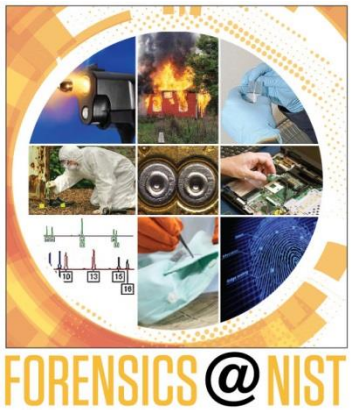


DNA Mixture Interpretation Webcast

April 12, 2013

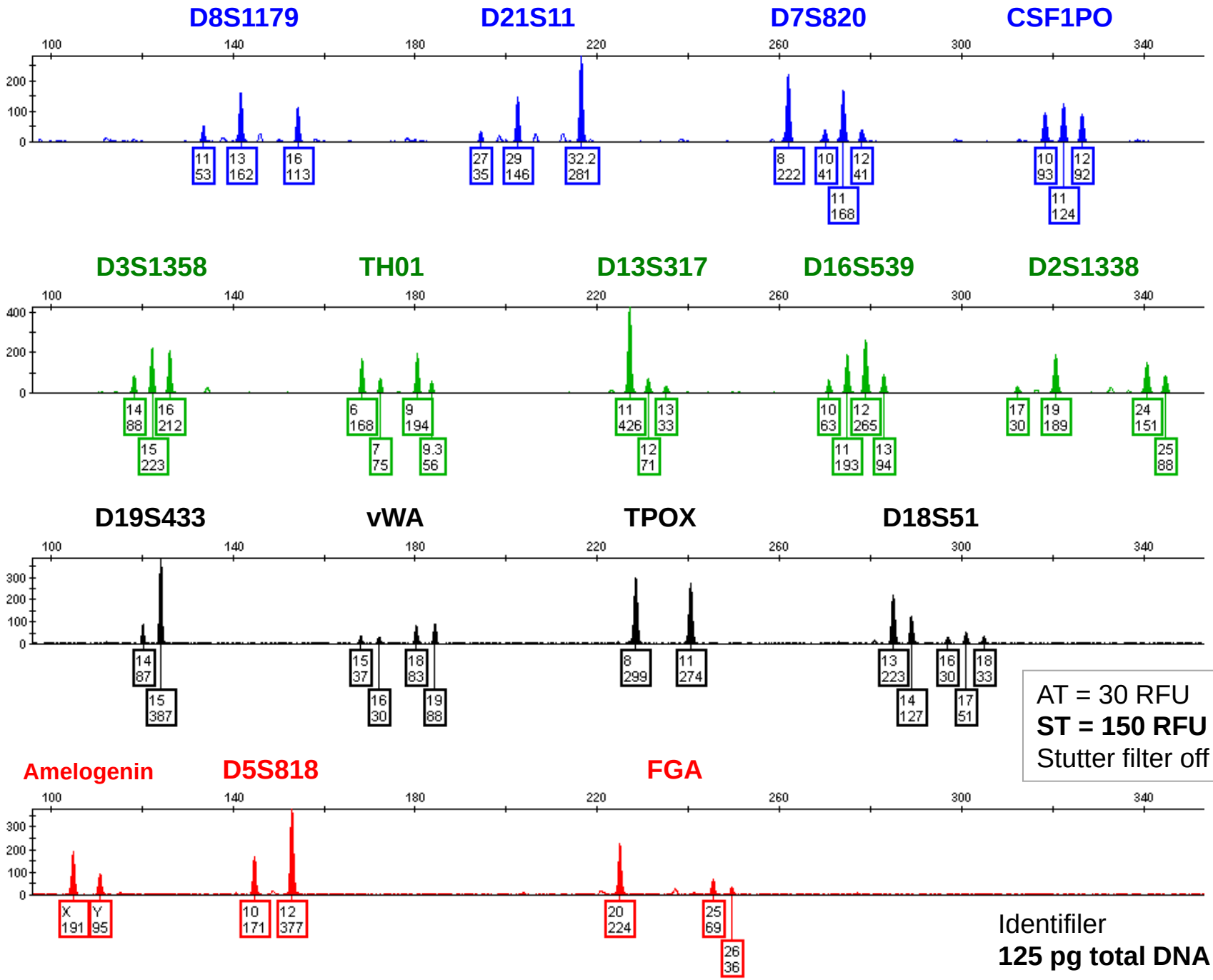
http://www.nist.gov/oles/forensics/dna-analyst-training-on-mixture-interpretation.cfm

http://www.cstl.nist.gov/strbase/mixture.htm

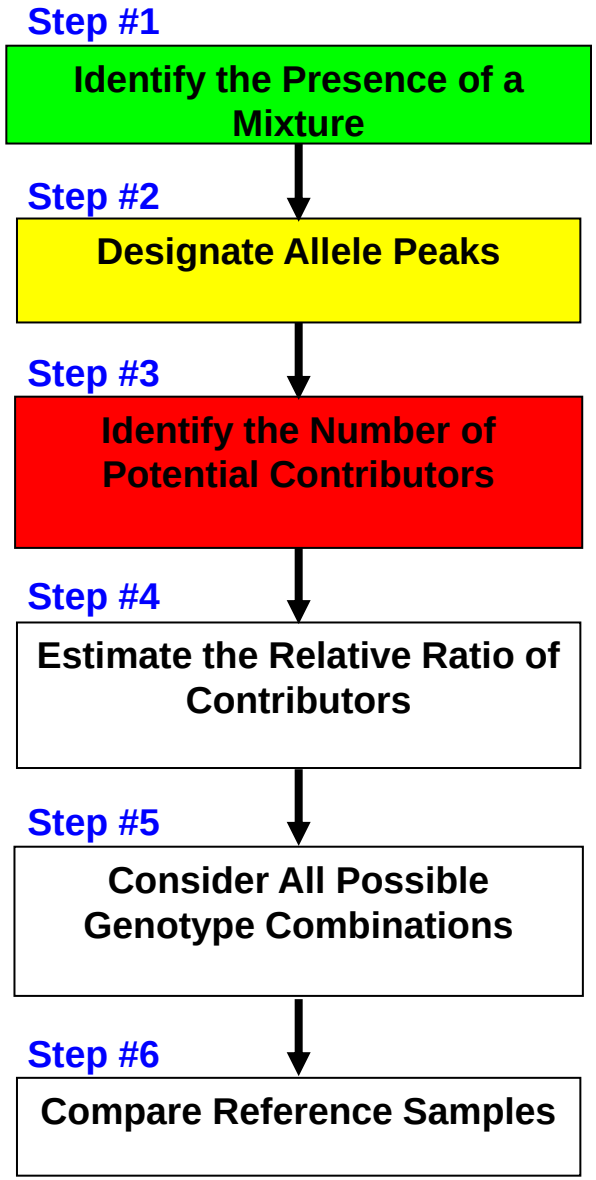
Low Template DNA Challenges and Validation Suggestions

