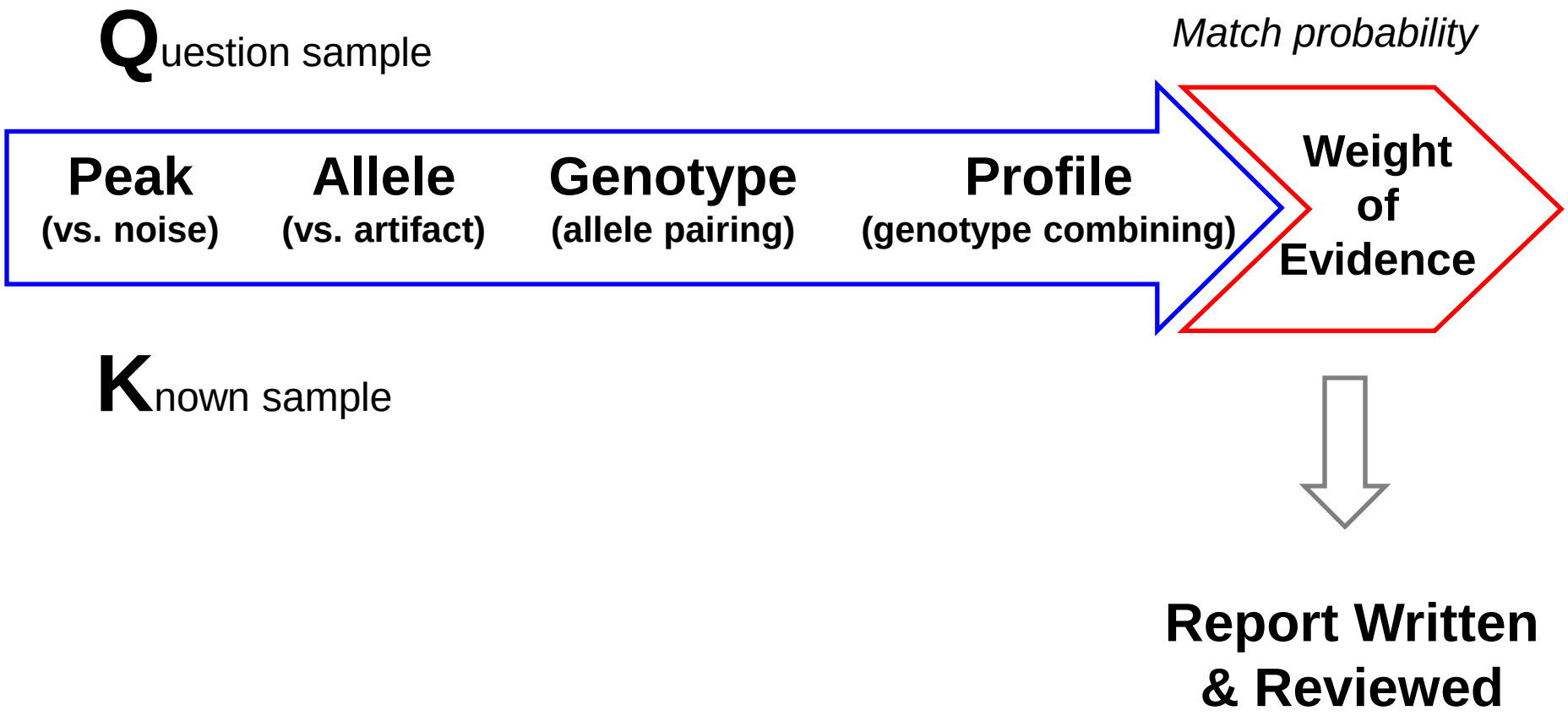
Challenges in real-world data

- **Stochastic (random) variation** in sampling each allele during the PCR amplification process
  - This is highly affected by DNA quantity and quality
  - Imbalance in allele sampling gets worse with low amounts of DNA template and higher numbers of contributors
- **Degraded DNA** template may make some allele targets unavailable
- **PCR inhibitors** present in the sample may reduce PCR amplification efficiency for some alleles and/or loci
- **Overlap of alleles** from contributors **in DNA mixtures**
  - Stutter products can mask true alleles from a minor contributor
  - Allele stacking may not be fully proportional contributor contribution

Overview of Data Interpretation Process



Steps in DNA Interpretation



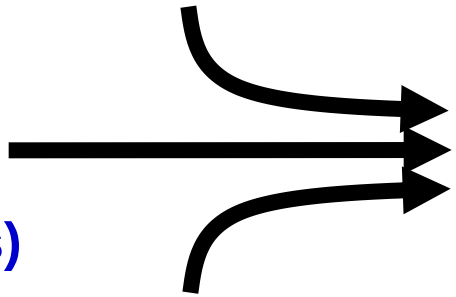
Elements Going into the Calculation of a Rarity Estimate for a DNA Sample

2

Population allele frequencies

1

DNA Profile
(with specific alleles)



Rarity estimate of the specific DNA profile

Appropriate genetic formulas

3

There are different ways to express the profile rarity

Table 11.3 Random match probability for a 13-locus STR profile using the U.S. Caucasian allele frequencies found in Table 11.1.

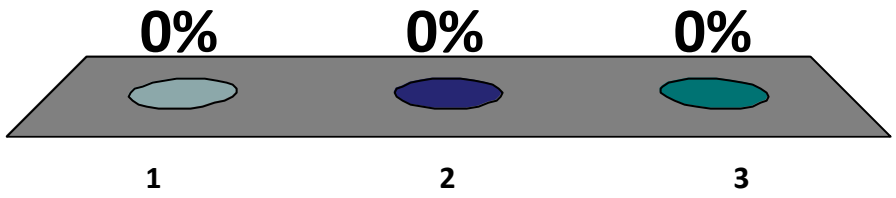
	Allele 1	Allele 2	Allele 1 Frequency (*p*)	Allele 2 Frequency (*q*)	Formula	Expected Genotype Frequency
D13S317	11	14	0.33940	0.04801	2pq	0.0326
TH01	6	6	0.23179		p^2	0.0537
D18S51	14	16	0.13742	0.13907	2pq	0.0382
D21S11	28	30	0.15894	0.27815	2pq	0.0884
D3S1358	16	17	0.25331	0.21523	2pq	0.1090
D5S818	12	13	0.38411	0.14073	2pq	0.1081
D7S820	9	9	0.17715		p^2	0.0314
D8S1179	12	14	0.18543	0.16556	2pq	0.0614
CSF1PO	10	10	0.21689		p^2	0.0470
FGA	21	22	0.18543	0.21854	2pq	0.0810
D16S539	9	11	0.11258	0.32119	2pq	0.0723
TPOX	8	8	0.53477		p^2	0.2860
VWA	17	18	0.28146	0.20033	2pq	0.1128
AMEL	X	Y				
Product rule						1.20×10^{-15}
Combined frequency						1 in 8.37×10^{14}

1 in 837 trillion

John M. Butler (2009) *Fundamentals of Forensic DNA Typing*, Table 11.3

Have you read the 2010 SWGDAM STR Interpretation Guidelines?

1. Yes
2. No
3. Never heard of them before!



Overview of the SWGDAM 2010 Interp Guidelines

1. Preliminary evaluation of data – **is something a peak and is the analysis method working properly?**
2. Allele designation – **calling peaks as alleles**
3. Interpretation of DNA typing results – **using the allele information to make a determination about the sample**
    1. Non-allelic peaks
    2. Application of peak height thresholds to allelic peaks
    3. Peak height ratio
    4. Number of contributors to a DNA profile
    5. Interpretation of DNA typing results for mixed samples
    6. Comparison of DNA typing results
4. Statistical analysis of DNA typing results – **assessing the meaning (rarity) of a match**

Other supportive material: statistical formulae, references, and glossary

DNA Interpretation Process

Sample

Extraction

Quantitation

PCR

Amplification

CE

Separation/ Detection

Locus specific

Stats

Peak (vs. noise)	**Allele** (vs. artifact)	**Any Missing Alleles?**	**Genotype** (allele pairing)	**Profile** (genotype combining)
	2.1, 3.1	**Amp variation** (potential allele dropout?)		
	3.1.1.1	**3.2**	**3.3**	**3.4**
1.1 Analytical threshold	Stutter threshold	Stochastic threshold	Peak height ratio threshold	Number of contributors
3.1.1.2 Sensitivity	Off-scale data threshold	**3.2.1**	Mixture ratio	
	3.1.1.3			

SWGDAM Guidelines (2010)

DNA Interpretation Process (cont.)

Profile
(genotype combining)

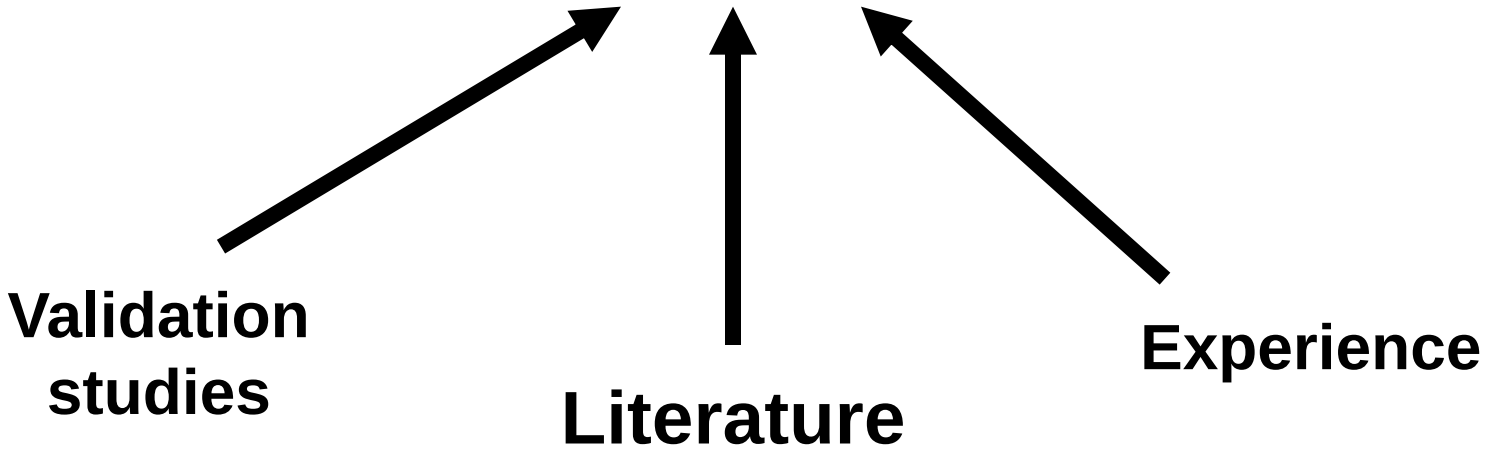
Statistical Rarity

Q → K Comparison

Report Issued with conclusions
(inclusion, exclusion, inconclusive)

