Forensic DNA Mixture Interpretation

Thresholds & Low-Level Mixtures

MAFS Workshop

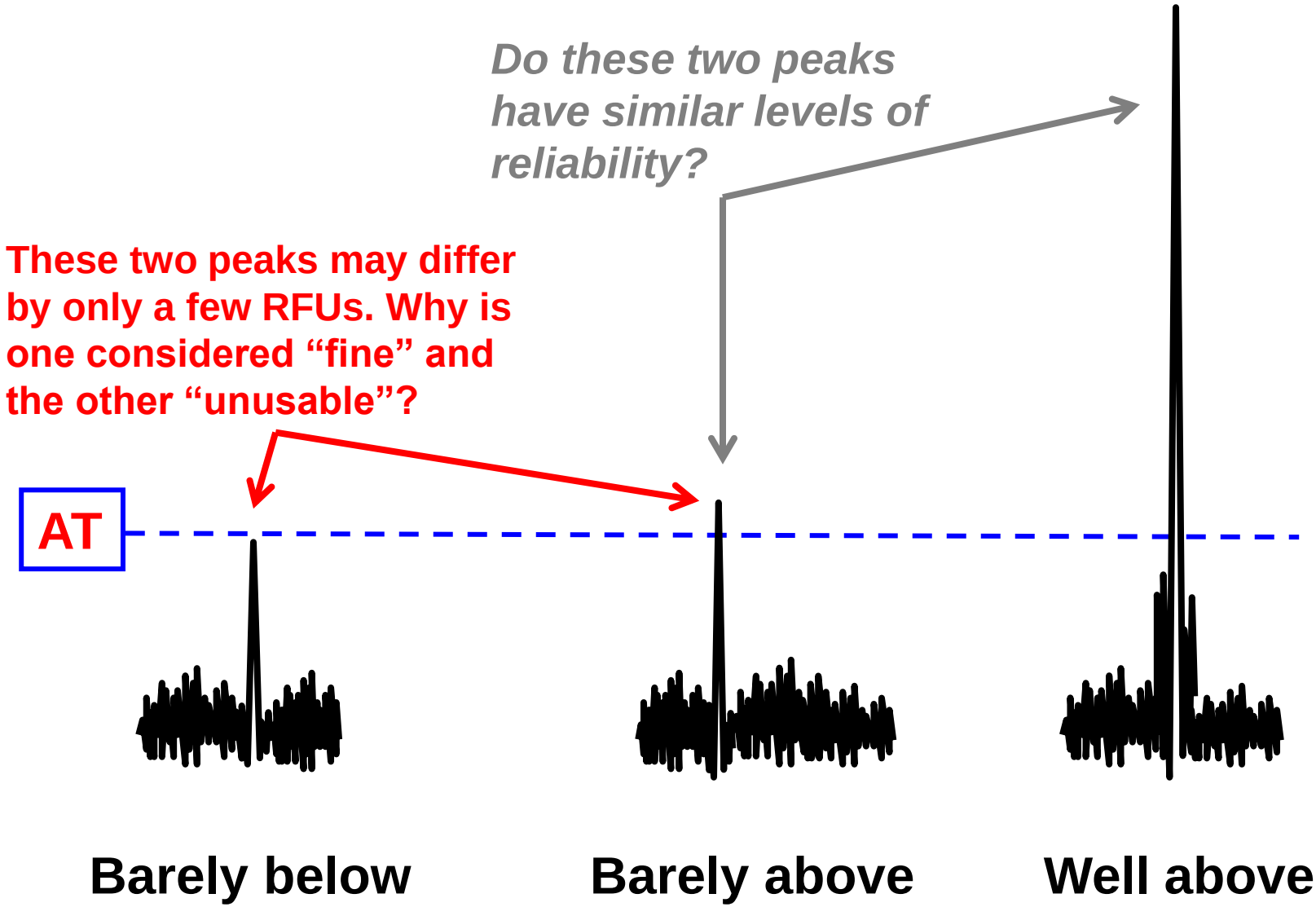
Milwaukee, WI

September 25, 2012

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

john.butler@nist.gov

What is the meaning of a threshold?

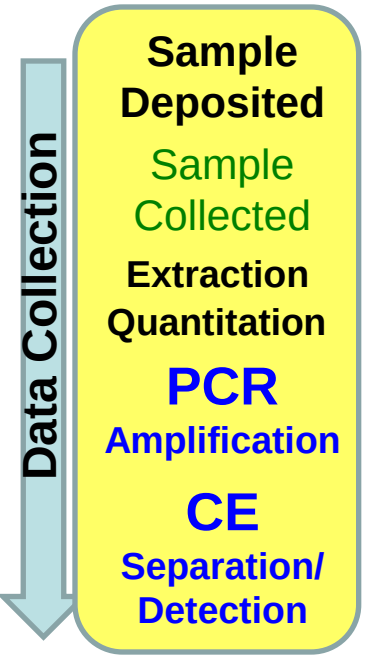


Keep in Mind…

"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.

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)

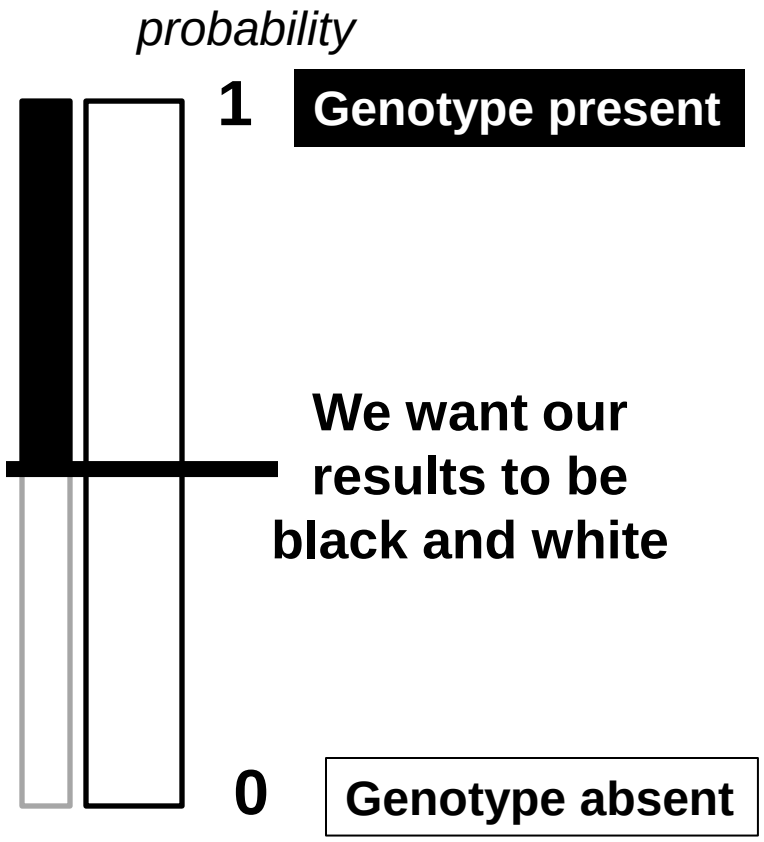
Principles Behind Thresholds

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

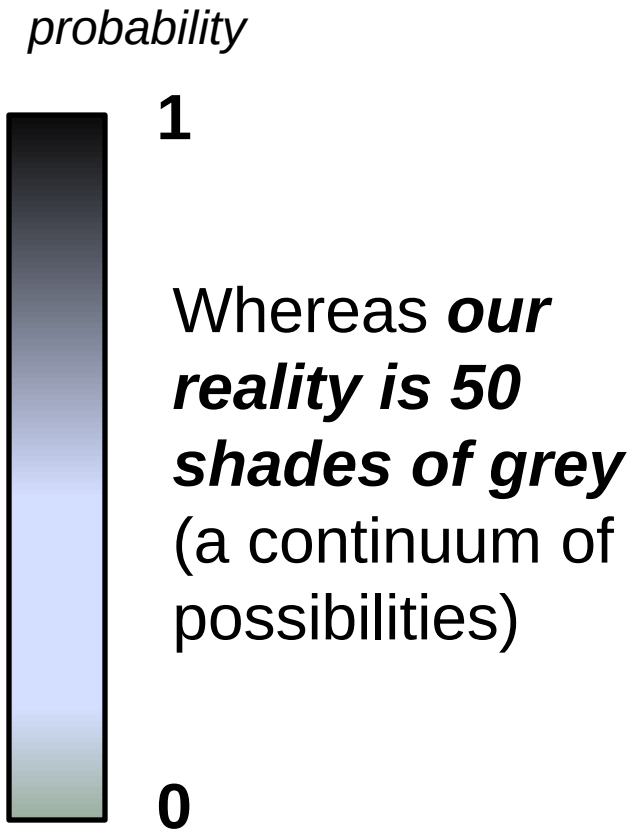
Threshold Decisions

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 Data Interpretation:
Binary vs Probabilistic



Binary Approach



Probabilistic Approach

Conference Held in Rome Earlier This Year

http://www.oic.it/ForensicGenetics/immagini/img-home.jpg

Bruce Budowle

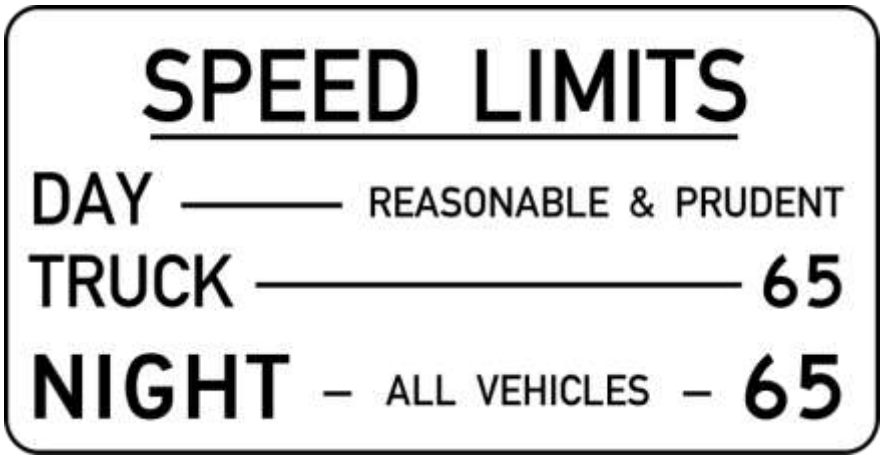
University of North Texas Health Science Center

- "**We put thresholds in place to help protect us from risk of making wrong decisions**. They have value."