John M. Butler

National Institute of Standards and Technology


D8S1179
D21S11
D7S820
CSF1PO
D3S1358
TH01
D13S317
D16S539
D2S1338
D19S433
vWA
TPOX
D18S51
Amelogenin
D5S818
FGA
Profile #10
AT = 30 RFU
ST = 150 RFU
Stutter filter off
Identifiler
125 pg total DNA

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



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

Identifiler

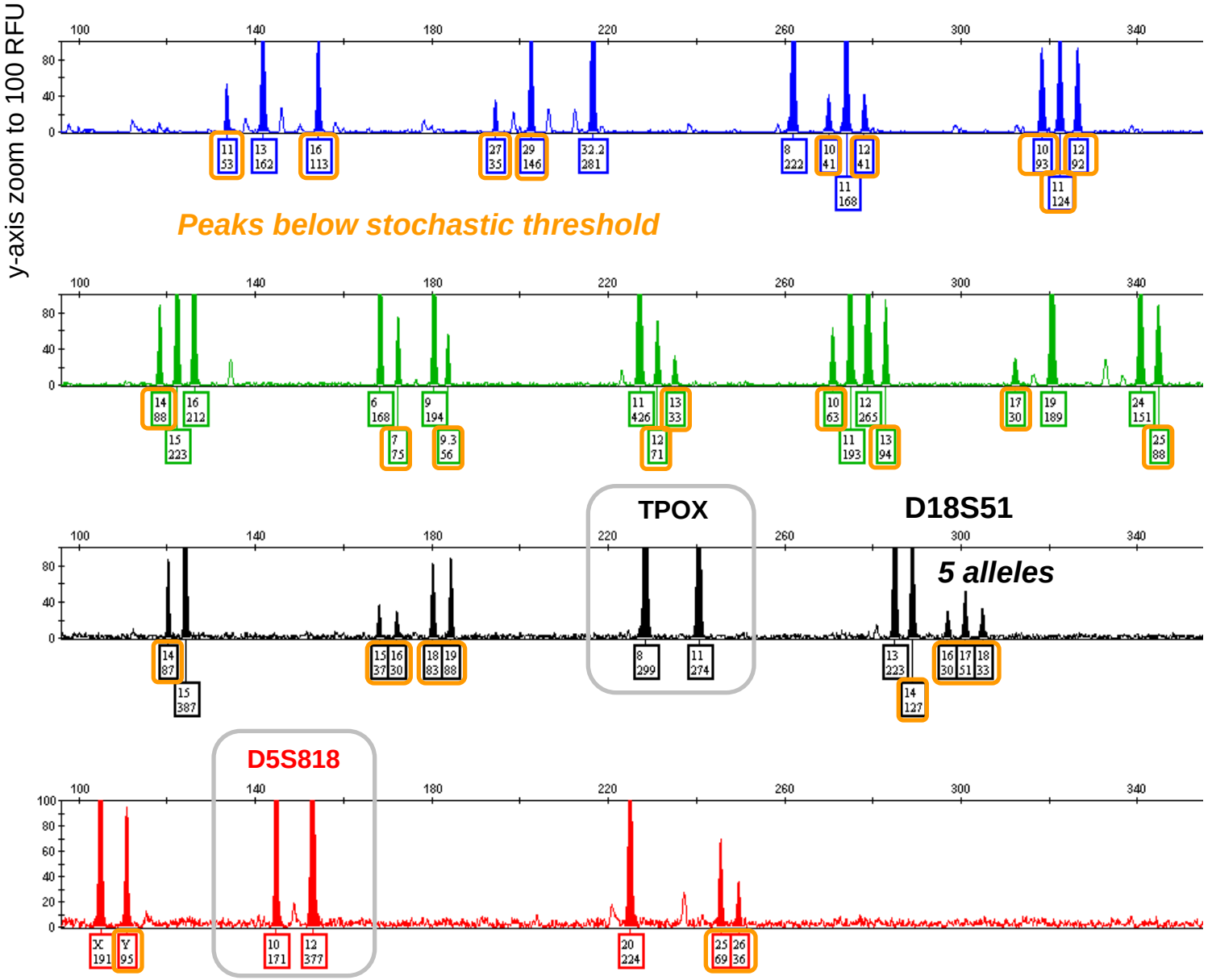
125 pg total DNA

Profile #10

AT = 30 RFU

ST = 150 RFU

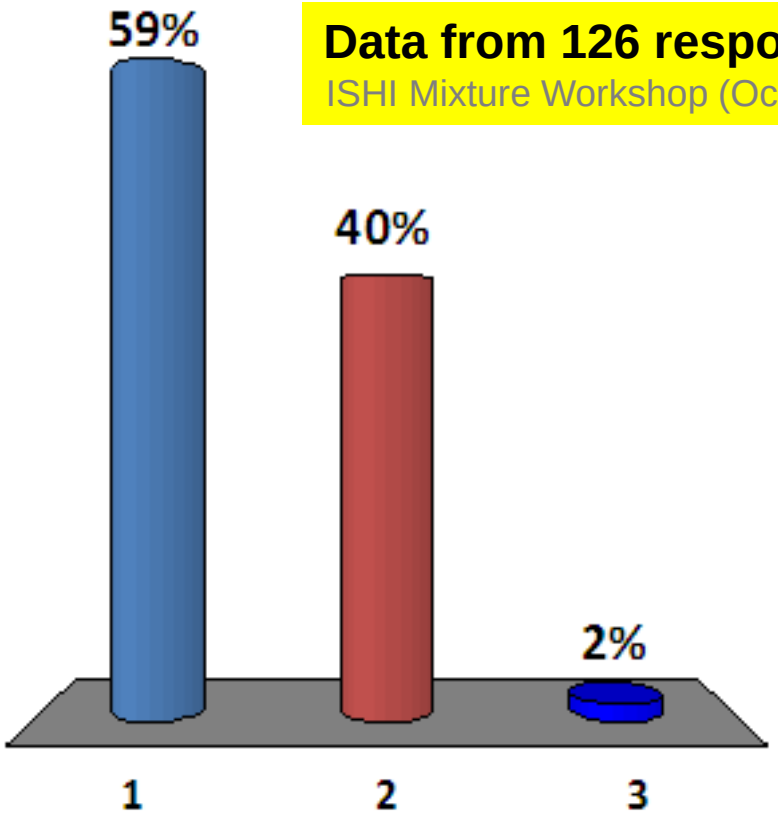
Stutter filter off



Previous Response to This Question

Would you do a CPE/CPI statistic on TPOX and D5S818 because all alleles are above the stochastic threshold?

