**ISHI 2010 Mixture Interpretation Workshop:
Principles, Protocols, and Practice
October 11, 2010 – San Antonio, TX**

Promega
NIJ National Institute of Justice

Case Example #3

John M. Butler

BOSTON UNIVERSITY
NIST

Clayton et al. (1998)
ISFG (2006) Rec. #4

Step #1
Identify the Presence of a Mixture

Step #2
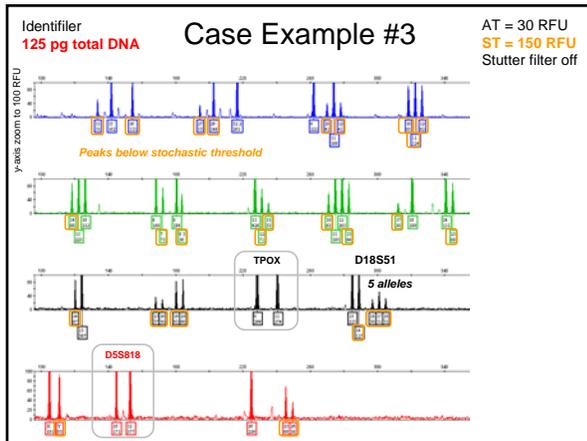Designate Allele Peaks

Step #3
Identify the Number of Potential Contributors

Step #4
Estimate the Relative Ratio of Contributors

Step #5
Consider All Possible Genotype Combinations

Step #6
Compare Reference Samples

## Impact of Results with Low Level DNA

When amplifying low amounts of DNA (e.g., 125 pg), allele dropout is a likely possibility leading to **higher uncertainty** in the potential number of contributors and in the possible genotype combinations

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Identifiler
125 pg total DNA

Case Example #3

AT = 30 RFU
ST = 150 RFU
Stutter filter off

y-axis zoom to 100 RFU

Peaks below stochastic threshold

TPOX   D18S51
5 alleles

D5S818

What Can We Say about this Result?

- Low level DNA (only amplified 125 pg total DNA)
  - likely to exhibit stochastic effects and have allele dropout
- Mixture of at least 3 contributors
  - Based on detection of 5 alleles at D18S51
  - If at equal amounts, ~40 pg of each contributor (if not equal, then less for the minor contributors); **we expect allele dropout**
- At least one of the contributors is male
  - Based on presence of Y allele at amelogenin
- Statistics if using CPI/CPE
  - Would appear that we can only use TPOX and D5S818 results with a stochastic threshold of 150 RFU *(will explore this further)*
- **Due to potential of excessive allele dropout, we are unable to perform any meaningful Q-K comparisons**


Uncertainty in the Potential Number of Contributors with this Result

D18S51

300

13 223   16 30   18 33
14 127   17 51
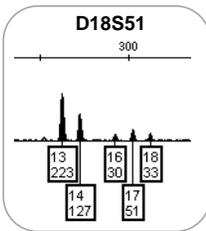
5 alleles observed

- Several of the peaks are barely above the analytical threshold of 30 RFU
  - In fact, with an analytical threshold of 50 RFU or even 35 RFU, there would only be three detected alleles at D18S51
- Stochastic effects could result in a high degree of stutter off of the 17 allele making alleles 16 and 18 potential stutter products
- No other loci have >4 alleles detected

## All Detected Alleles Are Above the Stochastic Threshold – **Or Are They?**
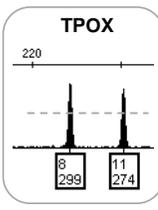
Does this result guarantee no allele drop-out?