- **Compares thresholds to speed limits**, which are set for safety reasons

Do you leave thresholds and protocols up to “analysts’ discretion”?

http://korsgaardscommentary.blogspot.com/2011/10/its-time-to-put-brakes-on-speed-limit.html



http://en.wikipedia.org/wiki/File:MONTANA-PR.svg

Typical speed limit sign that one would see at the Montana state line from December 1995 to June 1999

http://1.bp.blogspot.com/-5gagI4xZbT0/TdvMBGODBZI/AAAAAAAAJYo/Pj9MRqANvvs/s400/speed-limit-change-sign-537.jpg

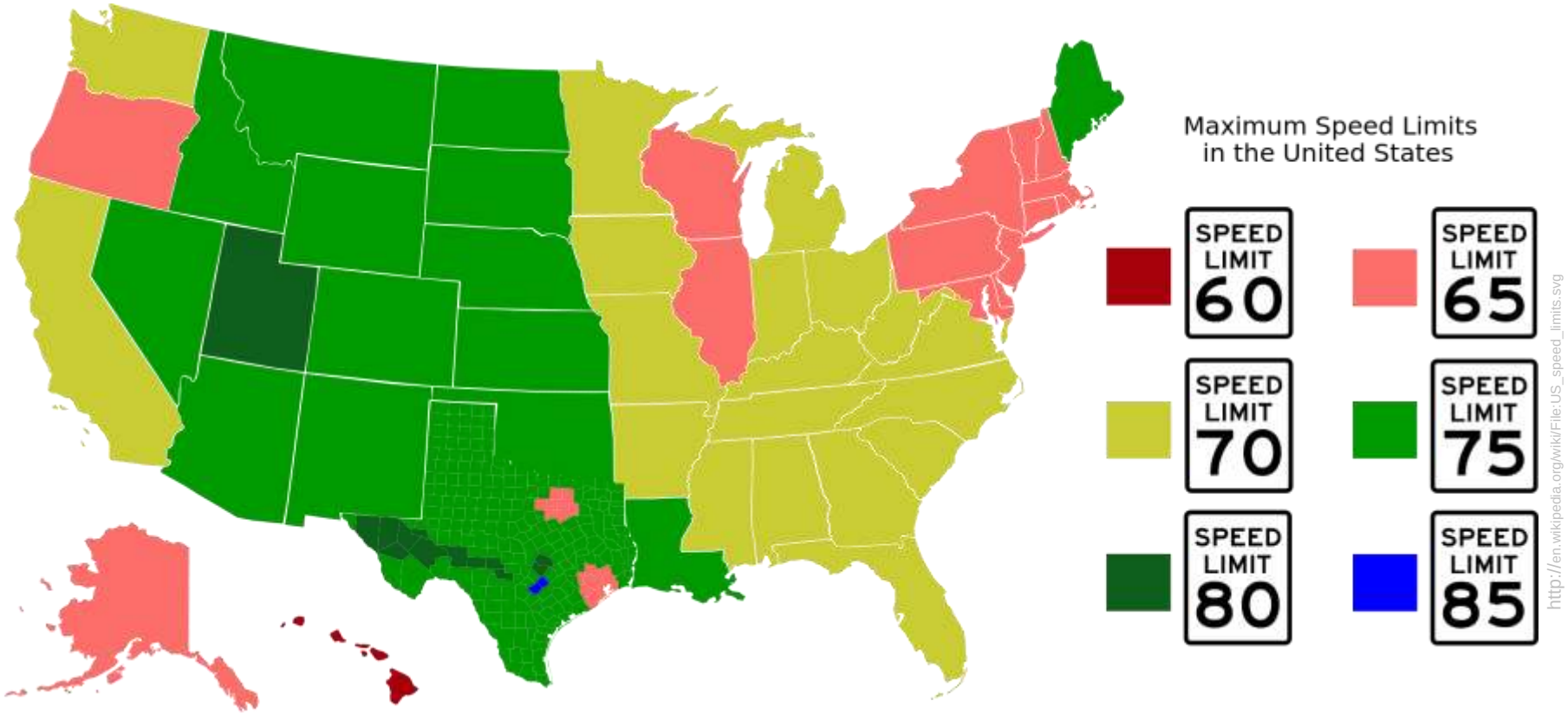
A Potential Outcome!

Do you carefully try to regulate everything with specific protocols?

Truly **a protocol with specificity**…. we even have **an auditor**, the local chief of police!

http://www.lostrepublic.us/archives/category/monetary-policy/privatized-roads

A variety of approaches exist for how protocols and thresholds are set…



http://en.wikipedia.org/wiki/Speed_limits_in_the_United_States

Would you prefer specific protocols for every detail in your lab?

1. Yes!
2. No!

How Speed Limits Are Set?

http://www.crab.wa.gov/LibraryData/REPORTS/EngineerAnswers/Article03-04SpeedLimits.pdf

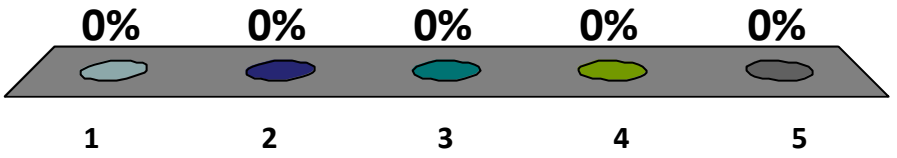
The posted speed limit for a road is set in slightly different ways in different counties. The most common way though, is to use **the "85th percentile" speed**. 85 out of 100 drivers will choose this speed no matter what the signs say. Many studies have shown this method to be safe, practical and enforceable. It also doesn't depend on the opinion of one person.

The 85th percentile speed is easily determined with special traffic counters that check the traffic on the roadway. The speed limit can then be set at the next lower 5 miles per hour. For example, if the traffic counters show 38 mph, the limit would be set at 35 mph. The speed limit may be set another 5 mph lower if there are features not obvious to the driver. These may include unusual roadside or traffic conditions including a high number of accidents.

How were the RFU levels set for your laboratory stochastic threshold?

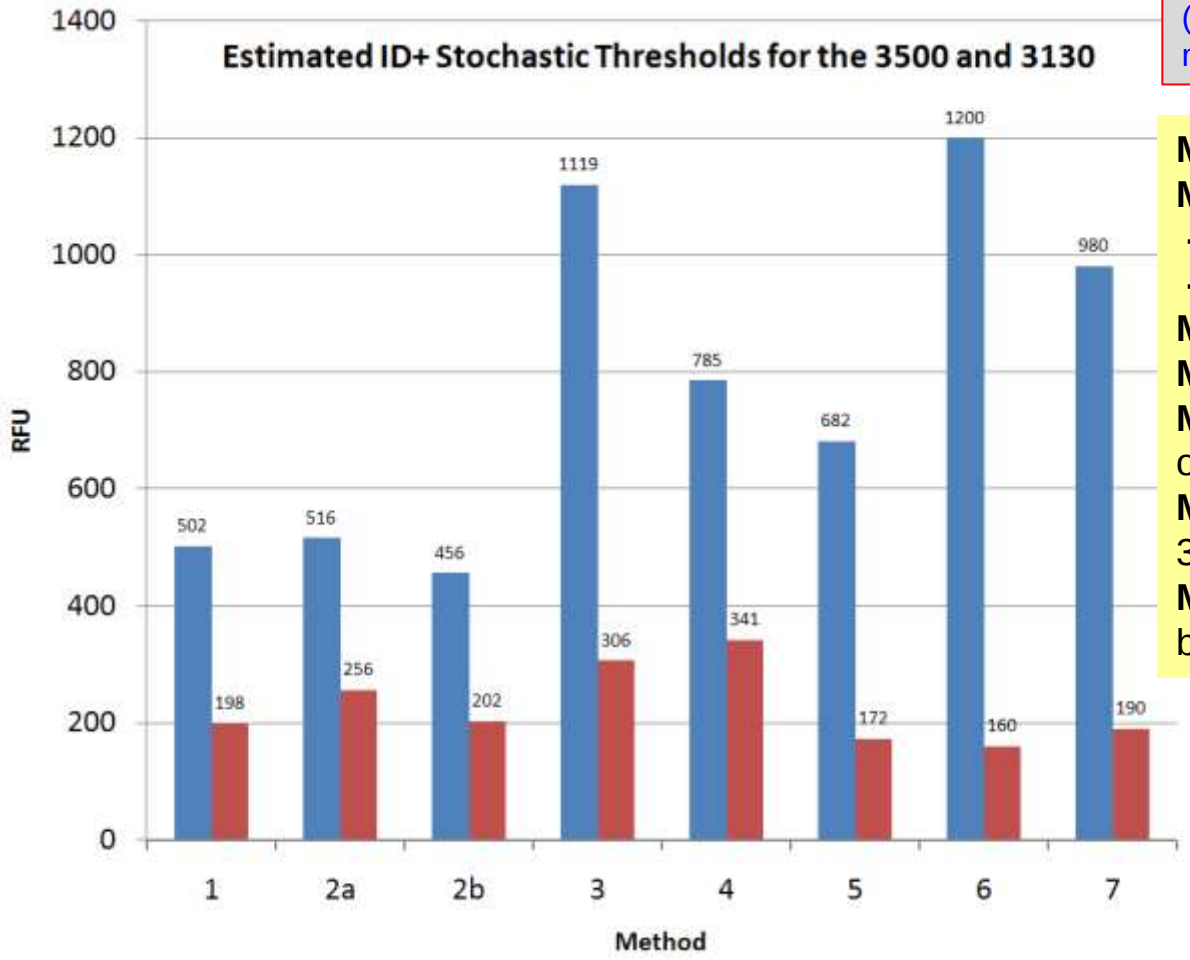
(select only one)

1. +2 SD
2. +3 SD
3. Above all dropout data
4. My TL established; I have no idea how
5. We do not use a stochastic threshold



Different approaches to determining a stochastic threshold

Results from CA DOJ Identifiler Plus validation experiments



Studied 3 DNA samples with serial dilutions (1, 0.5, 0.25, 0.125, 0.062, 0.031, 0.016 ng), multiple amps of each template quantity

Method 1: tallest false homozygote
Method 2: false homo. ave. +3SD
- **2a:** using most relevant input amount
- **2b:** using all observed false homo.