1. Yes
2. No
3. I don't work in a lab

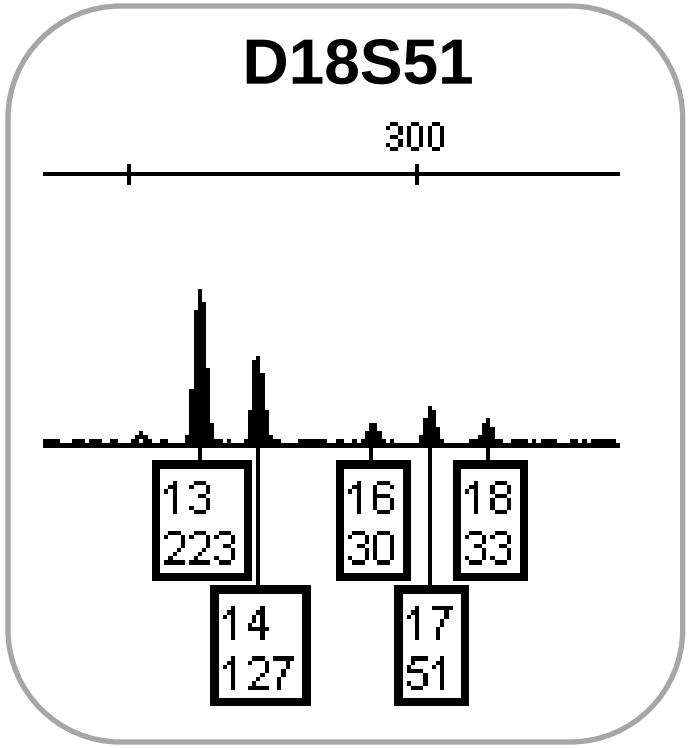


Data from 126 responses
ISHI Mixture Workshop (Oct 2011)

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**

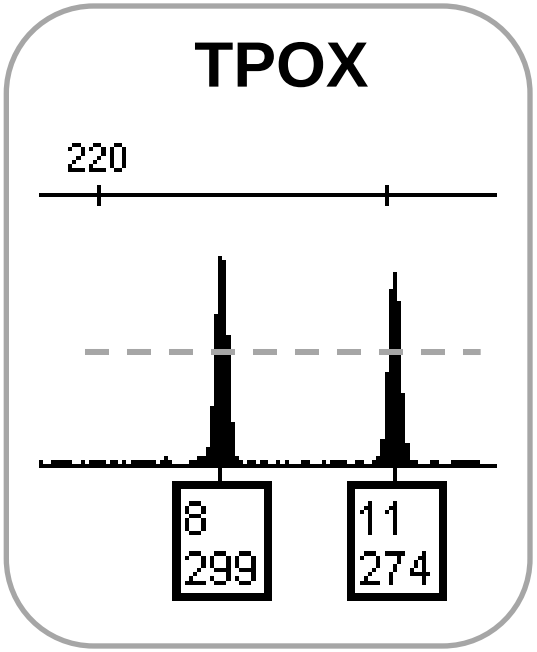
Uncertainty in the Potential Number of Contributors with this Result



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?**



Stochastic threshold = 150 RFU

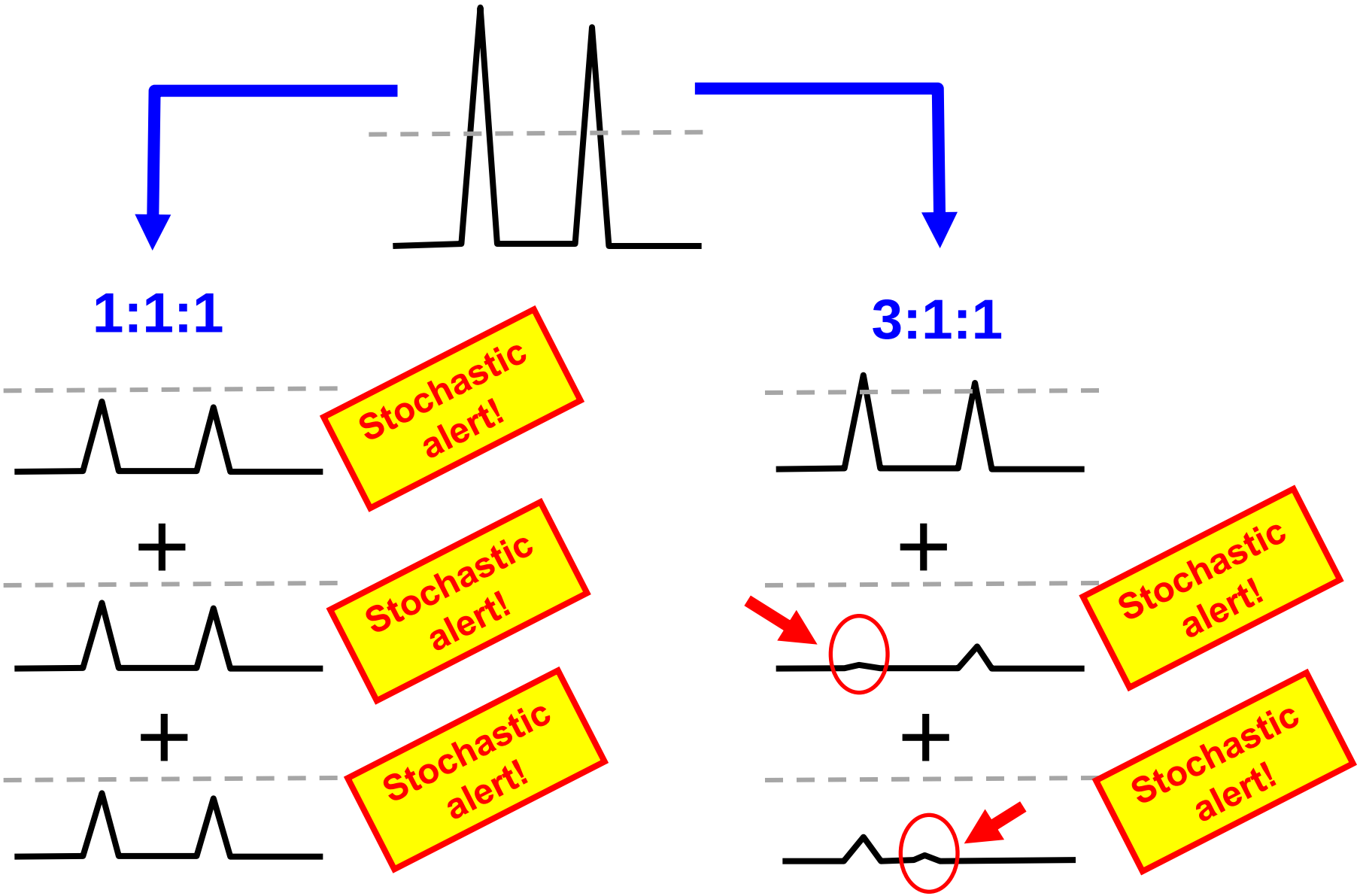
Does this result guarantee no allele drop-out?