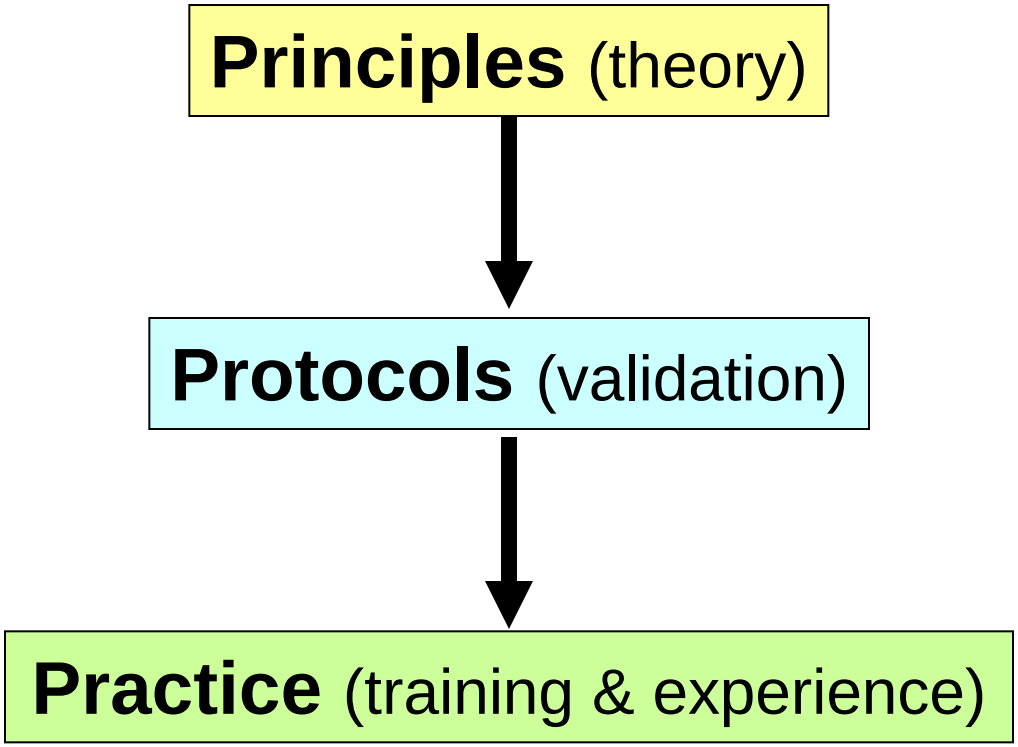
Your Laboratory Interpretation Protocols

Standard Operating Procedures (SOPs)



SWGDAM Guidelines (2010) Introduction: *…the laboratory should utilize written procedures for interpretation of analytical results with the understanding that specificity in the standard operating protocols will enable greater consistency and accuracy among analysts within a laboratory. It is recommended that standard operating procedures for the interpretation of DNA typing results be sufficiently detailed that other forensic DNA analysts can review, understand in full, and assess the laboratory's policies and practices. The laboratory's interpretation guidelines should be based upon validation studies, scientific literature, and experience.*

Elements of DNA Mixture Interpretation



Consistency across analysts

ISFG Recommendations
SWGDAM Guidelines

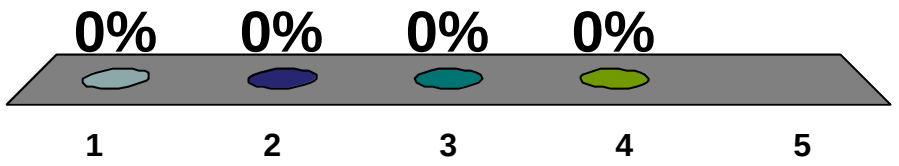
Your Laboratory SOPs

Training within Your Laboratory

Periodic training will aid accuracy and efficiency within your laboratory.

Has your lab implemented changes to your SOPs based on the new guidelines?

1. Yes
2. No
3. Reviewed SOPs but no changes needed
4. Working on it



Interpretation of Evidence Completed before Comparison to Known(s)

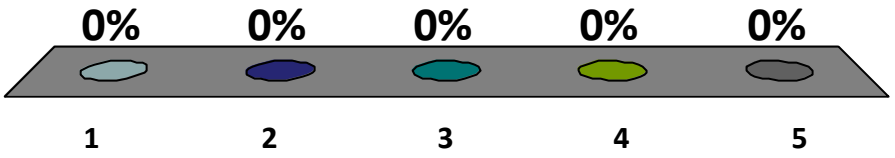
- "3.6.1. The laboratory **must establish guidelines** to ensure that, to the extent possible, **DNA typing results from evidentiary samples are interpreted before comparison with any known samples**, other than those of assumed contributors."

Q (question) before K (known)

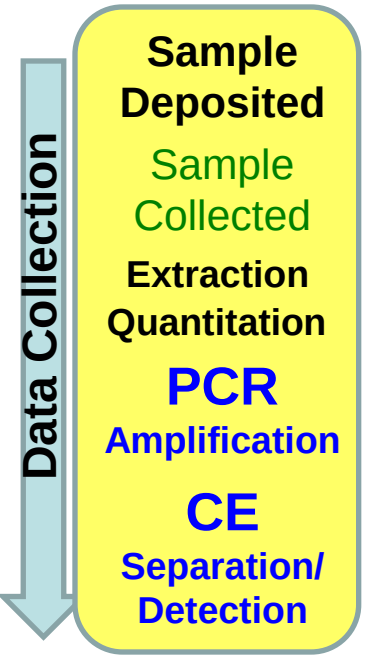
  - While the FBI QAS do not address this issue, this is an example of an issue felt by the committee members to be of such importance that it warranted a "must."

Do you interpret your evidence (lock down your inferred genotypes) independent of your alleged contributor?

1. Always
2. Most of the time
3. Sometimes
4. Rarely
5. Never



Steps in DNA Interpretation



Signal observed

Peak (vs. noise) → **Allele** (vs. artifact) → **Genotype** (allele pairing) → **Profile** (genotype combining)

A threshold is a value used **to reflect reliability of information** (generally you are more confident of data above a threshold than below)

Data Interpretation

Analytical Threshold — Peak

Stutter Threshold — Allele

Stochastic Threshold — ***All Alleles Detected?***

Peak Height Ratio — Genotype(s)

Mixture Ratio — Contributor profile(s)

Comparison to Known(s)
Weight of Evidence (Stats)

Principles Behind Thresholds

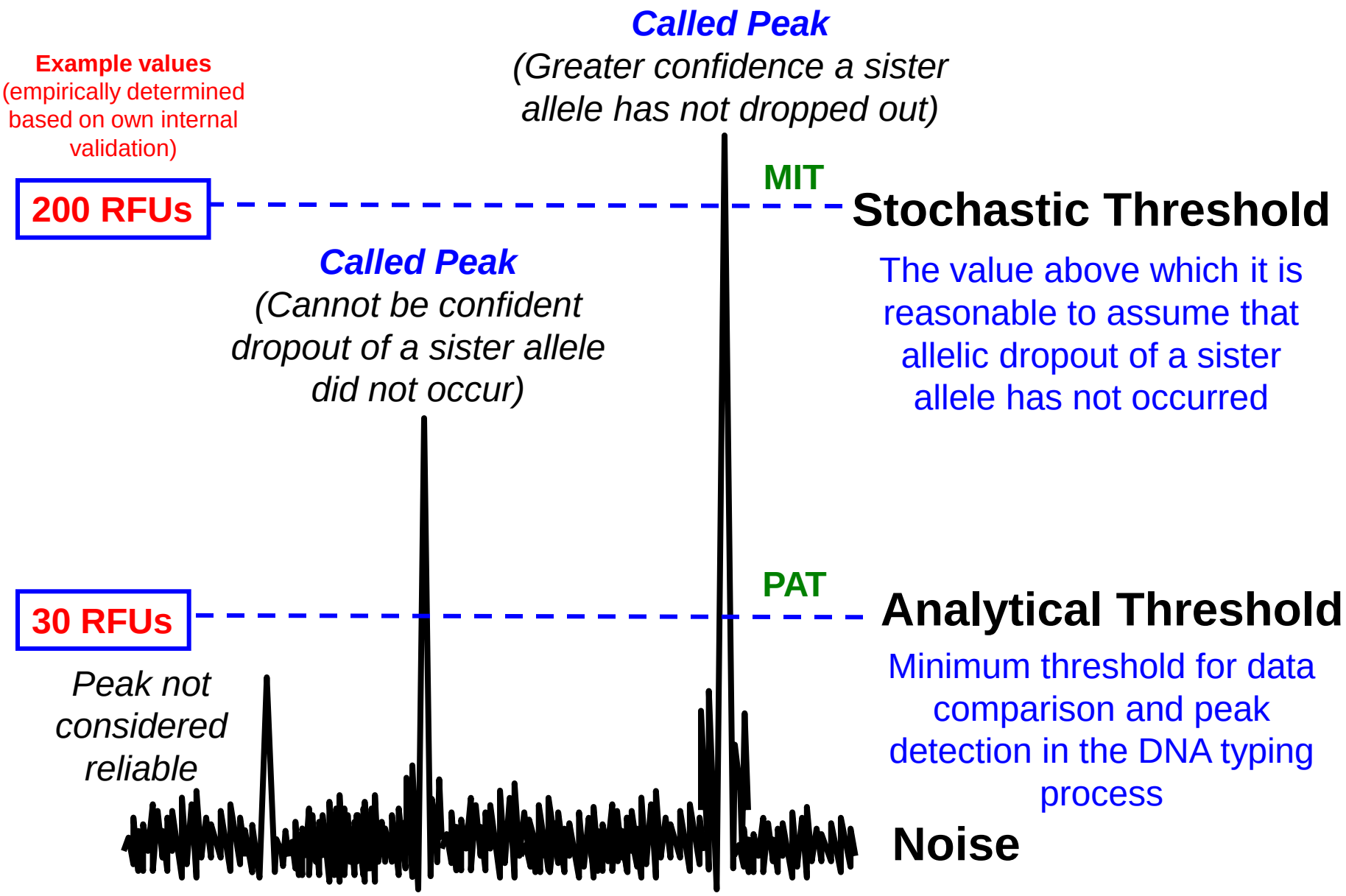
Thresholds (example values)	**Principles Behind** *(if properly set based on lab- & kit-specific empirical data)*
Analytical Threshold (e.g., 50 RFU)	Below this value, observed peaks cannot be reliably distinguished from instrument noise (baseline signal)
Limit of Linearity (e.g., 5000 RFU)	Above this value, the CCD camera can become saturated and peaks may not accurately reflect relative signal quantities (e.g., flat-topped peaks) and lead to pull-up/ bleed-through between dye color channels
Stochastic Threshold (e.g., 250 RFU)	Above this peak height value, it is reasonable to assume that allelic dropout of a sister allele of a heterozygote has not occurred at that locus; single alleles above this value in single-source samples are assumed to be homozygous
Stutter Threshold (e.g., 15%)	Below this value, a peak in the reverse (or forward) stutter position can be designated as a stutter artifact with single-source samples or some mixtures (often higher with lower DNA amounts)
Peak Height Ratio (e.g., 60%)	Above this value, two heterozygous alleles can be grouped as a possible genotype (often lower with lower DNA amounts)
Major/Minor Ratio (e.g., 4:1)	When the ratio of contributors is closer than this value in a two-person mixture, it becomes challenging and often impossible to correctly associate genotype combinations to either the major or minor contributor