Method 3: average PH het. +3 SD
Method 4: ave. PHR -3 SD vs. signal
Method 5: AT divided by minimum observed PHR
Method 6: partial profile at ~150 pg and 3x AT
Method 7: where majority of PHRs fall below 60%

Blue bars: 3500 ST
Red bars: 3130 ST

Sonja Klein (CA DOJ) presentation at the CAC meeting (Sacramento, CA), October 25, 2011: "Approaches to estimating a stochastic threshold"

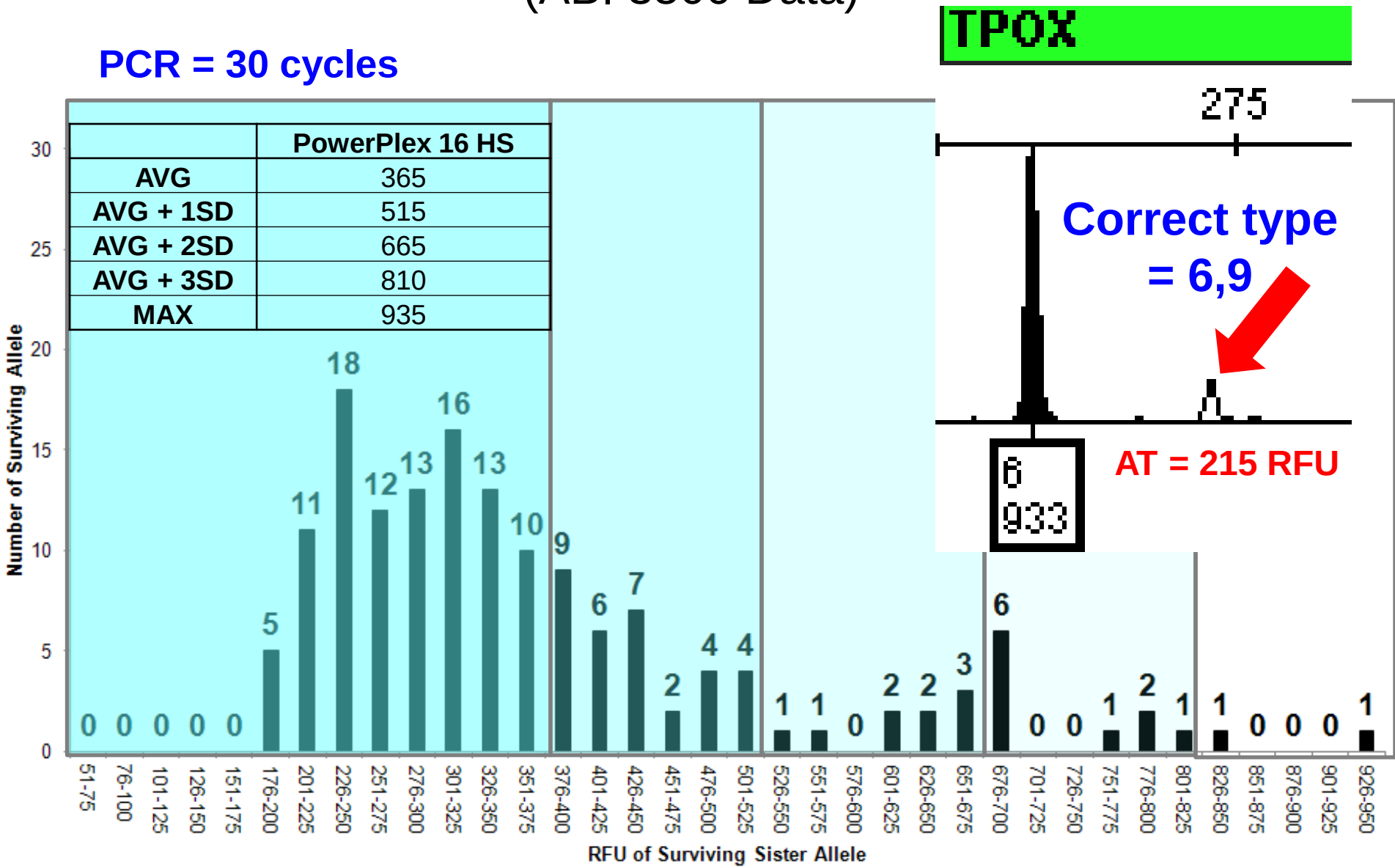
Data from Erica Butts (NIST), ISHI 2011 poster

PowerPlex 16 HS Stochastic Threshold

(ABI 3500 Data)