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

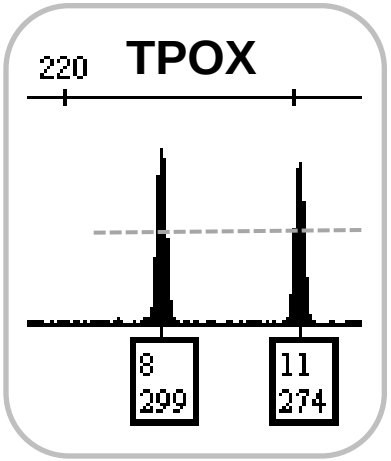


All Loci Are Not Created Equal
when it comes to mixture interpretation

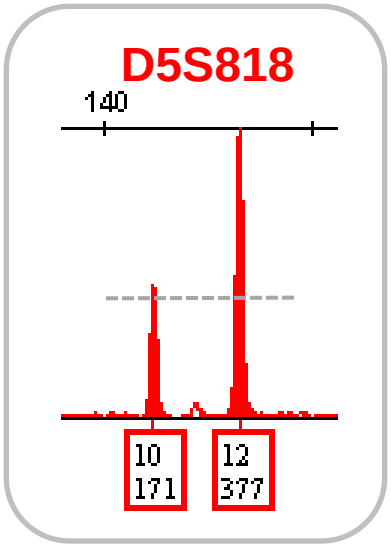
- In the case of less polymorphic loci, such as TPOX, there are fewer alleles and these occur at higher frequency. Thus, there is a greater chance of allele sharing (peak height stacking) in mixtures.

- **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

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%**



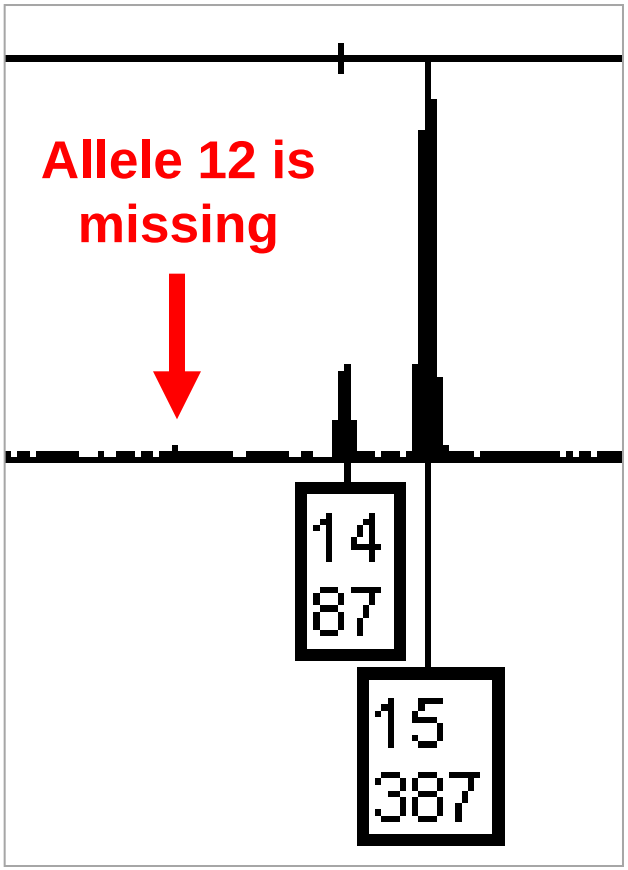
Combine loci = 0.59 x 0.18 = 0.11 or **11%**

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%**

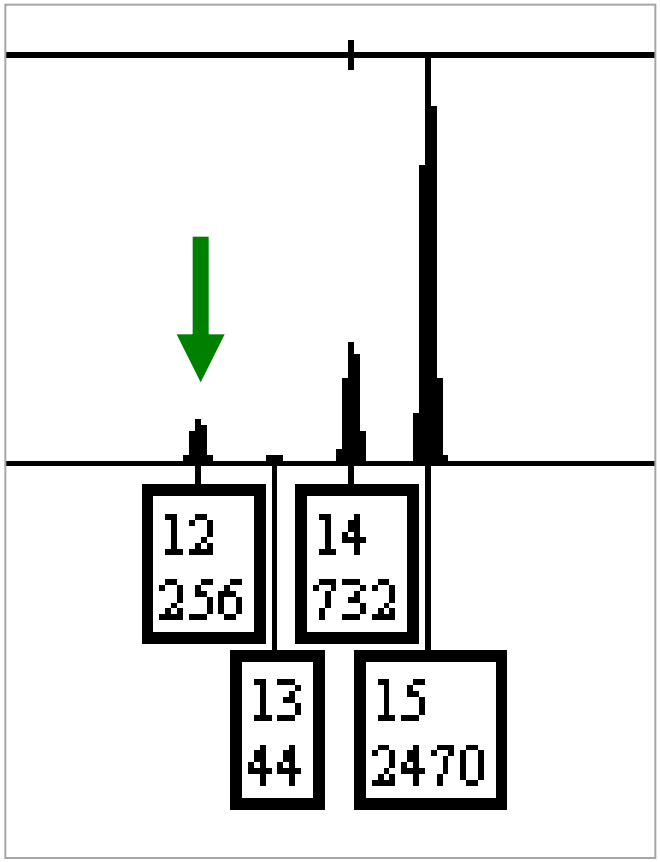
Impact of Amplifying More DNA

D19S433



125 pg total DNA amplified

D19S433



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

- **It would be best to declare the mixture result "inconclusive"**
  - Report wording could include an additional phrase to emphasize that low signal makes this result **inadequate for ANY comparisons to potential reference sample(s)** using currently available techniques

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

Example worksheet available at **http://www.cstl.nist.gov/strbase/mixture.htm**

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

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

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**."

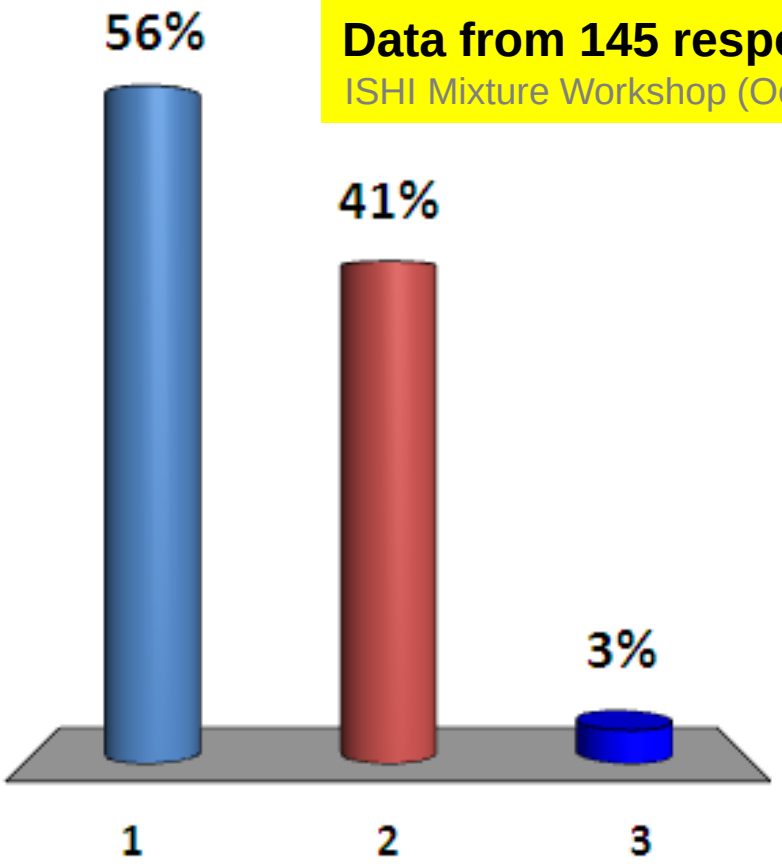
http://www.newscientist.com/article/mg20727743.300-how-dna-evidence-creates-victims-of-chance.html