TPOX

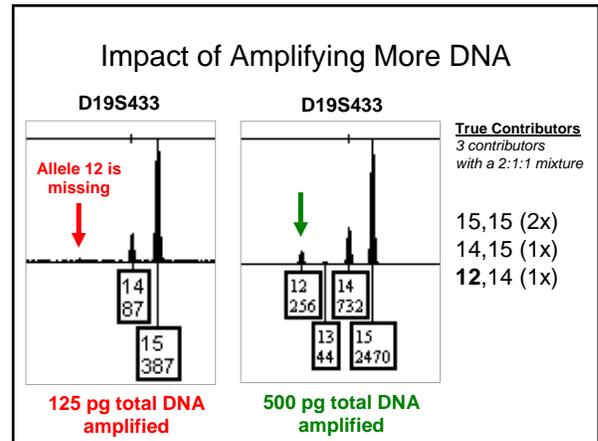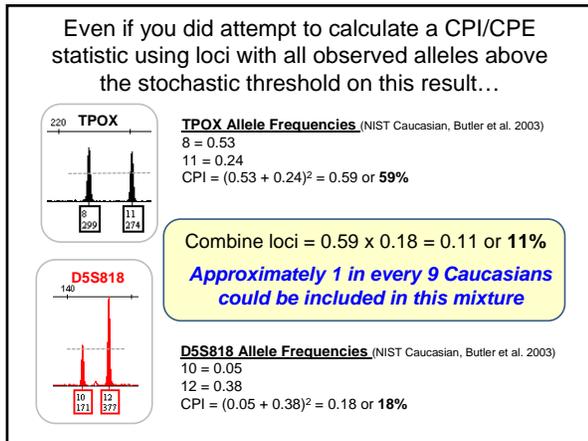220

8 299   11 274

Stochastic threshold = 150 RFU

We have assumed three contributors. If result is from an equal contribution of 3 individuals…

Then some alleles from individual contributors would be below the stochastic threshold and we could not assume that all alleles are being observed!

Assuming Three Contributors…
Some Possible Contributions to This Result

1:1:1   3:1:1

Stochastic alert!
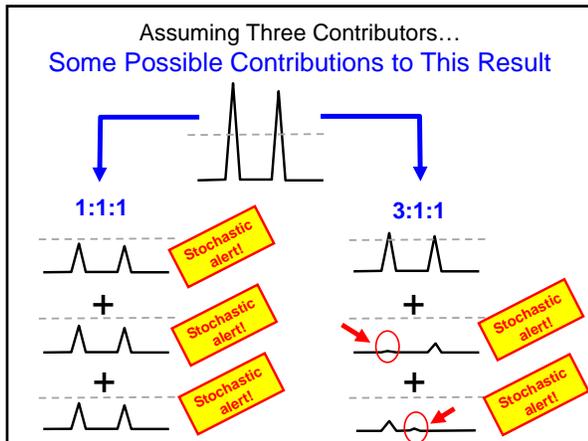
All Loci Are Not Created Equal
when it comes to mixture interpretation

- In the case of less polymorphic loci, such as TPOX, there are fewer alleles and these occur at higher frequency. Thus, there is a greater chance of allele sharing (peak height stacking) in mixtures.

- **Higher locus heterozygosity is advantageous for mixture interpretation** – we would expect to see more alleles (within and between contributors) and thus have a better chance of estimating the true number of contributors to the mixture

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Even if you did attempt to calculate a CPI/CPE statistic using loci with all observed alleles above the stochastic threshold on this result…

TPOX Allele Frequencies (NIST Caucasian, Butler et al. 2003)
8 = 0.53
11 = 0.24
CPI = $(0.53 + 0.24)^2$ = 0.59 or **59%**

Combine loci = 0.59 x 0.18 = 0.11 or **11%**
Approximately 1 in every 9 Caucasians could be included in this mixture

D5S818 Allele Frequencies (NIST Caucasian, Butler et al. 2003)
10 = 0.05
12 = 0.38
CPI = $(0.05 + 0.38)^2$ = 0.18 or **18%**

Impact of Amplifying More DNA

D19S433   D19S433

Allele 12 is missing

125 pg total DNA amplified   **500 pg total DNA amplified**

True Contributors
3 contributors with a 2:1:1 mixture

15,15 (2x)
14,15 (1x)
12,14 (1x)

How should you handle the suspect comparison(s) with this case result?