Threshold Decisions

Thresholds to Determine	**Decisions to Make (lab & kit specific)**	**Useful Validation Data**
Analytical = ____ RFU	Single overall value or color specific	Noise levels in negative controls or non-peak areas of positive controls
Stochastic = ____ RFU	Minimum peak height RFU value or alternative criteria such as quantitation values or use of a probabilitistic genotype approach	Level where dropout occurs in low level single-source heterozygous samples under conditions used (e.g., different injection times, post-PCR cleanup)
Stutter filter = ___%	Profile, locus, or allele-specific	Stutter in single-source samples (helpful if examined at multiple DNA quantities)
Peak Height Ratio = ___%	Profile, locus, or signal height (quantity) specific	Heterozygote peak height ratios in single-source samples (helpful if examined at multiple DNA quantities)
Major/Minor Ratio = ____	When will you attempt to separate components of a mixture into major and minor contributors for profile deductions?	Defined mixture ratios (e.g., 1:1, 1:3, 1:9) with known samples to observe consistency across loci and to assess ability to deduce correct contributor profiles

Approaches to Setting a Stochastic Threshold

Overview of Two Thresholds



Butler, J.M. (2010) *Fundamentals of Forensic DNA Typing*. Elsevier Academic Press: San Diego.

General Definition of Stochastic

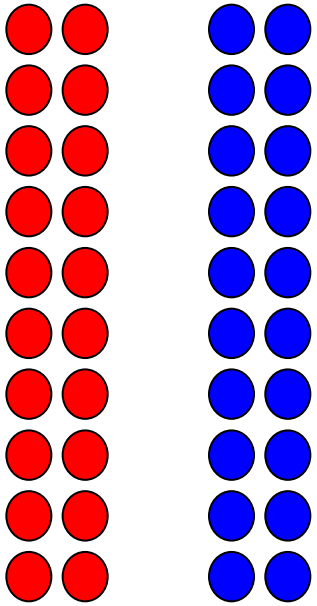
- Stochastic is synonymous with "random." The word is of Greek origin and means "pertaining to chance“. … Stochastic is often used as counterpart of the word "deterministic," which means that random phenomena are not involved. Therefore, stochastic models are based on random trials, while deterministic models always produce the same output for a given starting condition.

- http://mathworld.wolfram.com/Stochastic.html

High copy number

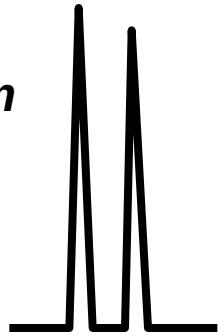
>20 copies per allele

Copies of allele 1 **Copies of allele 2**



What is sampled is consistent with the true amount present in the sample

Resulting electropherogram

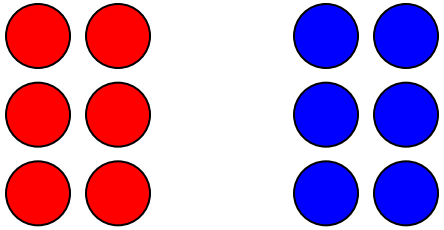


Complete (and correct) genotype

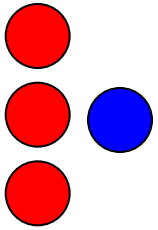
Low copy number

6 copies per allele

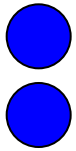
True amount



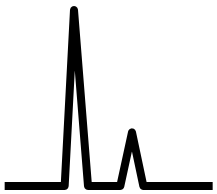
What might be sampled by the PCR reaction…



OR



Extreme allele imbalance



Allele imbalance

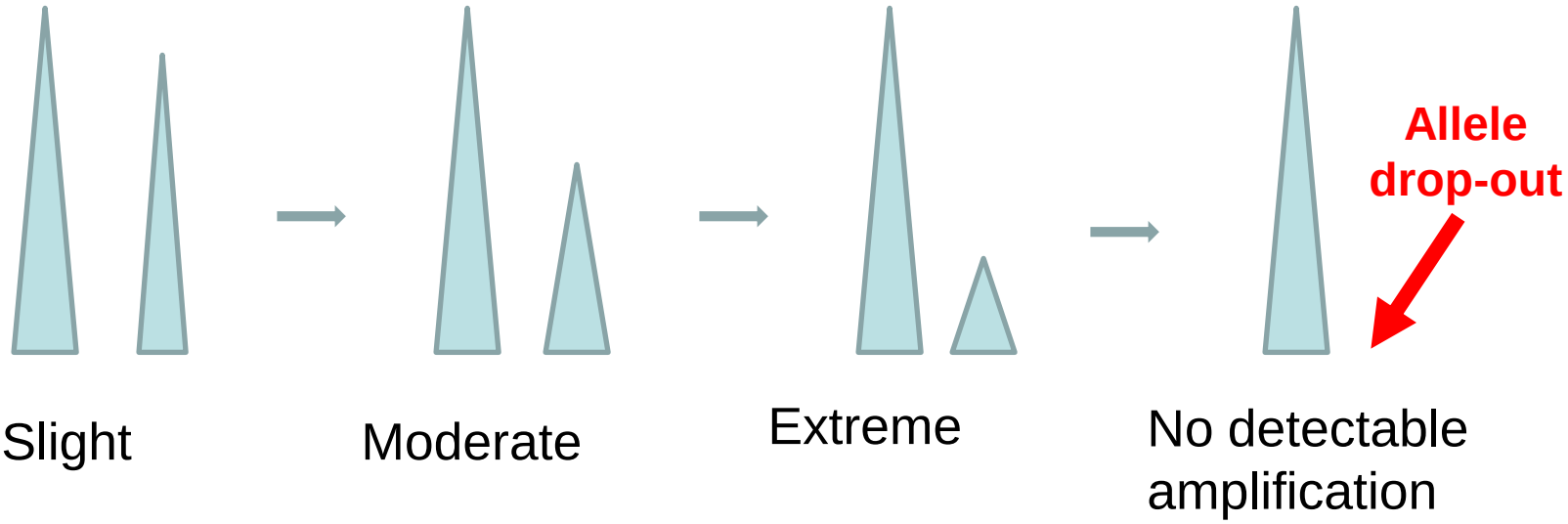
Allele dropout

How can we characterize variation?

- Look at total amount of variation at end of process
  - Follow the positive control over time
- Experimentally break process into components and characterize using appropriate statistics
  - e.g., separate amplification variation from injection variation
- Analyze existing or new validation data, training sample data, SRM data, kit QC data
- Use casework data
  - e.g., variation between knowns (victim's DNA profile within an intimate sample) and matching single-source evidence profiles

Problem with Stochastic Effects

- **Allele drop-out** is an extension of the amplification disparity that is observed when heterozygous peaks heights are unequal
  - Occurs in single-source samples and mixtures
  - Analyst is unable to distinguish complete allele drop-out in a true heterozygote from a homozygous state



What is Allele Drop Out?

- Scientifically
  - **Failure to detect** an allele within a sample or failure to amplify an allele during PCR. *From SWGDAM Guidelines, 2010*
  - Note that: Failure to detect ≠ failure to amplify
- Operationally
  - Setting a threshold(s) or creating a process, based on validation data and information in the literature, which allows assessment of the likelihood of drop-out of an allele or a locus.

Stochastic Effects and Stochastic Threshold

SWGDAM 2010 Interpretation Guidelines glossary:

- **Stochastic effects**: the observation of intra-locus peak imbalance and/or allele drop-out resulting from random, disproportionate amplification of alleles in low-quantity template samples

- **Stochastic threshold**: the peak height value above which it is reasonable to assume that, at a given locus, allelic dropout of a sister allele has not occurred

http://www.fbi.gov/about-us/lab/codis/swgdam-interpretation-guidelines

Important Principle: With many casework sample, we cannot avoid stochastic effects and allele or locus drop-out.

Why ?

We do not know the number of contributors to a sample or the true contributor ratio in a mixture!