Results from a Previous Training Workshop

Has your laboratory implemented a "stop testing" approach with complex and/or low-level DNA mixtures?

1. Yes
2. No
3. I don't work in a lab

Data from 145 responses
ISHI Mixture Workshop (Oct 2011)

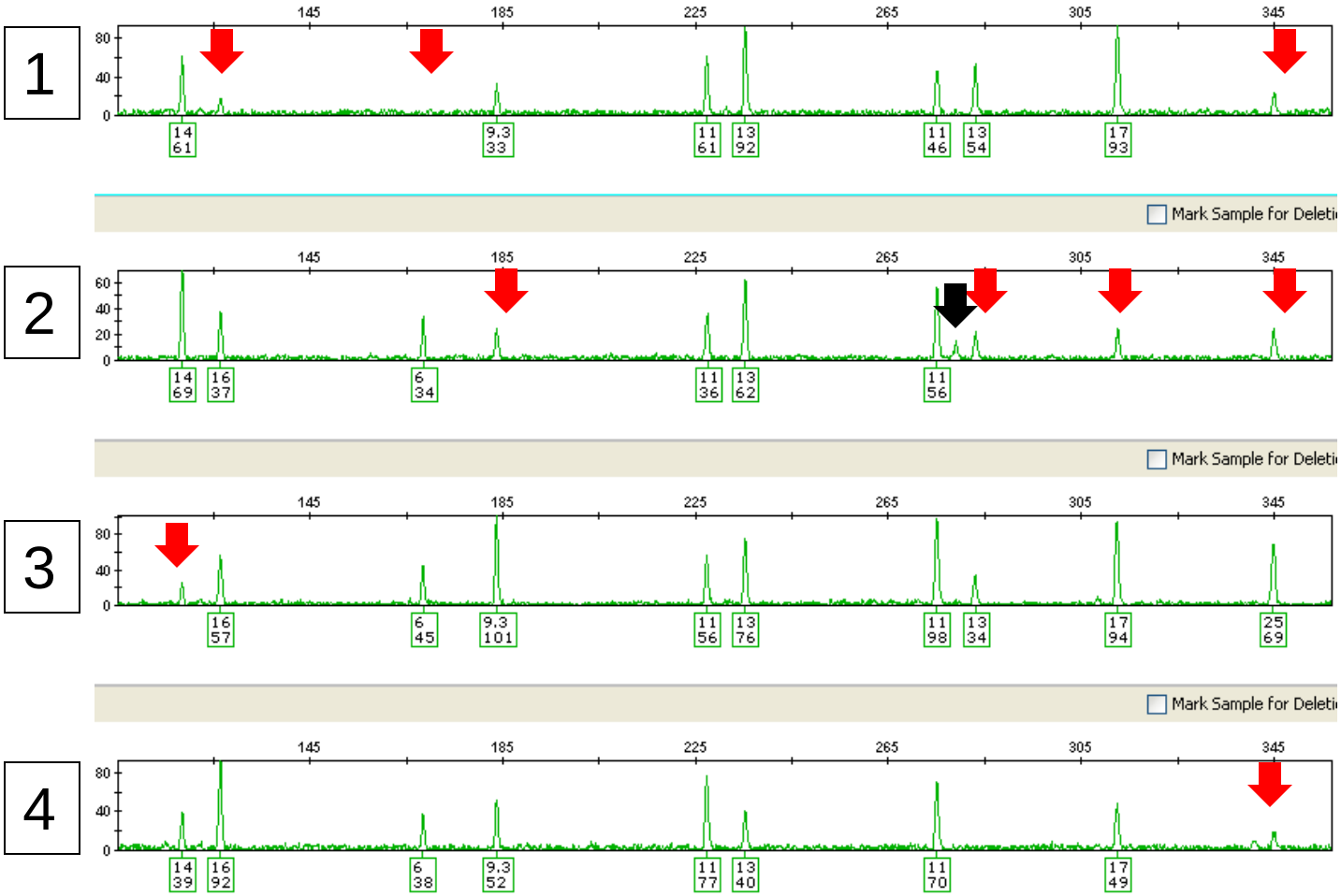


What “Stochastic” Means…

- Variability and allele dropout can occur anywhere in a DNA profile with low template DNA amounts…
- Peak height variability means that expected peak height ratios for paired alleles in heterozygotes quickly breaks down making mixture interpretation more challenging
- Confidence can be increased through replicate testing – but this requires splitting an already limited sample into smaller amounts

Stochastic Variation Observed

Same DNA – Amplified in Quadruplicate



Red arrows indicate allele drop-out (signal below analytical threshold)

Some observations

- in replicate #1 (top panel), lower size alleles drop-out (red arrows) more than larger size alleles
- variation exists between replicates: #3 and #4 had only a single missing allele while #2 is missing four alleles
- stutter peak (black arrow) in replicate #2 is almost as high as the second allele

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

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 are focused on **modeling uncertainty through probabilistic genotype approaches**

- Will require software to perform all of the calculations

- See articles included in STRBase mixture section literature listing: http://www.cstl.nist.gov/strbase/mixture.htm

December 2012 Issue of *FSI Genetics*

Contents lists available at SciVerse ScienceDirect

Forensic Science International: Genetics

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

Editorial

Focus issue—Analysis and biostatistical interpretation of complex and low template DNA samples

Contents lists available at SciVerse ScienceDirect

Forensic Science International: Genetics

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

DNA commission of the International Society of Forensic Genetics: Recommendations on the evaluation of STR typing results that may include drop-out and/or drop-in using probabilistic methods

P. Gill[a,b,*], L. Gusmão[c], H. Haned[d], W.R. Mayr[e], N. Morling[f], W. Parson[g], L. Prieto[h], M. Prinz[i], H. Schneider[j], P.M. Schneider[k], B.S. Weir[l]