PCR = 30 cycles

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



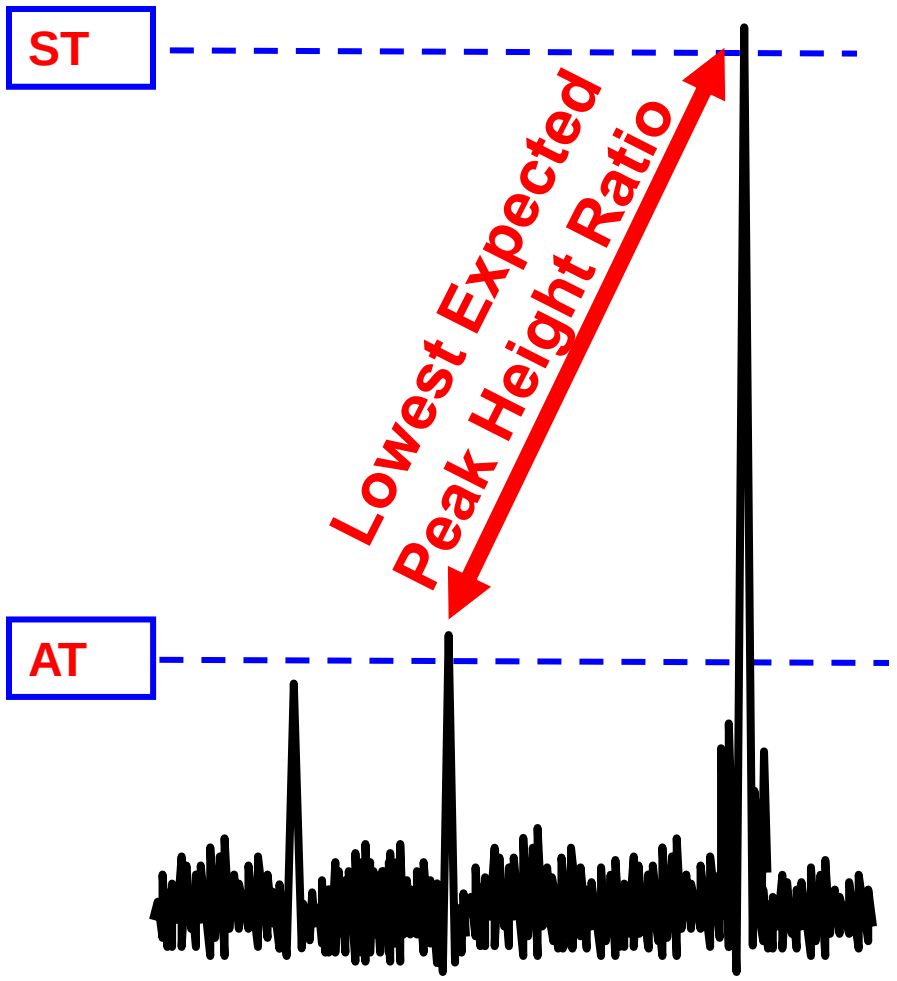
Limitations of Stochastic Thresholds

- 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**

Stochastic and Analytical Thresholds
Impact Lowest Expected Peak Height Ratio



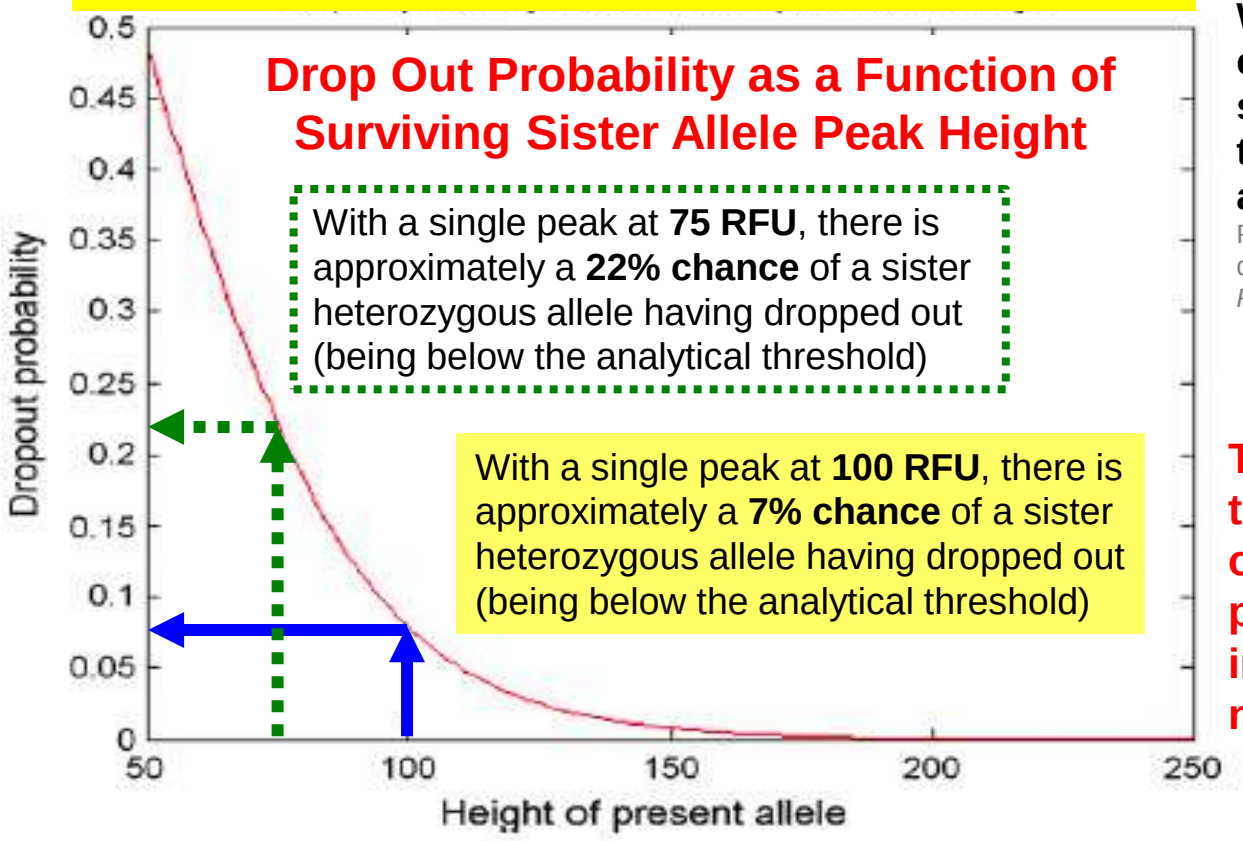
The lower you go trying to analyze low-level data… (i.e., more sensitive STR kits)

the worse your expected peak height ratios for single-source samples

Therefore, there is greater uncertainty with associating genotypes of contributors in mixtures (or even determining that you have a mixture)

Setting a Stochastic Threshold is Essentially Establishing a Risk Assessment

How much error are you willing to accept?



"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 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

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 for statistical purposes

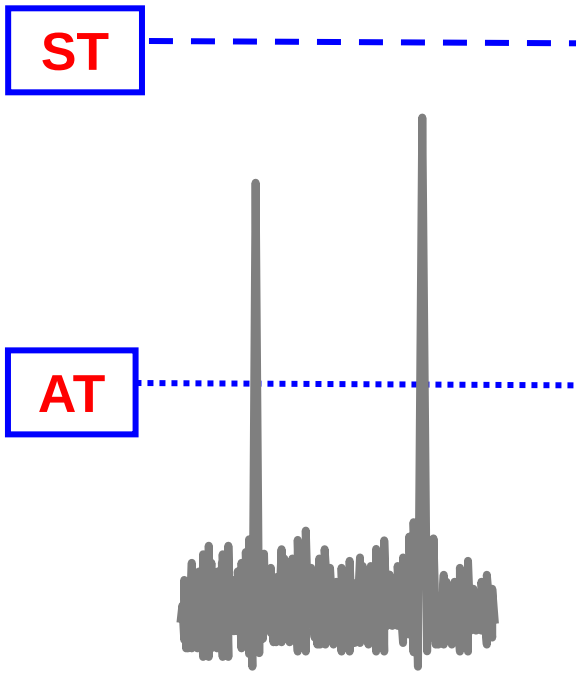
- If alleles are seen below the established stochastic threshold, then the locus is typically eliminated (“INC” – declared inconclusive) in many current lab SOPs

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)?

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.

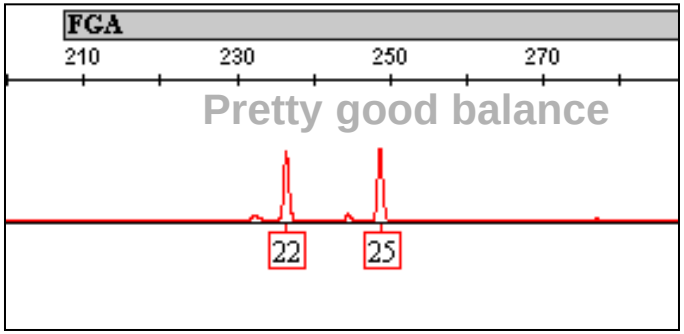
Peak Height Ratio Measurements

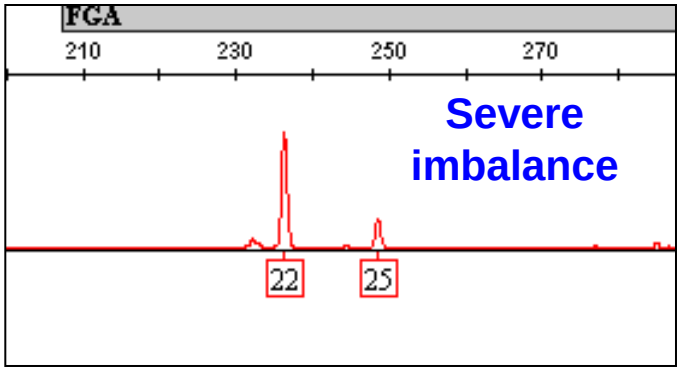
Identifiler STR Kit – only FGA shown

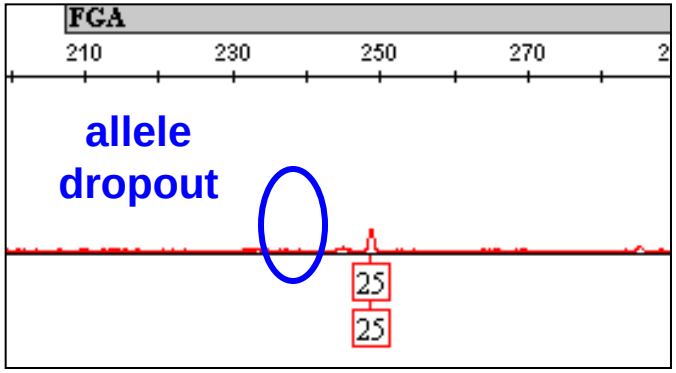
Signal aided with 31 PCR cycles

Peak Heights (RFUs)

		FGA-22	FGA-25	PHR	Average PHR
100 pg	(1)	1692	1517	**0.90**	**0.69** (0.23)
	(2)	1915	864	0.45	
	(3)	1239	909	0.73	
50 pg	(1)	992	260	**0.26**	**0.49** (0.36)
	(2)	1422	419	0.29	
	(3)	895	805	0.90	
10 pg	(1)	--	66	**0**	**0.37** (0.32)
	(2)	54	107	0.50	
	(3)	130	219	0.59	



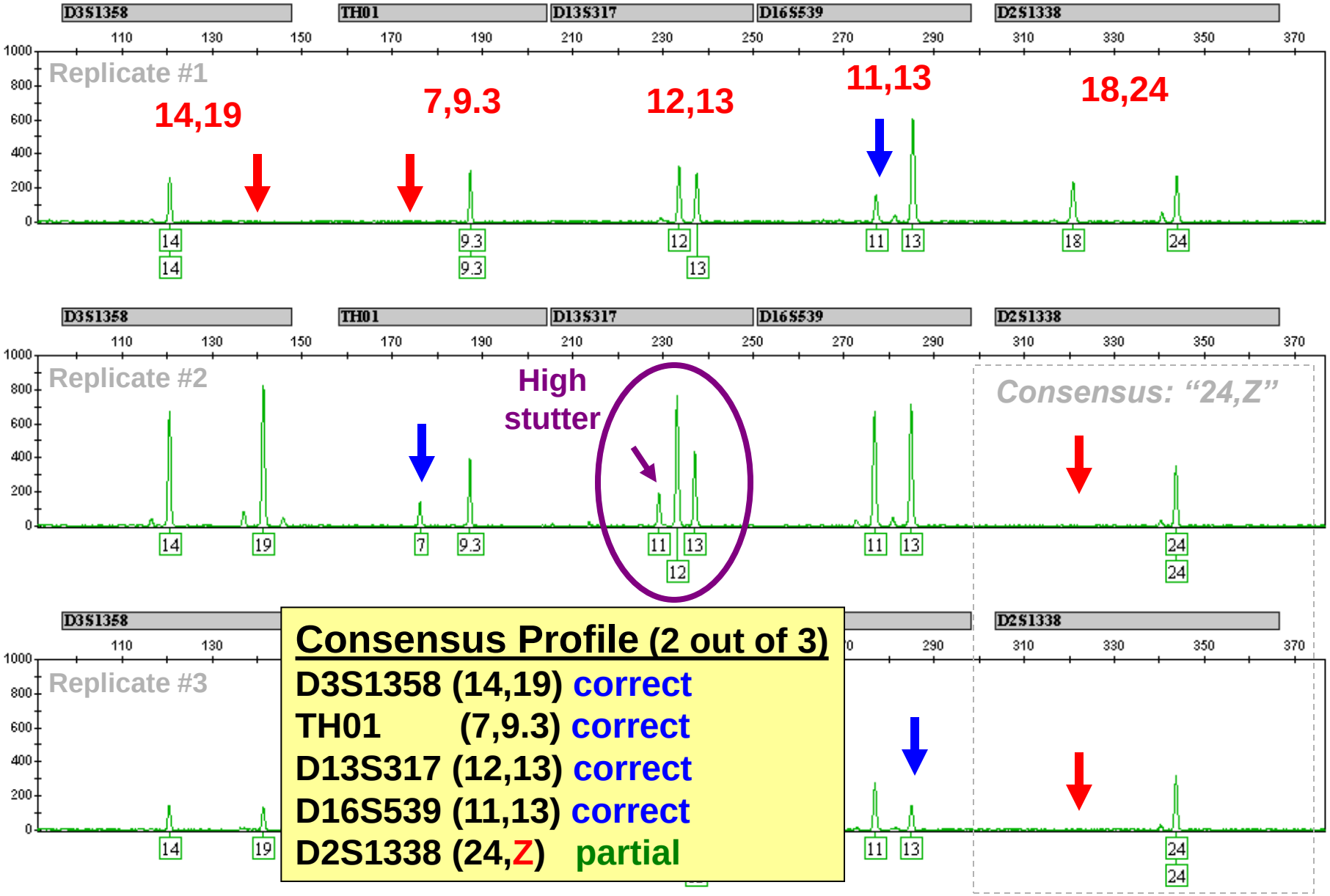




All levels performed in triplicate…

10 pg template DNA with 31 cycles of PCR - triplicates

Identifiler data
(green loci)