Sample Mixture Ratio Impacts Amount of DNA Available for PCR Amplification

Assume sample is a 1:3 mixture of two sources:

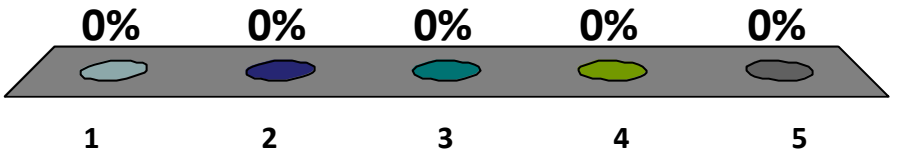
Amount of DNA	~ # of cells from major component	~ # of cells from minor component
1 ng	107	36
0.5 ng	53	18
0.25 ng	27	9
0.125 ng	12	4
0.063 ng	7	2

Stochastic effects expected with PCR amplification from <20 cells

If your laboratory uses a stochastic threshold (ST), it is:

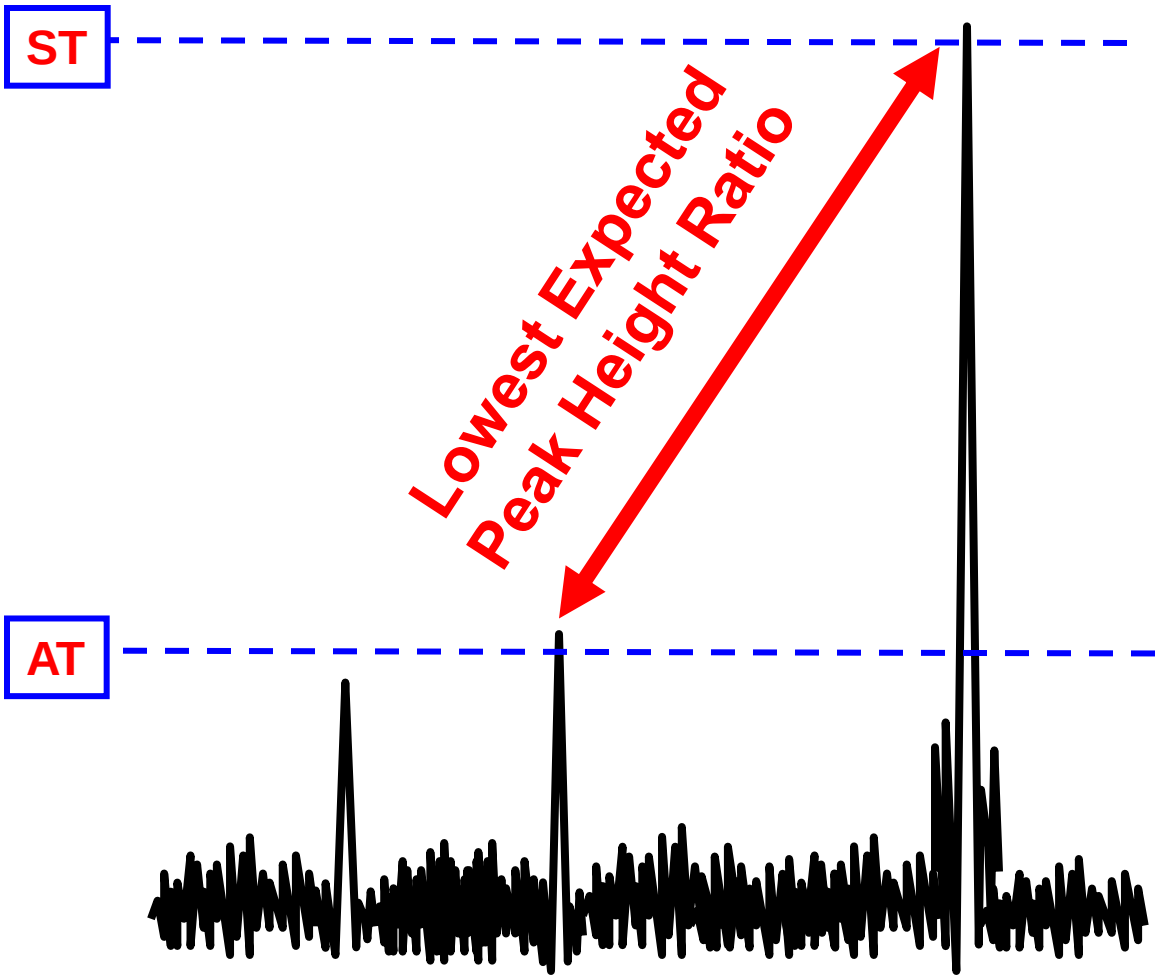
1. Same value as our analytical threshold (**we don't use a ST**)
2. About twice as high as our AT (e.g., AT = 50 and ST = 100 RFU)
3. Less than twice as high as our AT
4. Greater than twice as high as our AT
5. I don't know!

Data from 140 responses at ISHI Mixture Workshop (Oct 2011)



Stochastic and Analytical Thresholds

Impact Lowest Expected Peak Height Ratio



Determining the Dropout (Stochastic) Threshold

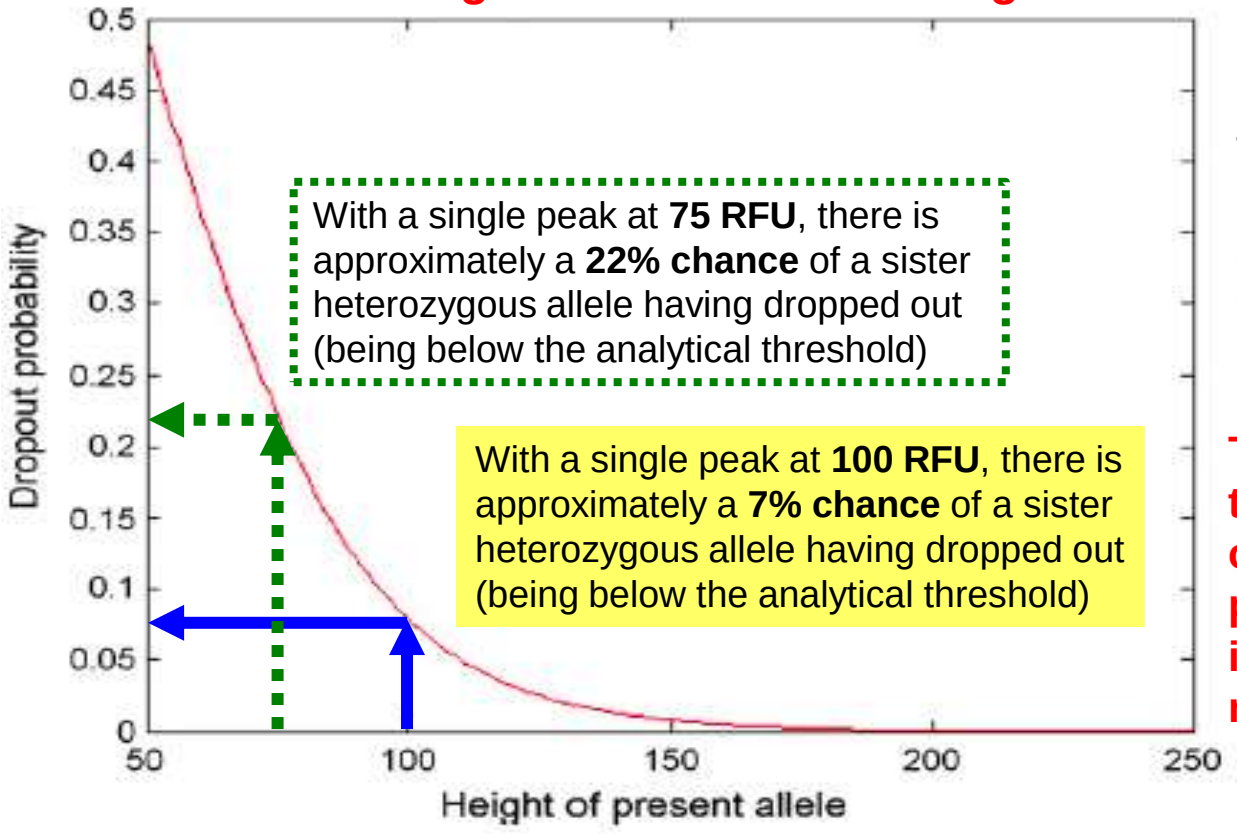
Gill *et al.* (2008) *FSI Genetics* 2(1): 76–82

- 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)

Setting a Stochastic Threshold is Essentially Establishing a Risk Assessment

Drop Out Probability as a Function of Surviving Sister Allele Peak Height



"Currently, most laboratories use an arbitrary stochastic threshold. **When a protocol is changed, especially if it is made more sensitive to low-level DNA, then the stochastic threshold must also change**."

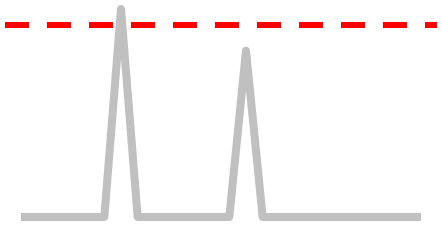
Puch-Solis R, et al. (2011). Practical determination of the low template DNA threshold. *Forensic Sci. Int. Genet.* 5(5): 422-427.

The position and shape of this curve may change based on anything that can impact peak detection (e.g., CE injection time, PCR cycle number, post-PCR cleanup).

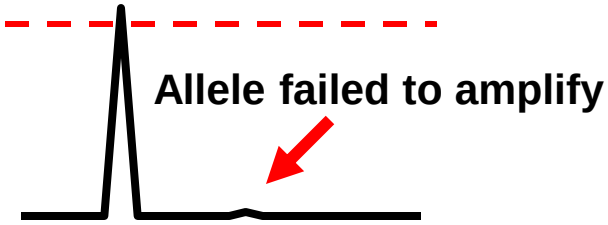
Gill, P., et al. (2009). The *low-template* (stochastic) threshold-Its determination relative to risk analysis for national DNA databases. *FSI Genetics*, 3, 104-111.