Some of the articles present in this issue…

Contents lists available at SciVerse ScienceDirect

Forensic Science International: Genetics

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

Exploratory data analysis for the interpretation of low template DNA mixtures

H. Haned [a,*], K. Slooten [a,b], P. Gill [c,d]

[a] Netherlands Forensic Institute, Department of Human Biological traces, The Hague, The Netherlands
[b] VU University Amsterdam, Amsterdam, The Netherlands
[c] Norwegian institute of Public Health, Oslo, Norway
[d] University of Oslo, Norway

Contents lists available at SciVerse ScienceDirect

Forensic Science International: Genetics

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

Validation of a DNA mixture statistics tool incorporating allelic drop-out and drop-in

Adele A. Mitchell *, Jeannie Tamariz, Kathleen O'Connell, Nubia Ducasse, Zoran Budimlija, Mechthild Prinz, Theresa Caragine

Department of Forensic Biology, Office of Chief Medical Examiner of The City of New York, 421 E 26th Street, New York, NY 10016, United States

A Statistical Modeling Approach

Kelly, H., et al. (2012). The interpretation of low level DNA mixtures. *Forensic Science International: Genetics, 6(2),* 191-197

Contents lists available at ScienceDirect

Forensic Science International: Genetics

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

The interpretation of low level DNA mixtures

Hannah Kelly [a,*], Jo-Anne Bright [a], James Curran [b], John Buckleton [a]

[a] ESR, PB 92021 Auckland, New Zealand
[b] Department of Statistics, University of Auckland, PB 92019 Auckland, New Zealand

Development of statistical models that account for the possibility of allele drop-out

A Simulation Approach

Forensic Science International: Genetics 5 (2011) 525–531

Contents lists available at ScienceDirect

Forensic Science International: Genetics

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

Estimating drop-out probabilities in forensic DNA samples: A simulation approach to evaluate different models

H. Haned [a,*], T. Egeland [b], D. Pontier [a], L. Pène [c], P. Gill [b,d]

[a] *Université de Lyon, Université Lyon 1, CNRS, UMR 5558, Laboratoire de Biométrie et Biologie Evolutive, 69622 Villeurbanne, France*
[b] *Institute of Forensic Medicine, University of Oslo, 0027 Oslo, Norway*
[c] *Institut National de Police Scientifique, Laboratoire de Police Scientifique de Lyon, France*
[d] *University of Strathclyde, Royal College, 204 George Street, Glasgow G11XW, UK*

A Logistic Regression Model

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

Statistical model for degraded DNA samples and adjusted probabilities for allelic drop-out

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, Frederik V's Vej 11, DK-2100 Copenhagen East, Denmark

Allelic drop-out probabilities estimated by logistic regression—Further considerations and practical implementation

Torben Tvedebrink [a,*], Poul Svante Eriksen [a,1], Maria Asplund [b,2], Helle Smidt Mogensen [b,3], Niels Morling [b,4]

[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, Frederik V's Vej 11, DK-2100 Copenhagen East, Denmark

A Logistic Regression Model

G Model
FSIGEN-761; No. of Pages 5
ARTICLE IN PRESS
Forensic Science International: Genetics xxx (2011) xxx–xxx
Contents lists available at ScienceDirect
Forensic Science International: Genetics
journal homepage: www.elsevier.com/locate/fsig

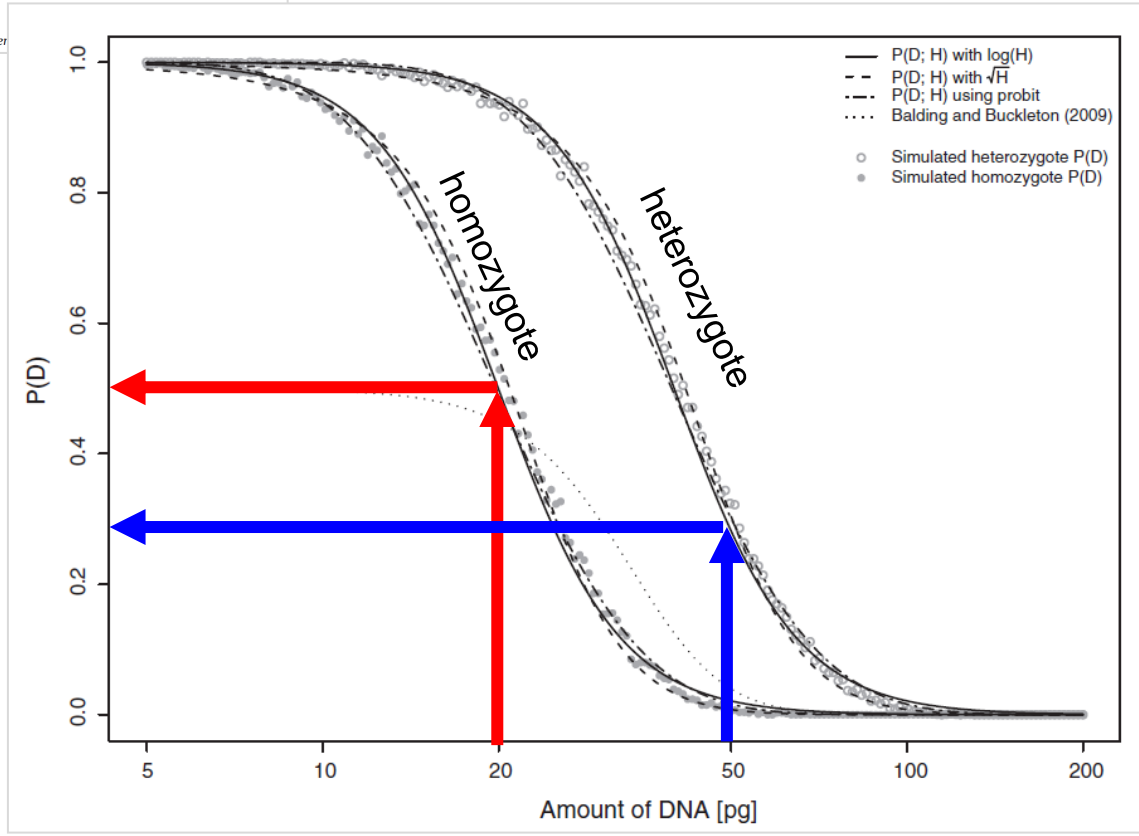
Allelic drop-out probabilities estimated by logistic regression—Further considerations and practical implementation

Torben Tvedebrink [a,*], Poul Svante Eriksen [a,1], Maria Asplund [b,2], Helle Smidt Mogensen [b,3], Niels Morling [b,4]

[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, Freder

At **20 pg**, approximately **50% of homozygote** alleles will have dropped out

At **50 pg**, approximately **30% of heterozygote** alleles will have dropped out



Validation Analogy

- Validation studies can be compared to efforts involved in learning to drive a car properly
- My 16-year old daughter recently obtained her driving permit and is learning how to drive
- Age thresholds must be passed before someone can be considered for a driving permit and license
- The ultimate success of obtaining a driver's license and staying accident-free is based on training and preparation