Consensus Profile (2 out of 3)

D3S1358 (14,19) correct
TH01 (7,9.3) correct
D13S317 (12,13) correct
D16S539 (11,13) correct
D2S1338 (24,Z) partial

Allele PHR imbalance

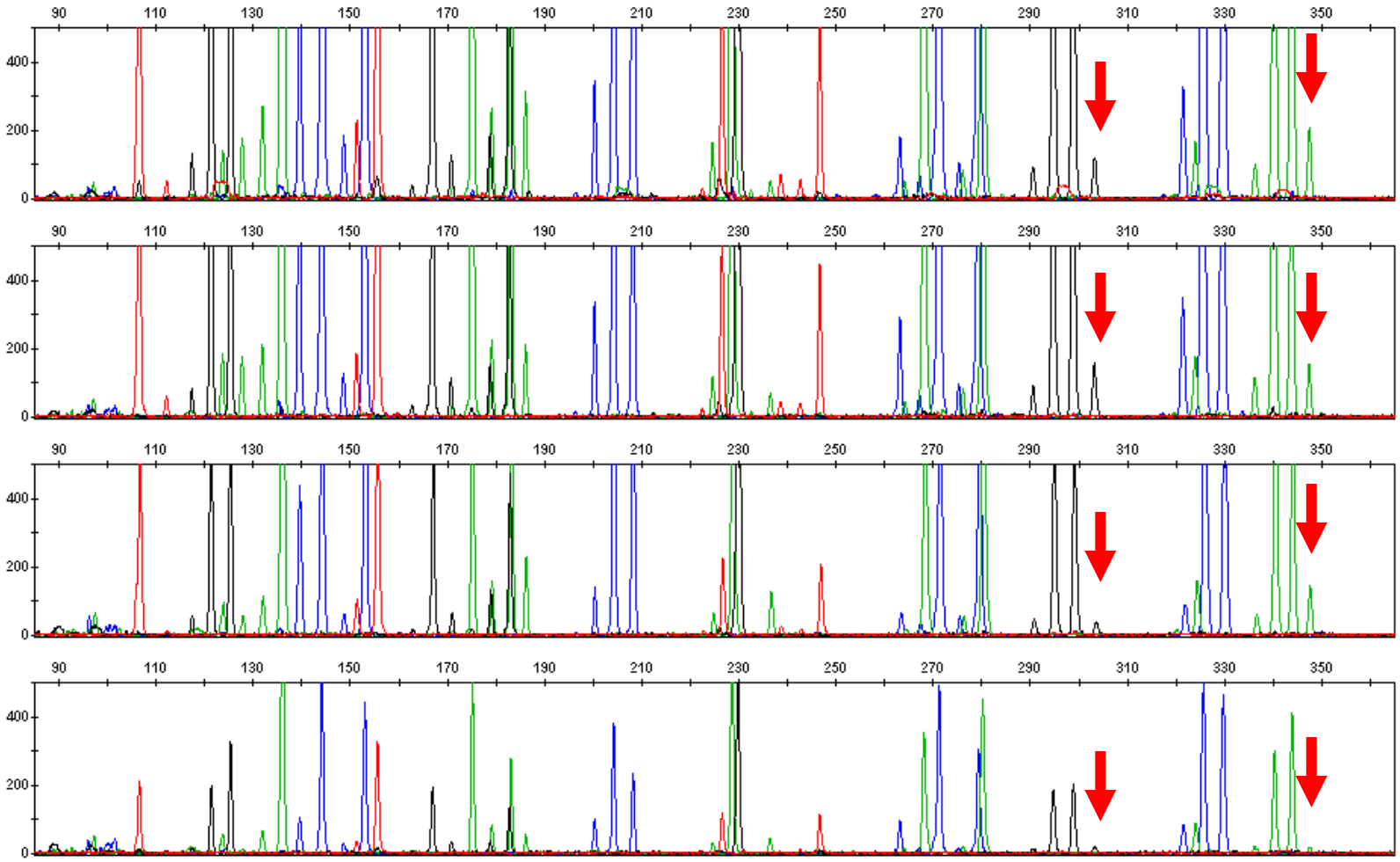
Allele dropout

10:1 Female: Male

Input DNA

Minor component amount

Identifiler Results: NEST I1, I2, I3, I4 (varying input DNA)

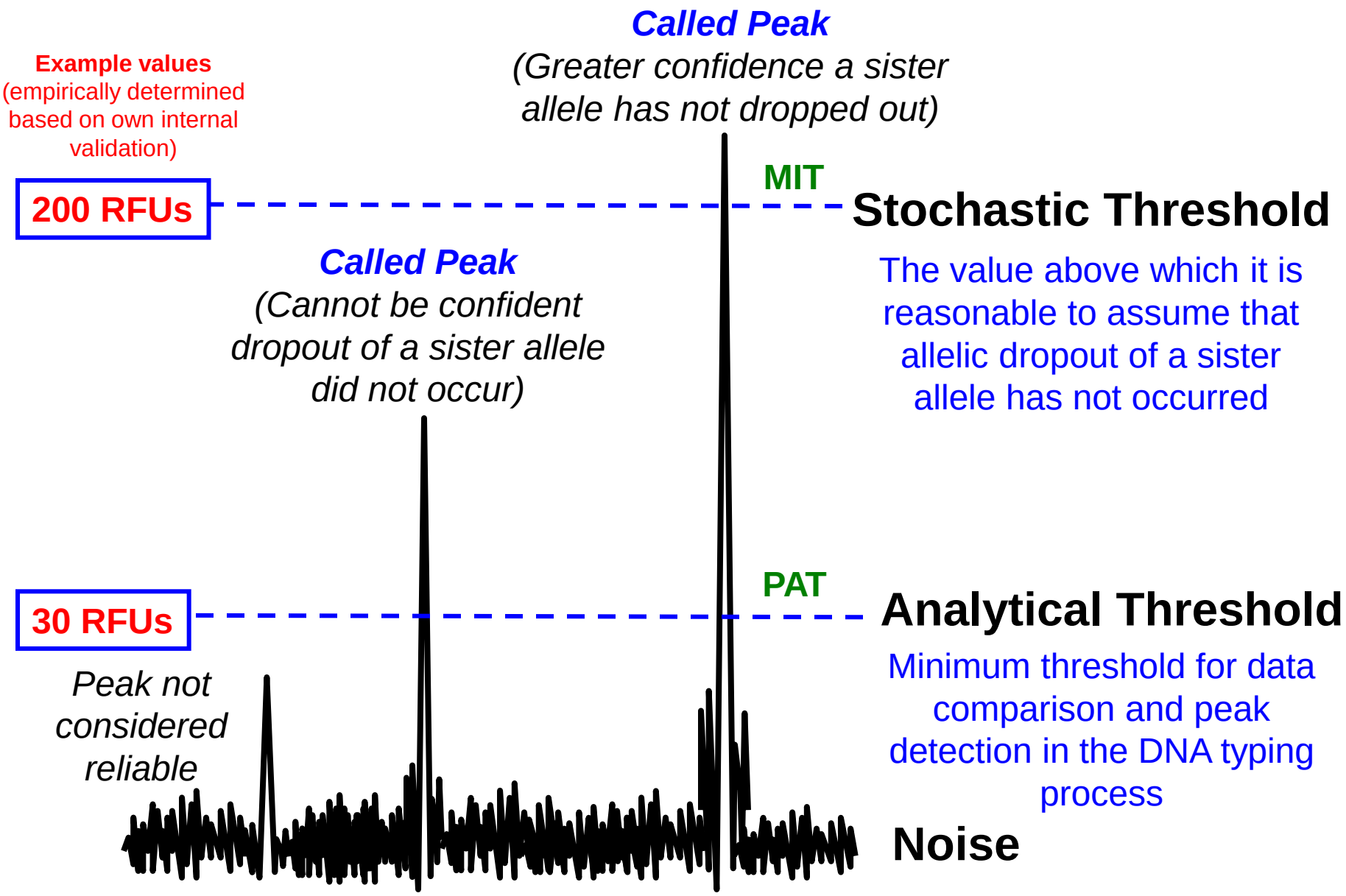


Minor components drop out at low levels due to stochastic effects

Data courtesy of Amy Christen, Marshall University NEST Project Team

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

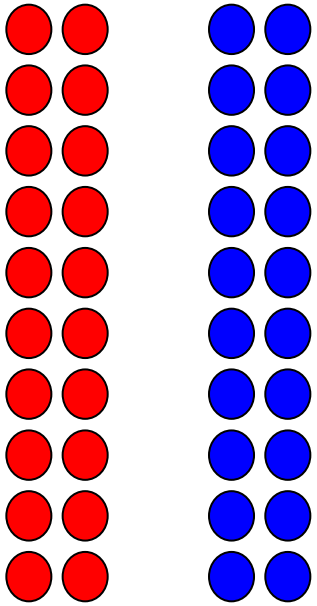
- Stochastic is synonymous with "random." The word is of Greek origin and means "pertaining to chance“. … Stochastic is often used as counterpart of the word "deterministic," which means that random phenomena are not involved. Therefore, stochastic models are based on random trials, while deterministic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