Stochastic Effects and Thresholds

Regular Injection

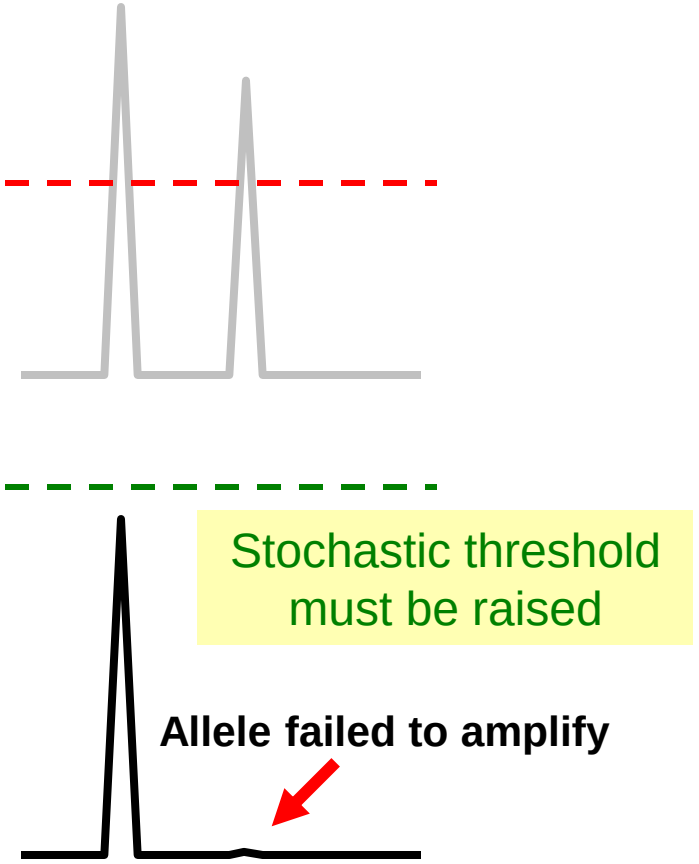


When PCR amplifying low levels of DNA, allele dropout may occur



False homozygote

Injection Following Desalting (MiniElute)



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

Setting Stochastic Methodology

- Calculated with data from the sensitivity study (DNA dilution series) analyzed with dye specific analytical thresholds

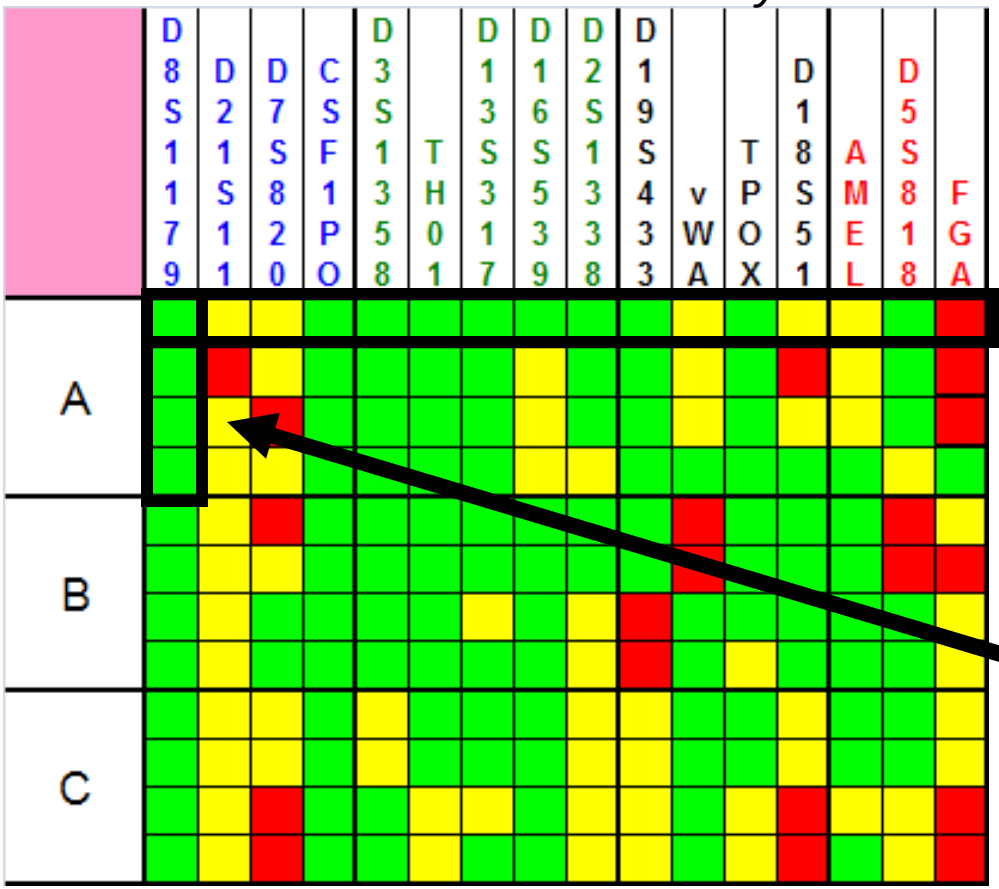
- Examination of sample amounts where dropout is observed (50 pg, 30 pg, 10 pg for Identifiler and Identifiler Plus)
  - Focus on sample amounts with dropout present to examine stochastic effects including severe imbalance of heterozygous alleles and allele dropout

- Stochastic Threshold: The RFU value of highest surviving false homozygous peak per dye channel

Heat Map Explanation

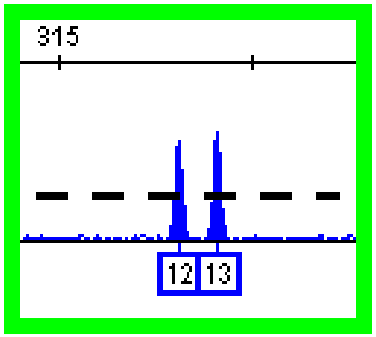
Green = full (correct) type
Yellow = allele dropout
Red = locus dropout

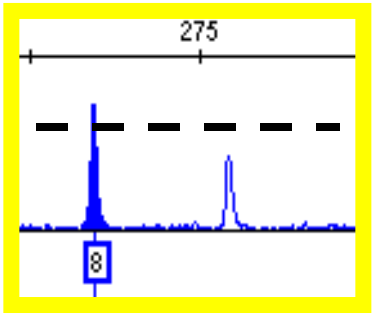
Results broken down by locus

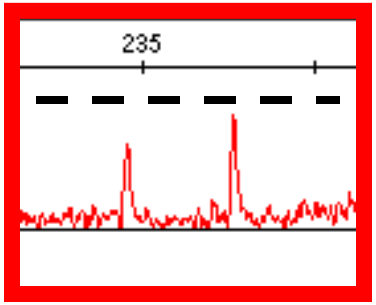


A single profile slice

A replicate slice







This is an easy way to look at a lot of data at once

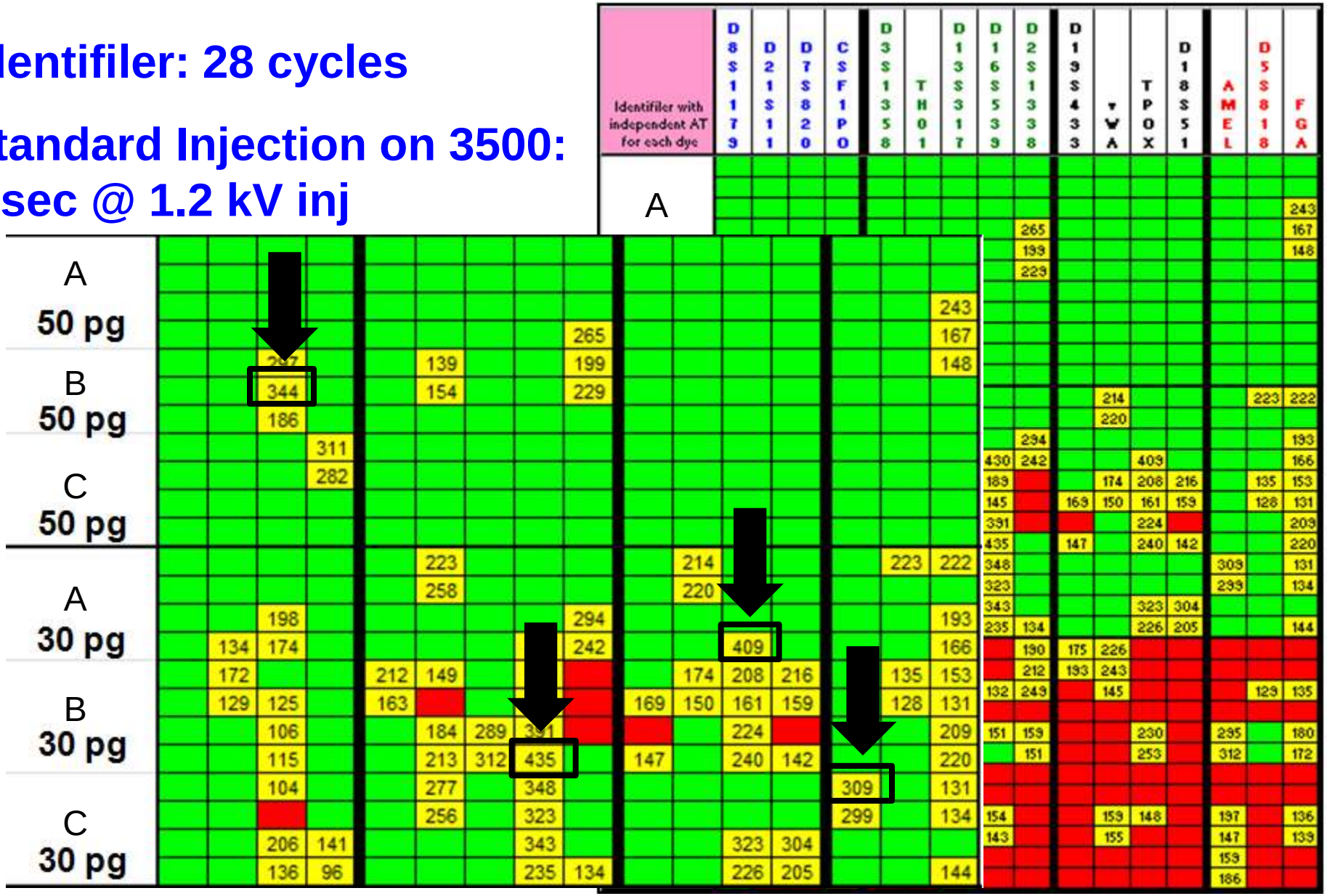
n=84 Samples

Slide from Erica Butts (NIST) 3500 presentation in Innsbruck, Austria (Sept 5, 2011)