Acquiring a Maryland Driver's License

- A knowledge test must first be passed to be eligible

  Allele peaks must first be observed to be interpreted…

- Three Stages for Rookie Drivers:

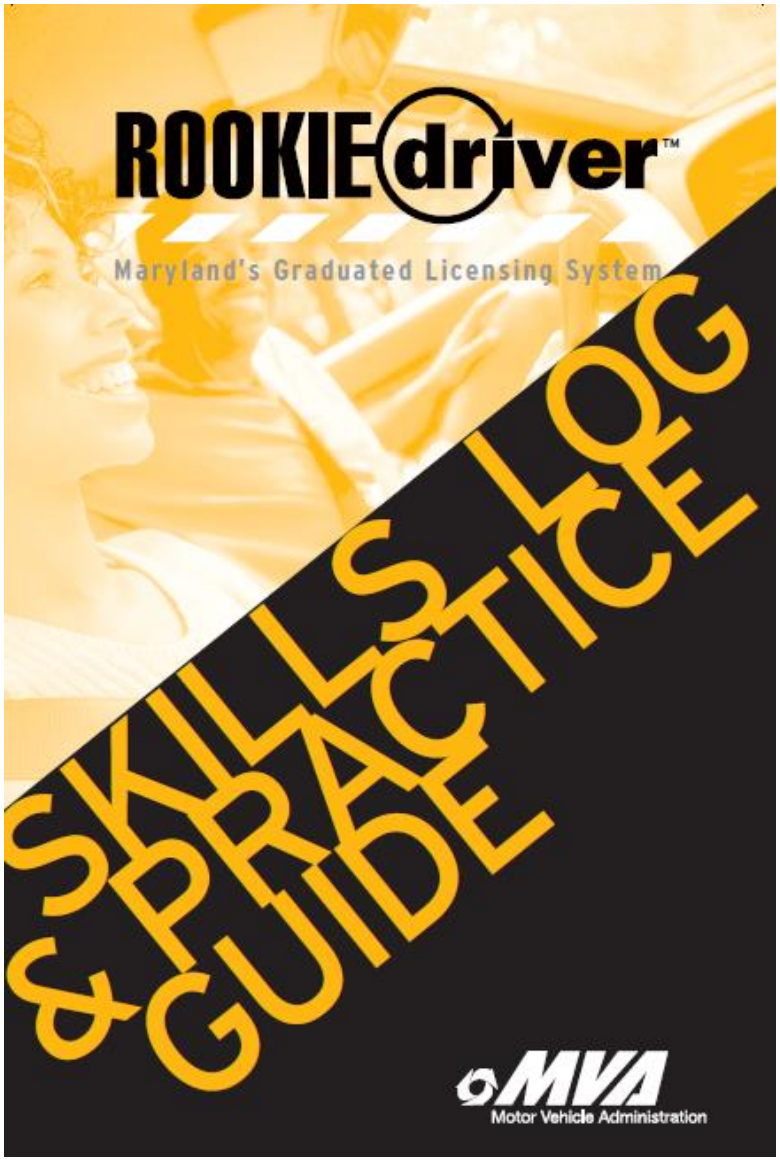
  1) Learner's Permit
     - Minimum age: 15 years 9 months old
     - Drives only with a qualified supervising driver
     - Must complete 60 hours of supervised driving experience

  2) Provisional License
     - Minimum age: 16 years 6 months old

  3) Full Driver's License
     - Minimum age: 18 years old

http://www.mva.maryland.gov/Driver-Services/RookieDriver/bgeneral.htm

Requirements for New Maryland Drivers



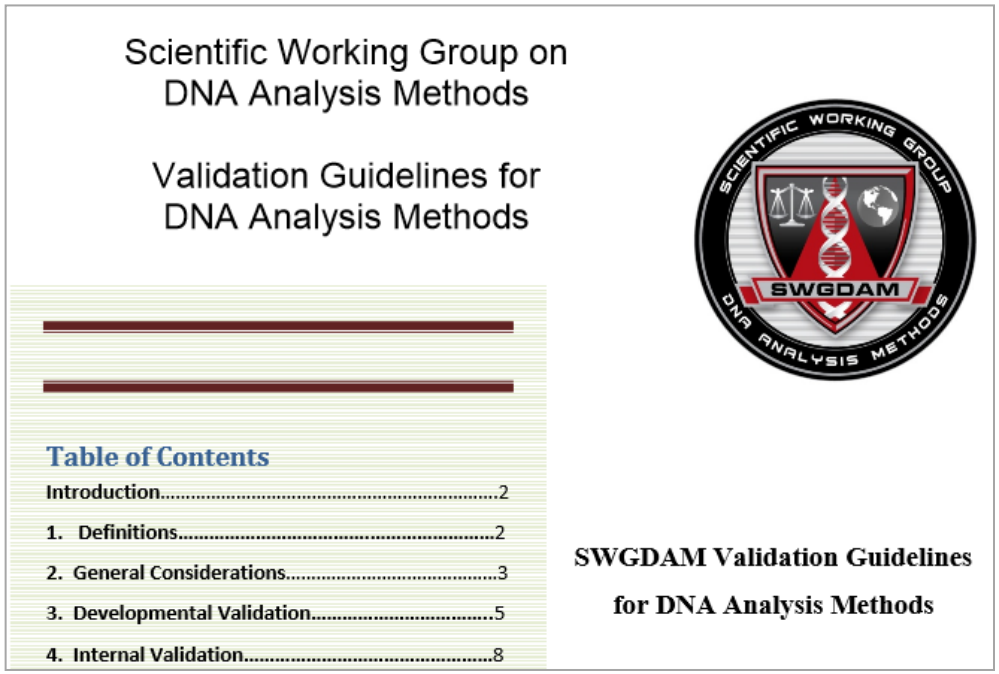
http://www.mva.maryland.gov/Resources/RD-006.pdf

New motor vehicle drivers (under 25 years old) must have:

- **60 hours of supervised driving experience** of which **10 hours** must be done **at nighttime**
- Must hold their **learner's permit for a minimum of 9 months**

New SWGDAM Validation Guidelines (2012)

http://swgdam.org/SWGDAM_Validation_Guidelines_APPROVED_Dec_2012.pdf


Scientific Working Group on
DNA Analysis Methods

Validation Guidelines for
DNA Analysis Methods

Table of Contents

Introduction....2
1. Definitions....2
2. General Considerations....3
3. Developmental Validation....5
4. Internal Validation....8

SWGDAM Validation Guidelines
for DNA Analysis Methods

Available on SWGDAM website: www.swgdam.org

"Each laboratory seeking to evaluate a new system must determine **which validation studies are relevant to the methodology**, in the context of its application, and determine **the number of samples required to satisfy each study**."