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

High copy number

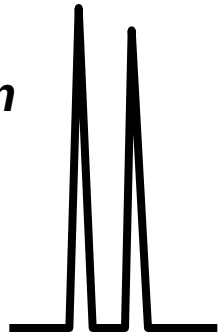
>20 copies per allele

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



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

Resulting electropherogram

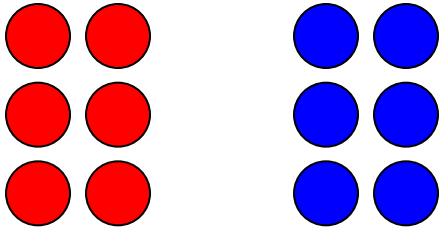


Complete (and correct) genotype

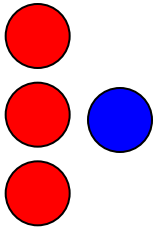
Low copy number

6 copies per allele

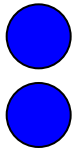
True amount



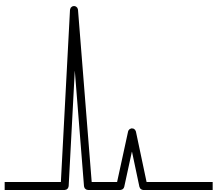
What might be sampled by the PCR reaction…



OR



Extreme allele imbalance



Allele imbalance

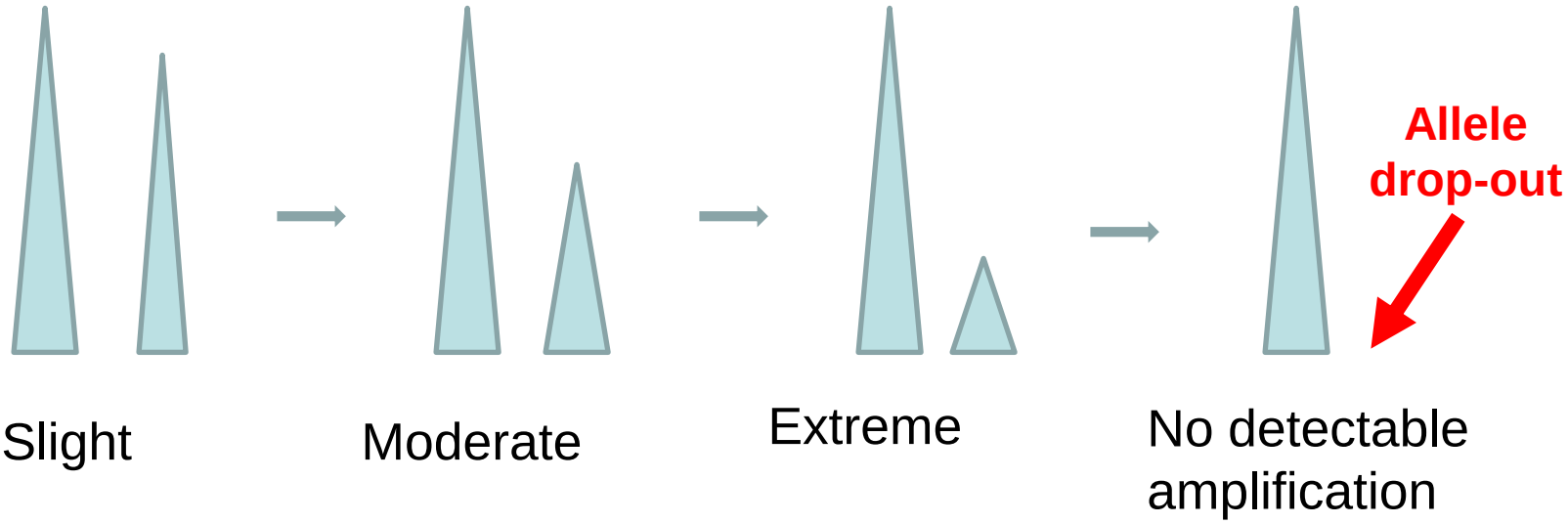
Allele dropout

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:

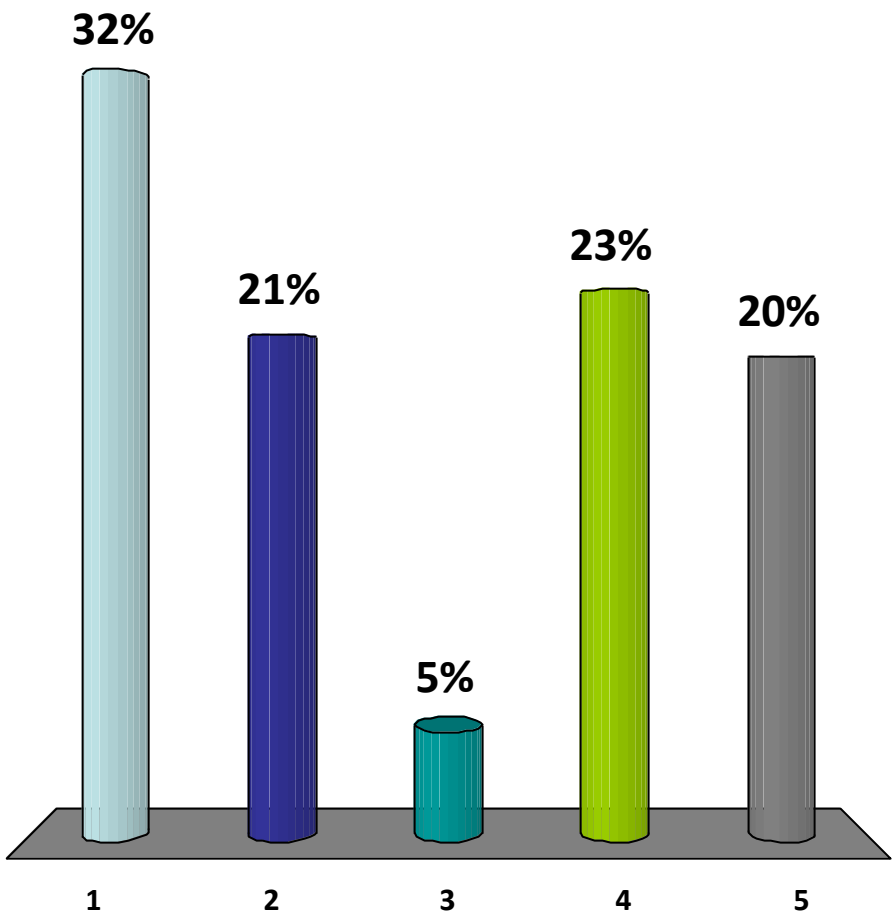
Amount of DNA	~ # of cells from major component	~ # of cells from minor component
1 ng	107	36
0.5 ng	53	18
0.25 ng	27	9
0.125 ng	12	4
0.063 ng	7	2

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)



Determining the Dropout (Stochastic) Threshold

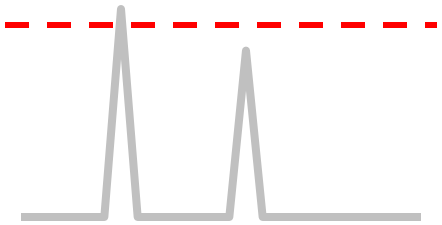
Gill *et al.* (2008) *FSI Genetics* 2(1): 76–82

- 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).

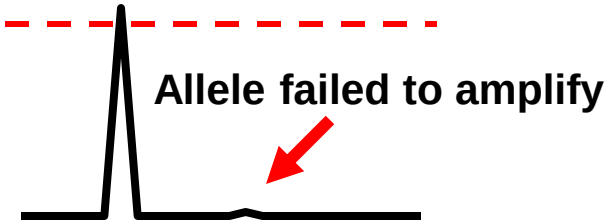
Dropout threshold will change depending on instrument and assay conditions (e.g., longer CE injection will raise dropout threshold)