- **No suspect comparisons should be made** as **the mixture result has too much uncertainty** with stochastic effects that may not account for all alleles being detected

- **Declare the result "inconclusive"**

How not to handle this result

- "To heck with the analytical and stochastic thresholds", **I am just going to see if the suspect profile(s) can fit into the mixture allele pattern observed** – and then if an allele is not present in the evidentiary sample try to explain it with possible allele dropout due to stochastic effects

- This is what Bill Thompson calls "painting the target around the arrow (matching profile)…"

Thompson, W.C. (2009) Painting the target around the matching profile: the Texas sharpshooter fallacy in forensic DNA interpretation. *Law, Probability and Risk* 8: 257-276

Value of Using a Profile Interpretation Worksheet

PROFILE INTERPRETATION WORKSHEET
IDENTIFILER

PROFILE NAME: *Case Example #3*

ANALYST: *John Butler*

DATE: *11 October 2010*

MIXTURE: ■ yes ☐ no ☐ unsure

Analytical threshold: *30 RFU*

Stutter % used: *0% (filter turned-off)*

Stochastic threshold: *150 RFU*

Peak height ratio: *60%*

Comments: *low level DNA (125 pg)*

Allele and Locus Assessments

ID LOCUS	Alleles called	Alleles above Stochastic Threshold	Stutter or other peaks to consider	Possible allele dropout? Y/N	Stochastic issues? (e.g., elevated stutter, PHR imbalance, drop-in, etc.) Y/N	Degradation / Inhibition (obvious)? Y/N	If mixture, restricted genotypes can be used? Y/N	Can this locus be interpreted? Y/N	Additional Comments
D8S1179	11,13,16	13	Maybe	Y	Y	N	N	N	

**Make decisions on the evidentiary sample and document them
prior to looking at the known(s) for comparison purposes**

What to do with low level DNA mixtures?

- **German Stain Commission "Category C"**
  (Schneider et al. 2006, 2009)
  – Cannot perform stats because stochastic effects make it uncertain that all alleles are accounted for

- **ISFG Recommendations #8 & #9** (Gill et al. 2006)
  – Stochastic effects limit usefulness

- *Fundamentals of Forensic DNA Typing* (2010)
  Butler 3rd edition (volume 1), chapter 18
  – Don't go "outside the box" without supporting validation

ISFG Recommendations on Mixture Interpretation

http://www.isfg.org/Publication;Gill2006

1. The likelihood ratio (LR) is the preferred statistical method for mixtures over RMNE

2. Scientists should be trained in and use LRs

3. Methods to calculate LRs of mixtures are cited

4. Follow Clayton et al. (1998) guidelines when deducing component genotypes

5. Prosecution determines H_p and defense determines H_d and multiple propositions may be evaluated

6. When minor alleles are the same size as stutters of major alleles, then they are indistinguishable

7. Allele dropout to explain evidence can only be used with low signal data

8. No statistical interpretation should be performed on alleles below threshold

9. Stochastic effects limit usefulness of heterozygote balance and mixture proportion estimates with low level DNA

Gill *et al.* (2006) DNA Commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Sci. Int.* 160: 90-101

A Complexity/Uncertainty Threshold

New Scientist article (August 2010)
- **How DNA evidence creates victims of chance**
  – 18 August 2010 by Linda Geddes
- From the last paragraph:
  – **In really complex cases, analysts need to be able to draw a line** and say "This is just too complex, I can't make the call on it," says Butler. "Part of the challenge now, is that every lab has that line set at a different place. But the honest thing to do as a scientist is to say: **I'm not going to try to get something that won't be reliable**."

http://www.newscientist.com/article/mg20727743.300-how-dna-evidence-creates-victims-of-chance.html

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Summary

- Do not blindly use a stochastic threshold with complex mixtures as assumptions regarding the number of contributors can impact interpretation

- Going back to try and get a better sample from the evidence (if available) is wiser than spending a lot of time trying to work with a poor quality DNA result

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Future of Complex, Low-level Mixtures

- **If you want to work in this area, you need supporting validation data** (collecting a few results at high DNA levels and extrapolating to greater complexity and smaller amounts of DNA will not be sufficient)

- Recent efforts in Europe are focused on **modeling uncertainty through probabilistic genotype approaches**

- Will require software to perform all of the calculations

- See articles included in the workshop reference list to learn more…