Stochastic Threshold

Identifiler: 28 cycles

Standard Injection on 3500:
7 sec @ 1.2 kV inj



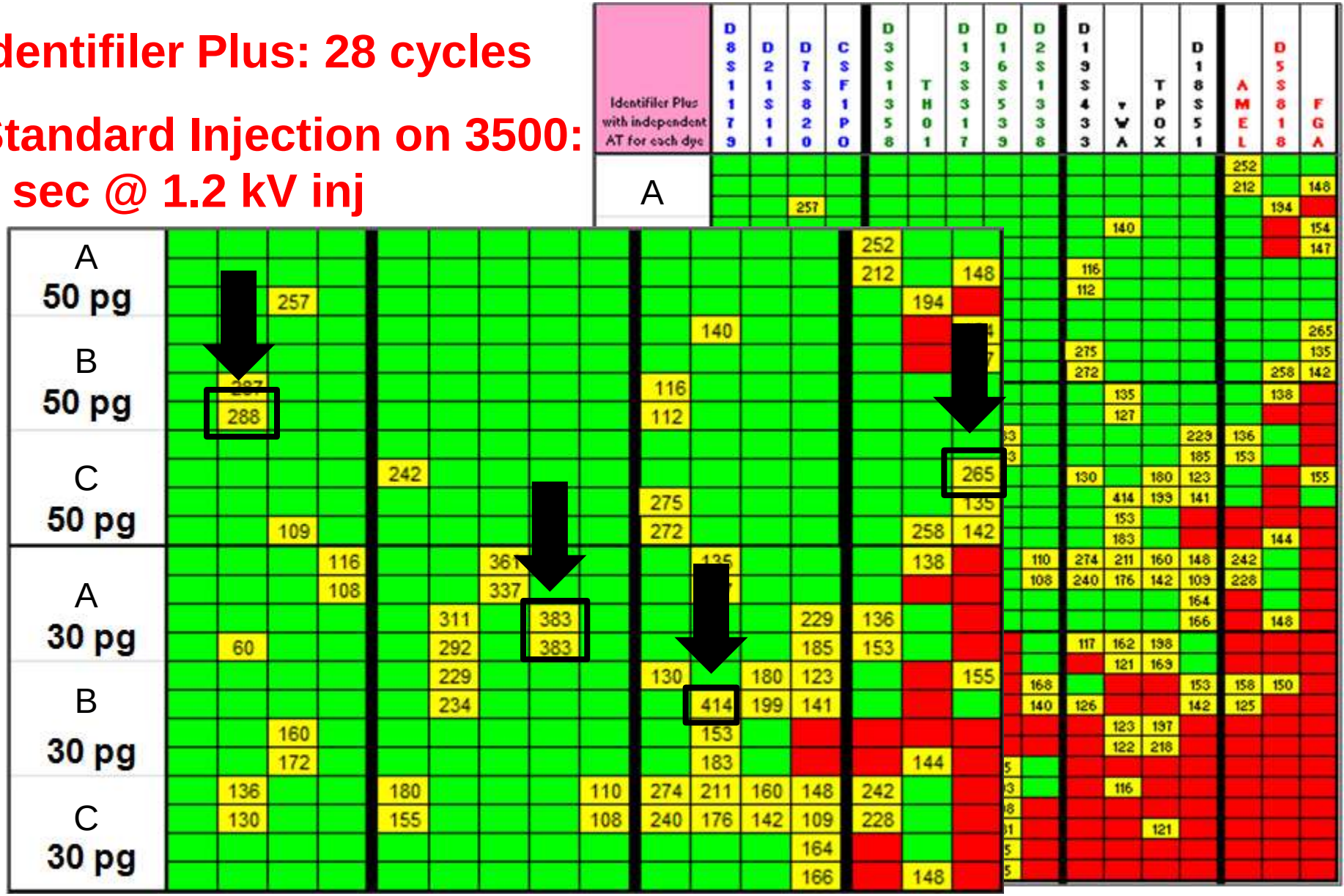
n=84 Samples

Slide from Erica Butts (NIST) 3500 presentation in Innsbruck, Austria (Sept 5, 2011)

Stochastic Threshold

Identifiler Plus: 28 cycles

Standard Injection on 3500: 5 sec @ 1.2 kV inj



n=84 samples

Summary of Thresholds

Both AT and ST values rounded to the nearest 5 RFU value

Expected peak height ratio (PHR) is assuming the possibility of having one peak at the AT and one peak at the ST

Expected PHR = AT/ST

Identifiler: 7 sec @ 1.2 kV (28 cycles)				
	AT (RFU)	**Highest Surviving Peak (RFU)**	**ST (RFU)**	**Expected PHR**
Blue	95	344	345	28%
Green	130	435	435	30%
Yellow	140	409	410	34%
Red	120	309	310	39%

Identifiler Plus: 7 sec @ 1.2 kV (28 cycles)				
	AT (RFU)	**Highest Surviving Peak (RFU)**	**ST (RFU)**	**Expected PHR**
Blue	55	288	290	19%
Green	75	383	385	19%
Yellow	105	414	415	25%
Red	120	265	265	45%

Keep in Mind…

"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**."

Bright, J.A., et al. (2010). Examination of the variability in mixed DNA profile parameters for the Identifiler multiplex. *Forensic Science International: Genetics, 4,* 111-114.

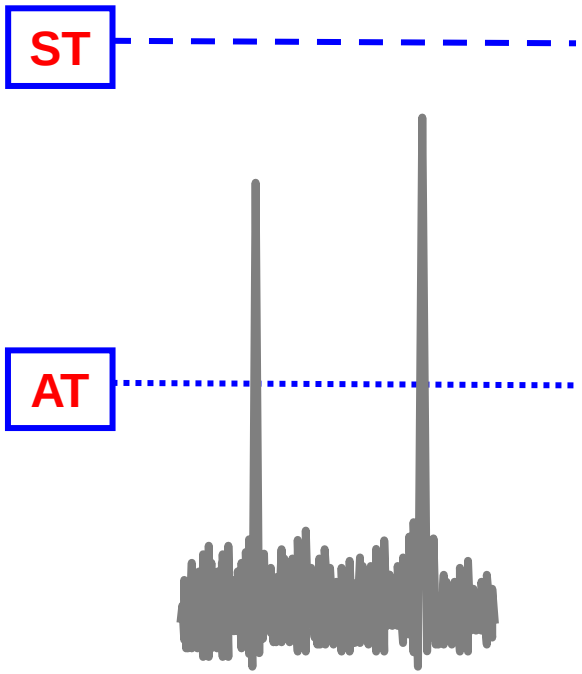
Coupling of Statistics and Interpretation

- **The CPE/CPI approach** for reporting an inclusionary statistic **requires that all alleles be observed** in the evidence sample

- If allele drop-out is suspected at a locus, then any allele is possible and the probability of inclusion goes to 100% -- in other words, the locus is effectively dropped from consideration

- If alleles are seen below the established stochastic threshold, then the locus is typically eliminated (“INC” – declared inconclusive) in many current lab SOPs

Can This Locus Be Used for Statistical Calculations?



It depends on your assumption as to the number of contributors!

If you assume <u>a single-source sample</u>, then you can assume that the detection of two alleles fully represents the heterozygous genotype present at this locus.

If you assume (from examining other loci in the profile as a whole) that the sample is a mixture of two or more contributors, then there may be allele drop-out and all alleles may not be fully represented.

Limitations of Stochastic Thresholds

- The possibility of allele sharing with a complex mixture containing many contributors may make a stochastic threshold meaningless

- “Enhanced interrogation techniques” to increase sensitivity (e.g., increased PCR cycles) may yield false homozygotes with >1000 RFU

- **New turbo-charged kits with higher sensitivity will need to be carefully evaluated to avoid allele drop-out and false homozygotes**