Stochastic Effects and Thresholds

Regular Injection

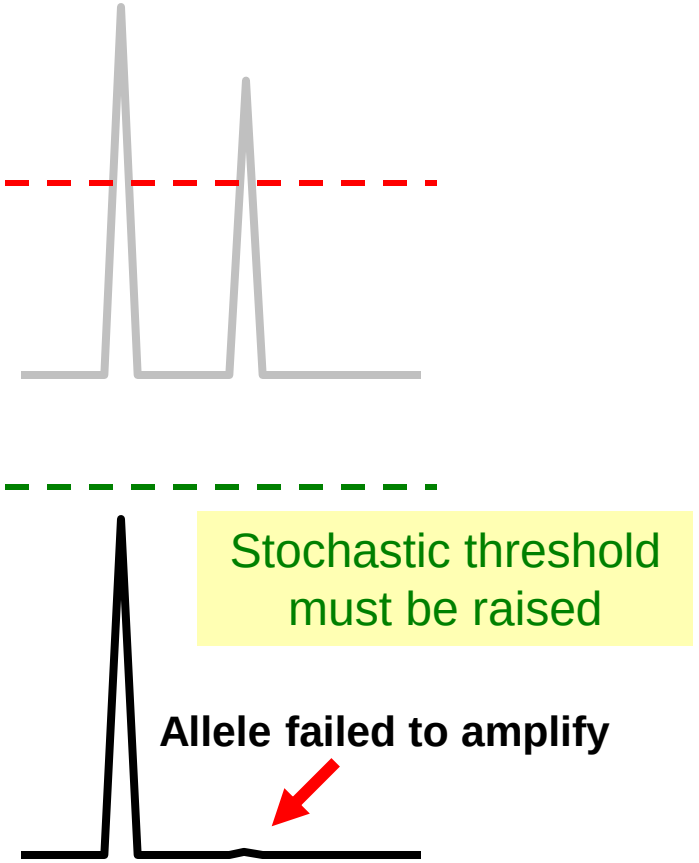


When PCR amplifying low levels of DNA, allele dropout may occur



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

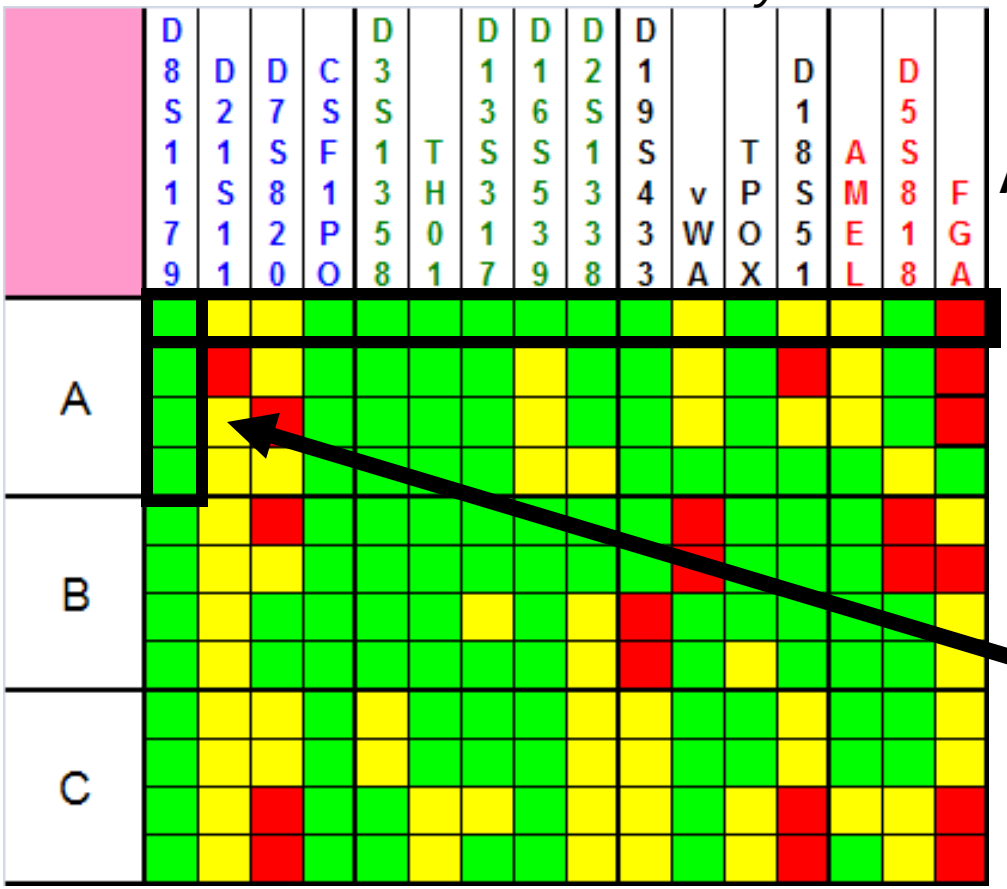
- Examination of sample amounts where dropout is observed (50 pg, 30 pg, 10 pg for Identifiler and Identifiler Plus)
  - Focus on sample amounts with dropout present to examine stochastic effects including severe imbalance of heterozygous alleles and allele dropout

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

Heat Map Explanation

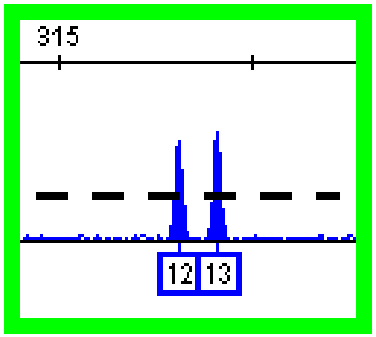
Green = full (correct) type
Yellow = allele dropout
Red = locus dropout