Internal Validation Data Should Drive Laboratory Interpretation Guidelines

SWGDAM Validation Guidelines – Approved December 2012

2.2.2.2 Quality assurance parameters and interpretation guidelines shall be derived from internal validation studies. For example, lower template DNA may cause extreme heterozygote imbalance; as such, empirical heterozygote peak-height ratio data could be used to formulate mixture interpretation guidelines and determine the appropriate ratio by which two peaks are determined to be heterozygotes. In addition to establishing an analytical threshold, results from sensitivity studies could be used to determine the extent and parameters of quality control tests that reagents require prior to their being used in actual casework.

Appropriate Samples Need to Be Evaluated During Validation Studies

3.6 **Case-type samples:** The ability to obtain reliable results should be evaluated using samples that are representative of those typically encountered by the testing laboratory. Where appropriate, consistency of typing results should be demonstrated by comparing results from the previous procedures to those obtained using the new procedure.

3.8 **Mixture studies:** The ability to obtain reliable results from mixed-source samples should be determined. These studies will assist the laboratory to establish guidelines for mixture interpretation, which may include determination of the number of contributors to the mixture, determination of the major and minor contributor profiles, and contributor ratios or proportions.

Important Things to Keep in Mind When Conducting Validation Studies

- Validation should establish the limits of a technique – thus **test in appropriate ranges**
  - PHR (Hb) variation tested at 1 ng will not apply to <100 pg data due to inherent stochastic variation with lower levels of DNA template
- **Replicate testing of the same DNA template**, especially at low levels, helps establish limits of reproducibility
- **Use known DNA samples** so reliability of genotypes and full profiles can be assessed
  - In the case of mixtures, plan specific ratios to evaluate
- **Test multiple DNA templates** as the quantitation of a single sample may not be what you think it is…

Experiment – Do Not Extrapolate

- It is not possible to fully apply concepts from single-source or 2-person mixtures like PHRs to more complex mixtures due to allele stacking possibilities

- If three person mixtures are being encountered regularly in your laboratory, then three person validation studies should be performed with known samples
  - Results of the validation study should be used to shape interpretation protocols
  - Establish the limits of reliable performance and stay within them (i.e., keep your car on the road)

Evaluate Reliability After Establishing Interpretation Guidelines

- Following validation experiments and establishment of specific parameters in the lab SOPs, challenge the new interpretation protocol with known samples to see if reliable results are obtained
  - For example, if the heterozygote peak height ratio has been set at 60%, then test multiple 2-person and 3-person mixtures with known genotypes and determine if reliable profiles can be deduced
  - **If an interpretation SOP does not work with known samples, how can it be expected to work reliably with casework samples?**

From Maryland Rookie Driver Information

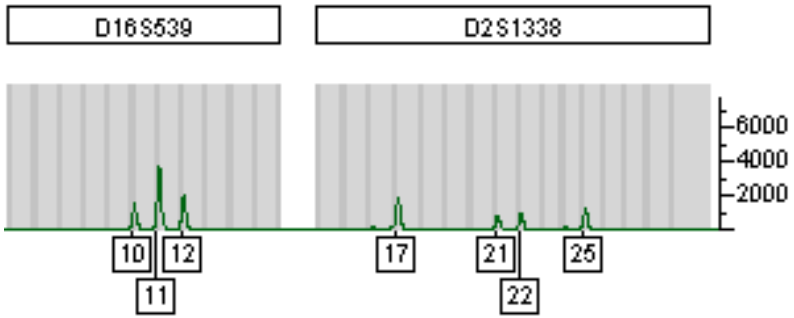
- "…Recording each driving and practice experience is an easy way to track the progress of the new driver. **Each practice experience should be planned and present challenges for the new driver. Simply having the new drivers drive around the neighborhood will not prepare them for the time when they have a license and are driving without a supervisor**. Take the time to make your new driver the best possible driver they can be."

http://www.mva.maryland.gov/Resources/RD-006.pdf

Validation Studies Should Correspond to Needed Levels of DNA Interpretation

http://1000awesomethings.files.wordpress.com/2012/04/visiting-old-home.jpg

Easy drive around the neighborhood



- **Are your laboratory validation studies like a simple "drive around the neighborhood" of DNA testing?**
  - If the mixture portion of your validation studies involved mixing 9947A and 9948 in five different mixture ratios (e.g., 1:9, 1:3, 1:1, 3:1, & 9:1), then perhaps you should explore some more difficult scenarios as real-world casework is more complicated!

DNA Validation Should Prepare for Casework Situations to **Help Understand Limitations and to Develop Interpretation Protocols**

- **"Each practice experience should be planned and present challenges for the new driver…"**
  (Maryland Rookie Driver information)

http://farm6.staticflickr.com/5100/5591761716_57cf063d96_z.jpg

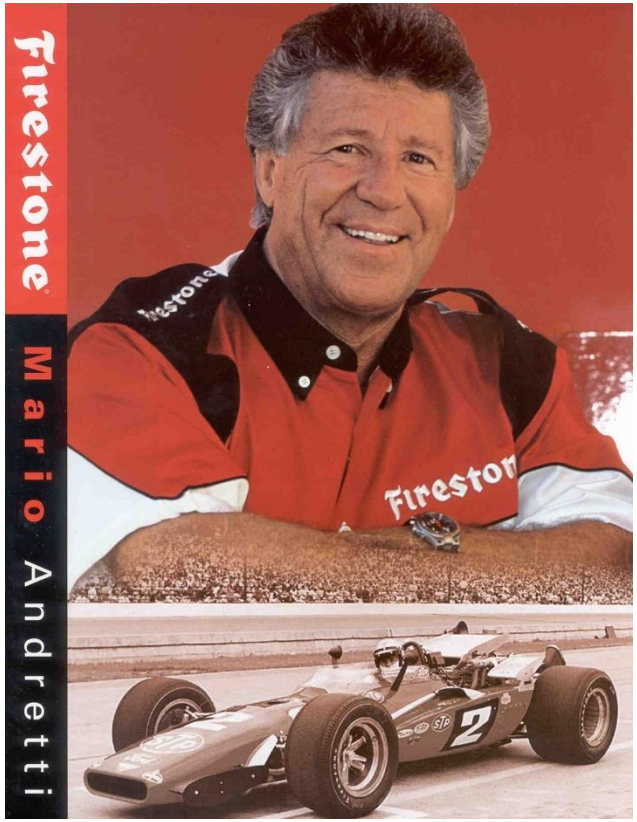
Coping with >2 contributors

http://cinemasights.files.wordpress.com/2009/11/speed-conflict.jpg

Under pressure with a "speed" case

Knowledge Obtained from Validation Studies Should Shape Interpretation SOPs and Benefit the Quality of Future Work

- "**<u>Take the time</u>** to make your new driver **the best possible driver they can be**…" (Maryland Rookie Driver information)



http://commons.wikimedia.org/wiki/File:Car_crash_1.jpg

Want to avoid accidents!

There are times when you should slow down or perhaps not drive at all…

Poor Quality Conditions

Large Numbers of Contributors