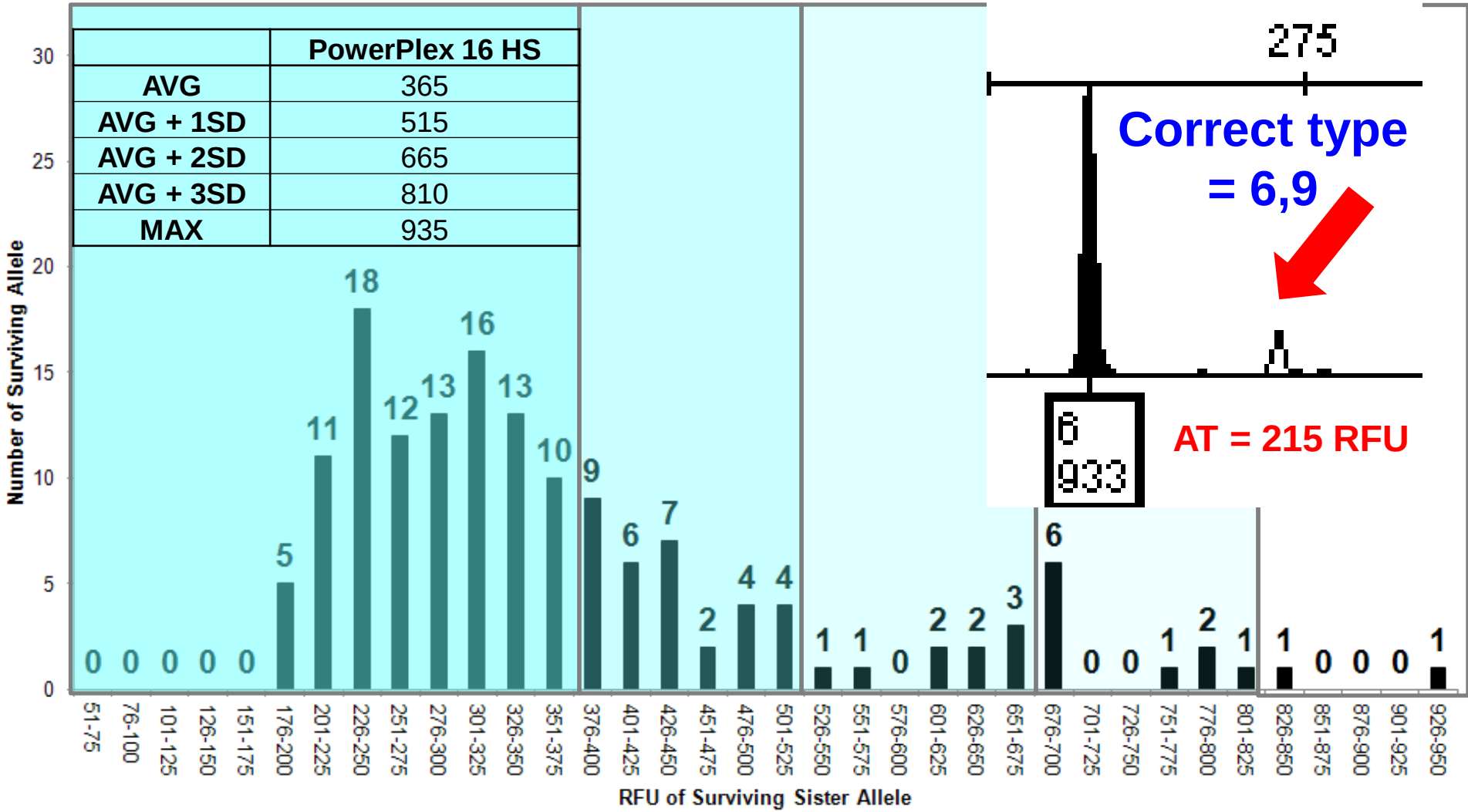
Data from Erica Butts (NIST)

PowerPlex 16 HS Stochastic Threshold

(ABI 3500 Data – **see Poster #42**)

PCR = 30 cycles

	PowerPlex 16 HS
AVG	365
AVG + 1SD	515
AVG + 2SD	665
AVG + 3SD	810
MAX	935



Stochastic Threshold Summary

- A stochastic threshold (ST) may be established for a specific set of conditions to reflect possibility of allele drop-out, which is essential for a CPE/CPI stats approach
- ST should be re-examined with different conditions (e.g., higher injection, sample desalting, increase in PCR cycles)
- ST will be dependent on the analytical threshold set with a method and impacts the lowest expected peak height ratio
- Assumptions of the number of contributors is key to correct application of ST

Stats Required for Inclusions

SWGDAM Interpretation Guideline 4.1:

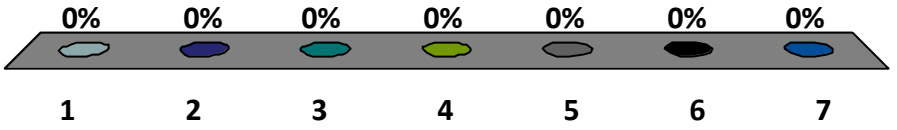
> "**The laboratory must perform statistical analysis in support of any inclusion** that is determined to be relevant in the context of a case, irrespective of the number of alleles detected and the quantitative value of the statistical analysis."

Buckleton & Curran (2008): "There is a considerable aura to DNA evidence. Because of this aura **it is vital that weak evidence is correctly represented as weak or not presented at all**."

Buckleton, J. and Curran, J. (2008) A discussion of the merits of random man not excluded and likelihood ratios. *Forensic Sci. Int. Genet.* 2: 343-348.

What kind of mixture statistic does your lab use?

1. LR
2. CPE (RMNE, CPI)
3. RMP
4. CPE or RMP
5. Other combinations
6. Probabilistic modeling (e.g., TrueAllele)
7. We don’t use stats (contradicting the guidelines – section 4.1)



DAB Recommendations on Statistics

February 23, 2000

Forensic Sci. Comm. 2(3); available on-line at
http://www.fbi.gov/hq/lab/fsc/backissu/july2000/dnastat.htm

"The DAB finds either one or both PE or LR calculations acceptable and strongly recommends that one or both calculations be carried out whenever feasible and a mixture is indicated"

- Probability of exclusion (PE)
  - Devlin, B. (1993) Forensic inference from genetic markers. *Statistical Methods in Medical Research* 2: 241–262.
- Likelihood ratios (LR)
  - Evett, I. W. and Weir, B. S. (1998) *Interpreting DNA Evidence*. Sinauer, Sunderland, Massachusetts.

CPE/CPI (RMNE) Limitations

- A CPE/CPI approach assumes that all alleles are present (i.e., cannot handle allele drop-out)
- Thus, statistical analysis of low-level DNA CANNOT be correctly performed with a CPE/CPI approach because some alleles may be missing
- Charles Brenner in his AAFS 2011 talk addressed this issue
- Research is on-going to develop allele drop-out models and software to enable appropriate calculations

Notes from Charles Brenner's AAFS 2011 talk

The Mythical "Exclusion" Method for Analyzing DNA Mixtures – Does it Make Any Sense at All?

1. The claim that is requires **no assumption about number of contributors** is mostly wrong.
2. The supposed **ease of understanding** by judge or jury is really an illusion.
3. **Ease of use** is claimed to be an advantage particularly for complicated mixture profiles, those with many peaks of varying heights. The truth is the exact opposite. **The exclusion method is completely invalid for complicated mixtures.**
4. The exclusion method is only **conservative** for guilty suspects.

- "Certainly no one has laid out an explicit and rigorous chain of reasoning from first principles to support the exclusion method. It is at best guesswork."

Brenner, C.H. (2011). The mythical "exclusion" method for analyzing DNA mixtures – does it make any sense at all? *Proceedings of the American Academy of Forensic Sciences*, Feb 2011, Volume 17, p. 79

Statistical Methods in Medical Research 1993; **2**: 241–262

Forensic inference from genetic markers

B Devlin Department of Epidemiology and Public Health, Yale University School of Medicine

Section 5.1 Exclusion probability

- Discussion about exclusion probabilities in Paternity cases.

Two types:

(1) Conditional Exclusion Probability - excluding a random man as a possible father, given the mother-child genotypes for a particular case.

(2) Average Exclusion Probability – excluding a random man as a possible father, given a randomly chosen mother-child pair.

Statistical Methods in Medical Research 1993; **2**: 241–262

Forensic inference from genetic markers

B Devlin Department of Epidemiology and Public Health, Yale University School of Medicine

Section 5.1 Exclusion probability

“The theoretical concept of exclusion probabilities, however, makes no sense within the framework of normal mixture models.”

“The interpretation of conditional exclusion probability is obvious, which accounts for its value in the legal arena. Unlike [LR], however, it is not fully efficient.”

Curran and Buckleton (2010)

JOURNAL OF FORENSIC SCIENCES

J Forensic Sci, September 2010, Vol. 55, No. 5
doi: 10.1111/j.1556-4029.2010.01446.x
Available online at: interscience.wiley.com

PAPER

CRIMINALISTICS; GENERAL

James M. Curran,[1] *M.Sc.(Hons.), Ph.D. and John Buckleton,*[2] *Ph.D.*

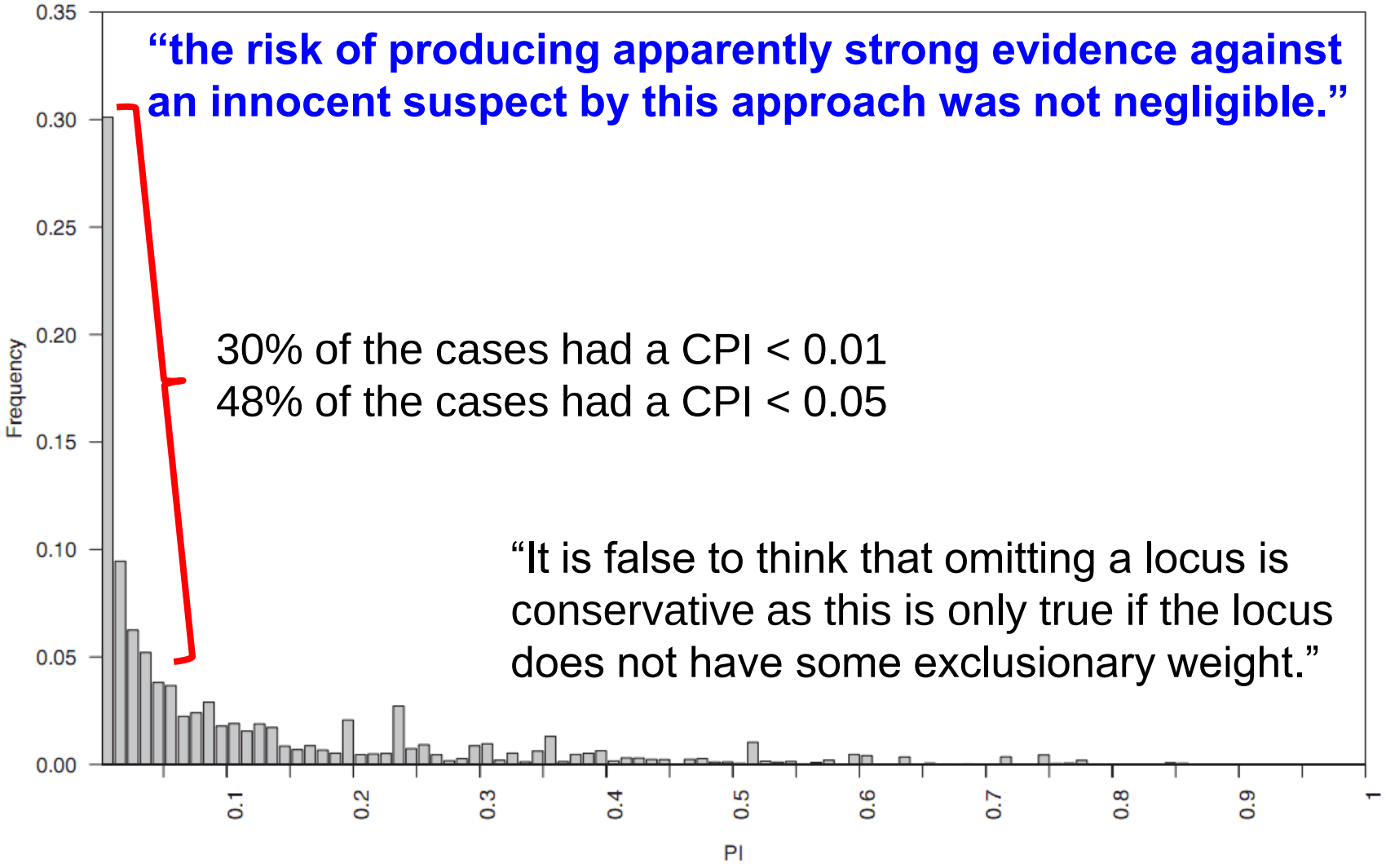
Inclusion Probabilities and Dropout

Created 1000 Two-person Mixtures (Budowle *et al.*1999 AfAm freq.).

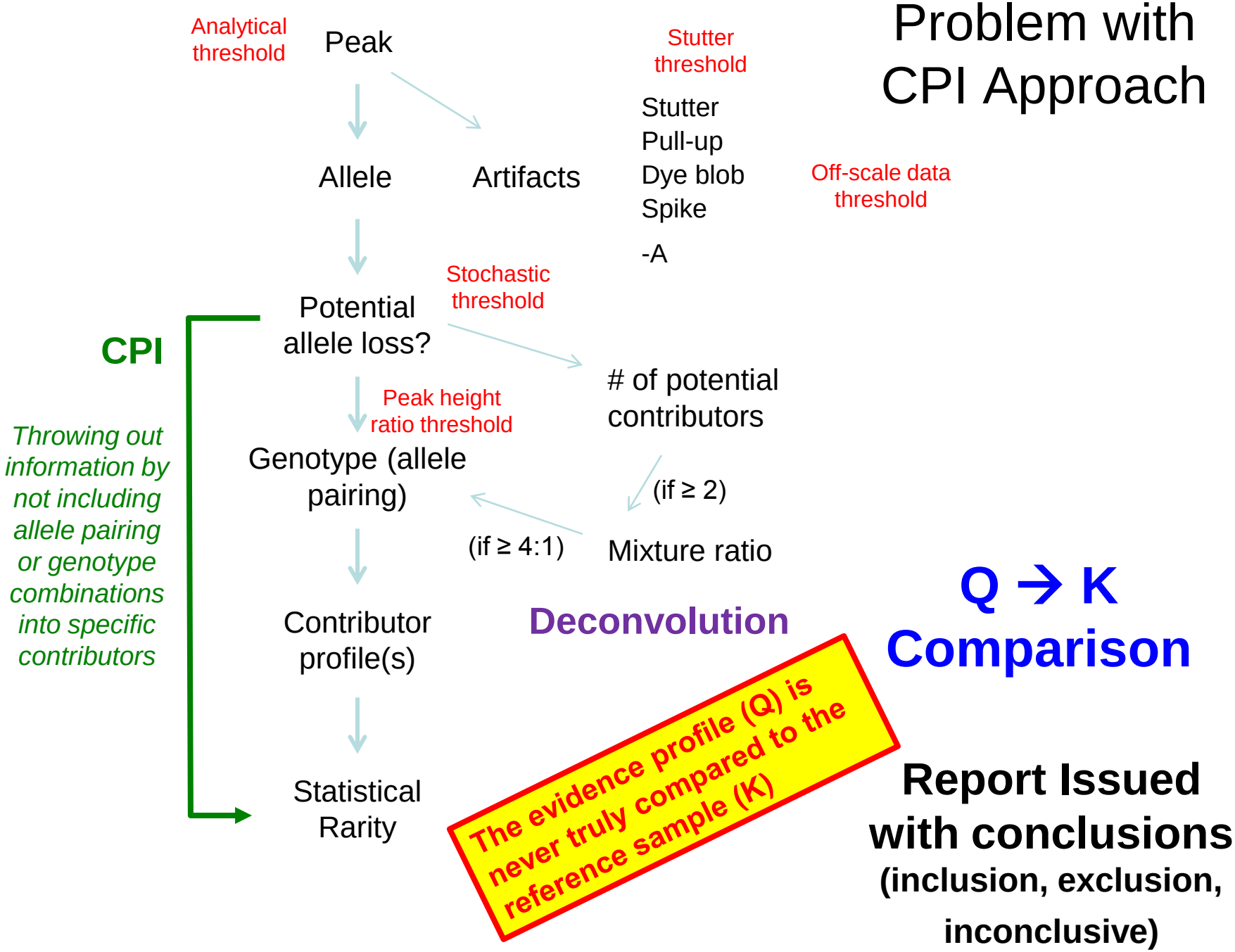
Created 10,000 “third person” genotypes.

Compared “third person” to mixture data, calculated PI for included loci, ignored discordant alleles.

Curran and Buckleton (2010)



Problem with CPI Approach



Impact of Dropping Loci

- The less data available for comparison purposes, the greater the chance of falsely including someone who is truly innocent

- Are you then being “conservative” (i.e., erring in favor of the defendant)?

Likelihood Ratio (LR)

- Provides ability to express and evaluate both the prosecution hypothesis, H_p (the suspect is the perpetrator) and the defense hypothesis, H_d (an unknown individual with a matching profile is the perpetrator)

$$LR = \frac{H_p}{H_d}$$

- **The numerator, H_p, is usually 1** – since in theory the prosecution would only prosecute the suspect if they are 100% certain he/she is the perpetrator

- The denominator, **H_d**, is typically the profile frequency in a particular population (based on individual allele frequencies and assuming HWE) – i.e., **the random match probability**

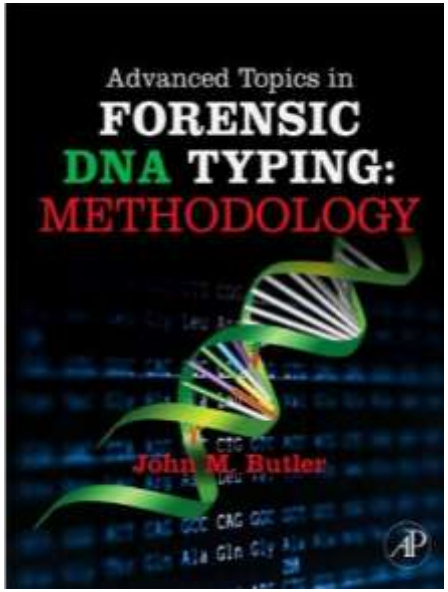
Steps Involved in Process
of Forensic DNA Typing

1) Data Interpretation
2) Statistical Interpretation

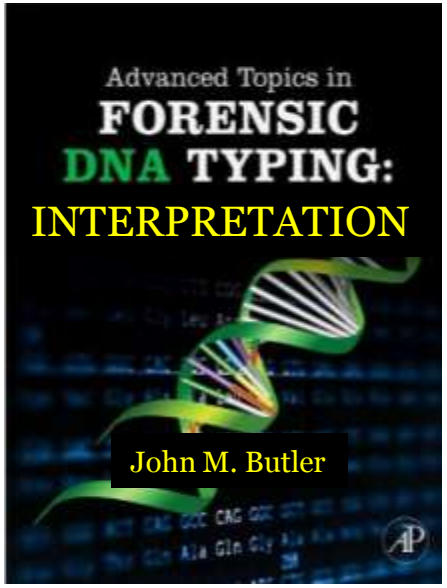
Gathering the Data **Understanding the Data**

Collection/Storage/ Characterization → **Extraction/ Quantitation** → **Amplification/ Marker Sets** → **Separation/ Detection** → **Interpretation** → **Report**

Advanced Topics: Methodology



Advanced Topics: Interpretation



Advanced Topics in Forensic DNA Typing: INTERPRETATION

Chapter	Topic (current planned chapters)
	Introduction
1	Data interpretation overview
2	Thresholds
3	STR alleles & artifacts
4	STR genotypes & dropout
5	STR profiles
6	**Mixture interpretation**
7	Low-level DNA and complex mixtures
8	CE troubleshooting
9	Statistical interpretation overview
10	STR population data analysis
11	Profile frequency estimates
12	**Mixture statistics**
13	Coping with potential missing alleles
14	**Kinship and parentage analysis**
15	Lineage marker statistics
16	Drawing conclusions & **report writing**
	Glossary
App 1	U.S. Population Data (24 loci with N=938)
App 2	Revised Forensic DNA QAS (Sept 2011)
App 3	DAB Recommendations on Stats (Feb 2000)
App 4	NRC II Recommendations (1996)
App 5	SWGDAM STR Interp Guidelines (Jan 2010)

Features in New Book

(planned for Spring 2013 release)

- Explanations of SWGDAM interpretation guidelines
- Interviews on report writing from multiple perspectives
- Mixture interpretation
- Kinship analysis
- CE troubleshooting
- Standard U.S. pop data
- Numerous D.N.A. Boxes (**D**ata, **N**otes, & **A**pplications)
  - Worked examples to show relevance of equations
  - “Better know a statistician”

"Better Know a Statistician…"

Purpose in Writing a Book on Interpretation

- Everyone may think that their way is correct – but misinterpretations have given rise to a variety of approaches being undertaken today, some of which are not correct…

- I believe that **a better understanding of general principles will aid consistency and quality of work being performed**

Take Home Messages

- Inclusionary statements (including “cannot exclude”) need statistical support to reflect the relevant weight-of-evidence
- Stochastic thresholds are necessary if using CPI statistics to help identify possible allele dropout
- CPI is only conservative for guilty suspects as this approach does a poor job of excluding the innocent
- Uncertainty exists in scientific measurements
- An increasing number of poor samples are being submitted to labs – labs may benefit from developing a complexity threshold