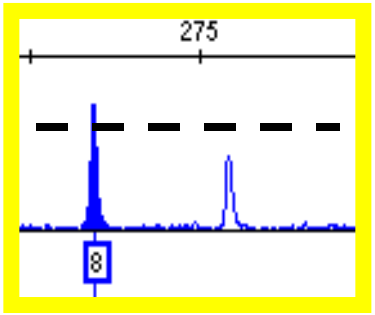
Results broken down by locus

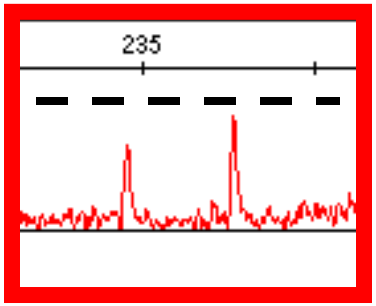


A single profile slice

A replicate slice







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

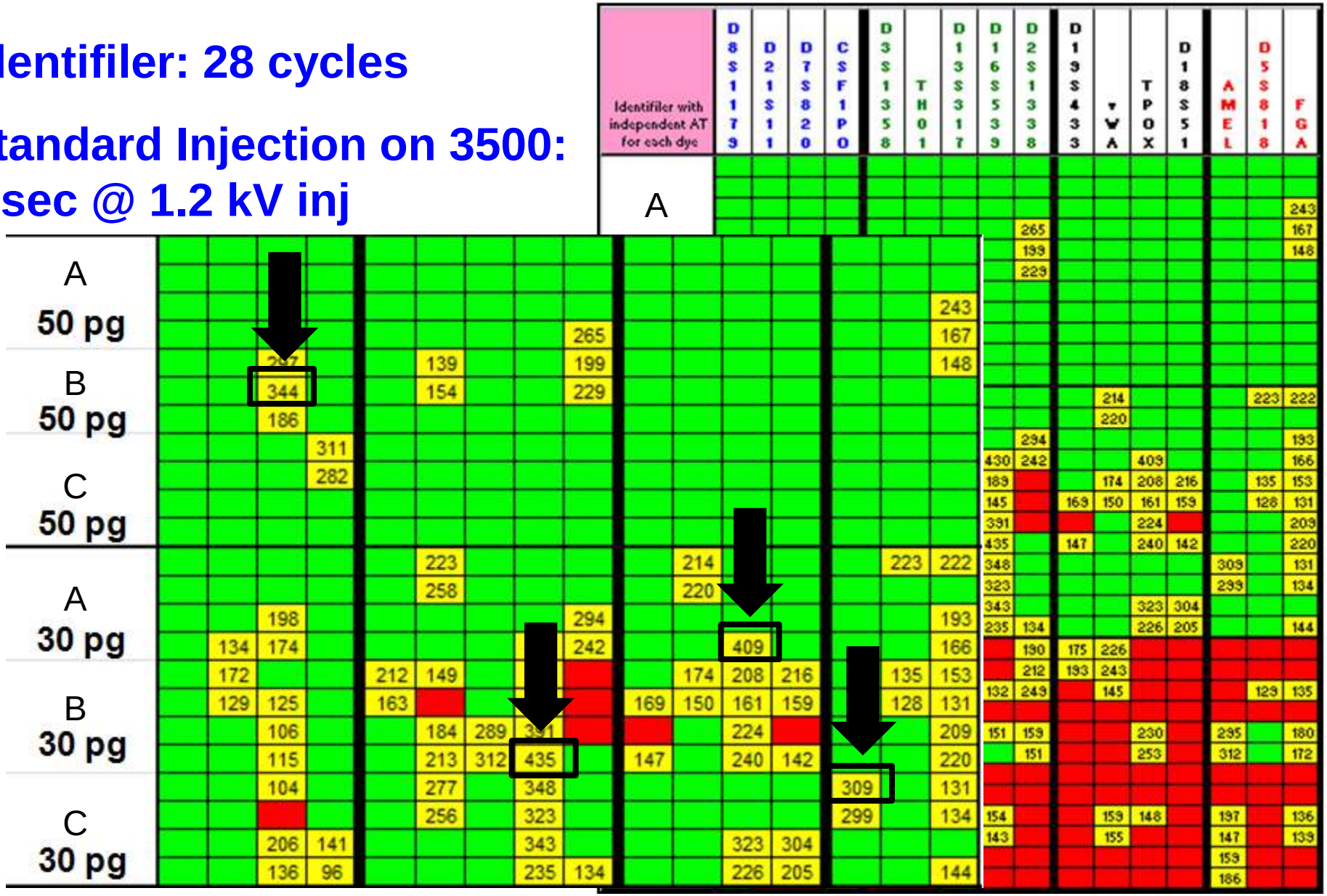
n=84 Samples

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



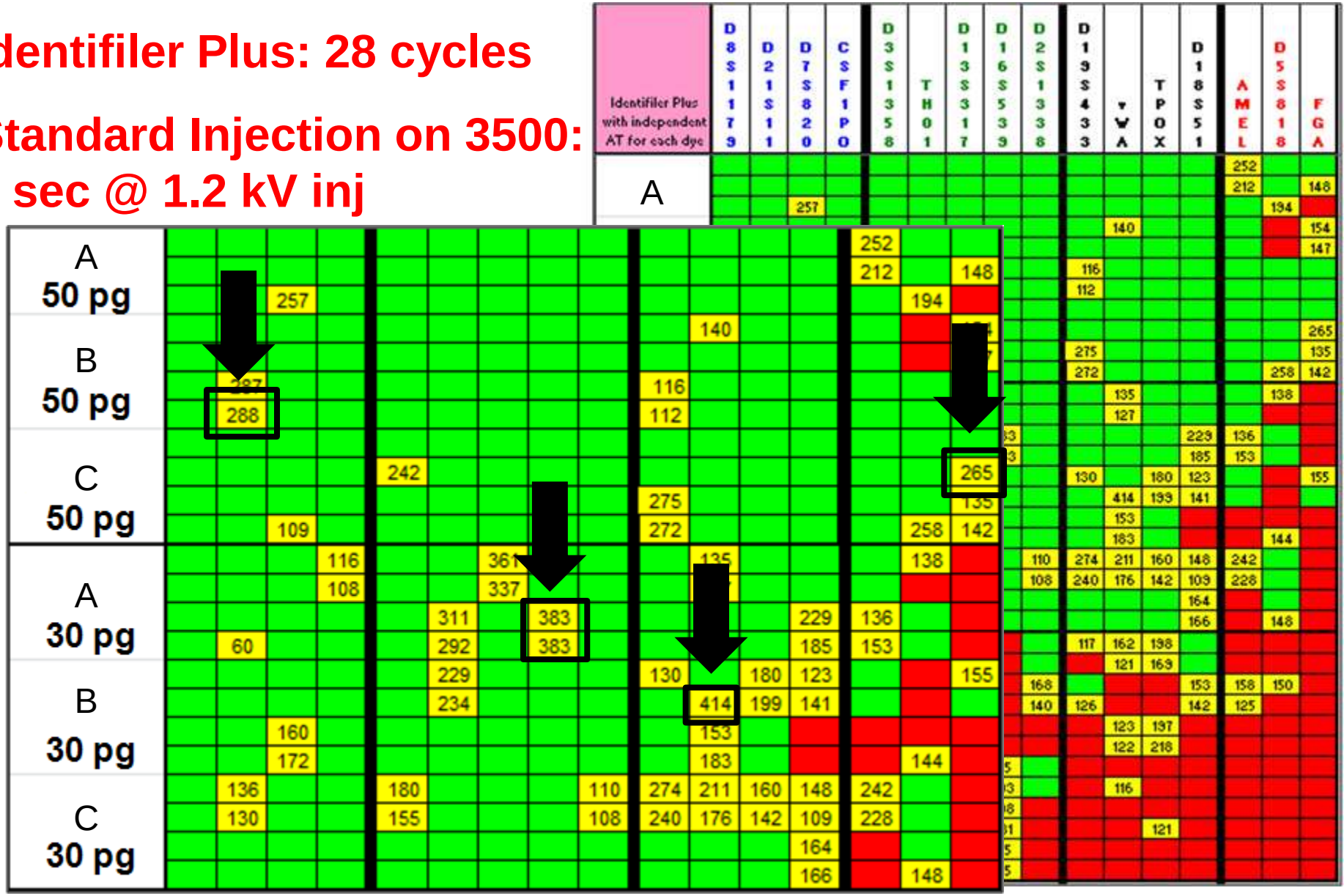
n=84 Samples

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%

Reliable Mixture Interpretation Cannot Usually Be Performed with Low Level DNA

- Intra-locus peak height ratios vary significantly
- Stutter products can be artificially high
- Allele dropout occurs
- Allele drop-in confuses results
  - can only be caught with replicate amplifications and analyses

Common Misunderstandings

- Using CPI stats is conservative to the defendant
  - The numerical stat is low but by throwing out information the ability to EXCLUDE innocent people is reduced
- Using CPI stats means that the potential number of contributors is not important
  - Higher numbers of contributors dilutes out the amount of DNA for each contributor which leads to more stochastic effects and the possibility of allele dropout (more uncertainty)
  - The CPI stat cannot handle allele dropout!

Handling Complex Mixtures

- Stochastic thresholds are necessary in combination with CPI statistics but may not apply for >2 person mixtures (due to potential allele sharing)

- Most labs are not adequately equipped to cope with complex mixtures
  - Extrapolating validation studies from simple mixtures will not be enough to create appropriate interpretation SOPs

David Balding (UK professor of statistical genetics): "LTDNA cases are coming to court **with limited abilities for <u>sound</u> interpretation**." (Rome, April 2012 meeting)

Thoughts on Where We Need to Go

- Away from CPI and towards likelihood ratio approaches
  - As noted in the Gill et al. (2006) ISFG DNA Commission recommendation #2
- This will require software to perform the calculations
  - This software will need to be validated
  - Peter Gill and others in Europe are pushing freeware solutions
- Still will require analysts to understand what is going on in the computer calculations!
  - Will require more significant engagement in mixture training
- The U.S. will be moving to more STR loci in the near future (from 13 to ~20 core STRs)
  - Using loci with better powers of discrimination will be helpful

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

- Some very useful points were made at a recent meeting by internationally renowned experts…

Peter Gill

University of Oslo, Norway

- “There is always an error rate associated with any threshold. You cannot have an error-free system because you are making binary decisions.”

- We need to get away from thinking in a binary way of 0 or 1

David Balding

- "**Low-template DNA cases are coming to court with limited abilities for <u>sound</u> interpretation**"

- "There are dangers with LTDNA but we know how to handle and manage them. Unfortunately, proper management is not a universal practice."

Rome meeting, April 27-28, 2012: The hidden side of DNA profiles: artifacts, errors and uncertain evidence

David Balding

- “In ideal analysis, we would never use thresholds, but in practice they are useful. I don’t think we have sophisticated enough models in many situations to understand all of the details of the data. **Thresholds provide a simplification.** That is reasonable as long as they are backed up by calibration evidence.”

Rome meeting, April 27-28, 2012: The hidden side of DNA profiles: artifacts, errors and uncertain evidence

Peter Gill

University of Oslo, Norway

- “If you are going to have a threshold, at least try to associate it with a level of risk. You can have a threshold any where you like, but the lower the [stochastic] threshold, the greater the risk is of wrongful designation [of genotypes]. The higher the threshold, the more likely you will have an inconclusive result.”

Peter Schneider

- “Stochastic events always imply that you see unexpected results. So if you have unexpected results you cannot have a strict interpretation protocol. What we have is a guideline.”

Rome meeting, April 27-28, 2012: The hidden side of DNA profiles: artifacts, errors and uncertain evidence

Peter Schneider

- “At present, we [in their lab in Cologne, Germany] do not carry out any statistical calculations on any profiles that have stochastic results. Probabilities of drop-out are not yet implemented in our laboratory… [even though] I think this is the way forward. For the time being, we are giving a verbal statement about the quality of the profile – and the possible strengths of the evidence. … In some cases, there are results that are worth reporting and that contain some evidentiary value but it must be clearly stated that the value may be quite limited.”

Rome meeting, April 27-28, 2012: The hidden side of DNA profiles: artifacts, errors and uncertain evidence

Bruce Budowle

- “We as forensic scientists have responsibility to address the errors or problems that might occur from wrongly interpreting evidence… Sometimes simplifying methods, where thresholds come into play, may be more practical now to reduce our chance of false associations… I am still a strong advocate [for thresholds] until we have a system that we can agree upon that is reasonable to be able to proceed that these are good ways to reduce false associations.”

Rome meeting, April 27-28, 2012: The hidden side of DNA profiles: artifacts, errors and uncertain evidence

Peter Gill

University of Oslo, Norway

- “All of the rules on thresholds do break down [at low levels of DNA]. This is not a problem as long as you understand the process…”
- “I think we have to keep the limit-of-detection threshold – typically between 30 and 50 RFUs. The stochastic threshold – typically about 150 RFU – is process-dependent. With the ABI 3500, the stochastic threshold will have to be increased to 300 or possibly 400 RFU to be equivalent.”

Rome meeting, April 27-28, 2012: The hidden side of DNA profiles: artifacts, errors and uncertain evidence

Peter Gill

University of Oslo, Norway

- "The use of the stochastic threshold has to be thought of in terms of risk analysis as well. The 2p rule isn't necessarily conservative. But what the threshold does do is give you an indication when it is unlikely that you will miscall a locus. **It doesn't mean to say that the risk is zero because it is never zero**… You can carry out a risk analysis and set your thresholds at 99.9% or 95%...So **thresholds are okay as long as you are okay with the idea of error**."

Rome meeting, April 27-28, 2012: The hidden side of DNA profiles: artifacts, errors and uncertain evidence

Peter Schneider

- "…As always, life is more complicated. There is no standard answer for these difficult samples because there are so many variables that go into the results – starting with the amount of DNA sample. If we can re-analyze it, it is much easier. If we only give you one or two PCRs, our information basis is much, much more limited…"

Rome meeting, April 27-28, 2012: The hidden side of DNA profiles: artifacts, errors and uncertain evidence

Peter Schneider

- “If you cannot explain your evidence to someone that is not from the field (like a judge) – and you need a lot of technical excuses to report something – then the result is not good. You should leave it on your desk and not take it to court. This is a very common sense approach to this problem.”

Rome meeting, April 27-28, 2012: The hidden side of DNA profiles: artifacts, errors and uncertain evidence

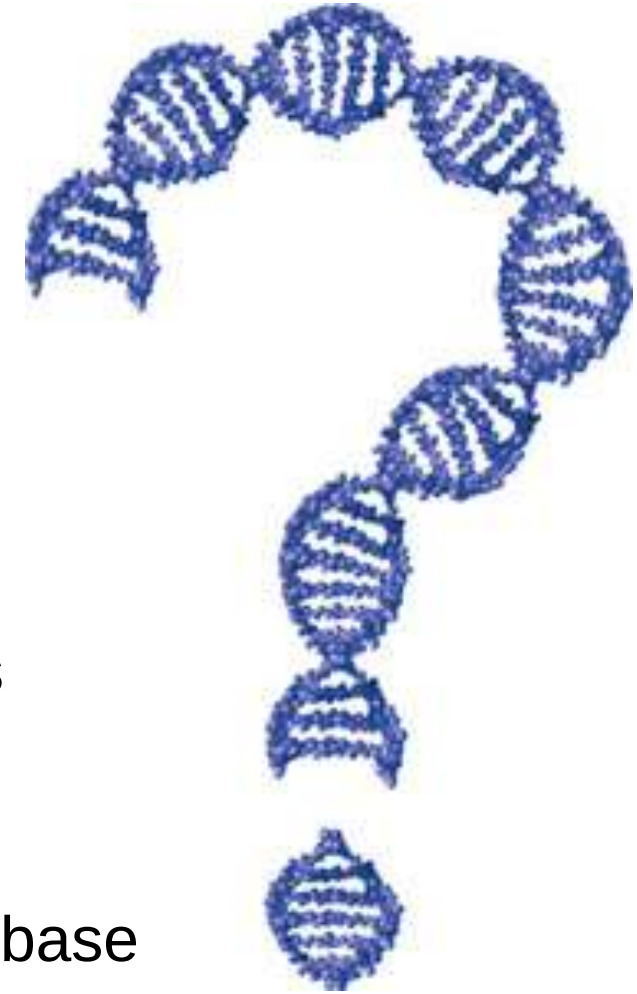
Perhaps We Should Slow Down with Some of the DNA Mixtures That We Are Taking On…

Poor Quality Conditions

Large Numbers of Contributors

Thank you for your attention

Acknowledgments: A great team of scientists within the NIST Applied Genetics Group and funding from the National Institute of Justice and the FBI



Contact Information

John Butler
NIST Fellow
Group Leader of Applied Genetics
john.butler@nist.gov
301-975-4049

http://www.cstl.nist.gov/biotech/strbase

Our team publications and presentations are available at:
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm