FORENSICS @ NIST
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Mixture Interpretation

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Forensics@NIST 2012 Meeting
Gaithersburg, MD
November 28, 2012

Outline

- What are mixtures?
- Why are mixtures difficult to interpret?
- NIST's role in mixture interpretation
- Workshops and training
- Software evaluation
- Conclusions

DNA Mixtures

- Mixtures arise when two or more individuals contribute to the sample being tested.
- Mixtures can be challenging to detect and interpret without extensive experience and careful training.
- Even more challenging with poor quality data when degraded DNA is present…

J.M. Butler (2005) Forensic DNA Typing, 2nd Edition, p. 154

Mixture Case Summaries

	minimum # of contributors
Crime Class	**1**
Sexual Assault	884
Major Crime	1261
High Volume	344
Total	2489
Single source	**54.8%**

http://www.cstl.nist.gov/biotech/strbase/pub_pres/Promega2008poster.pdf

Data Set from 14 Different Labs

NIST
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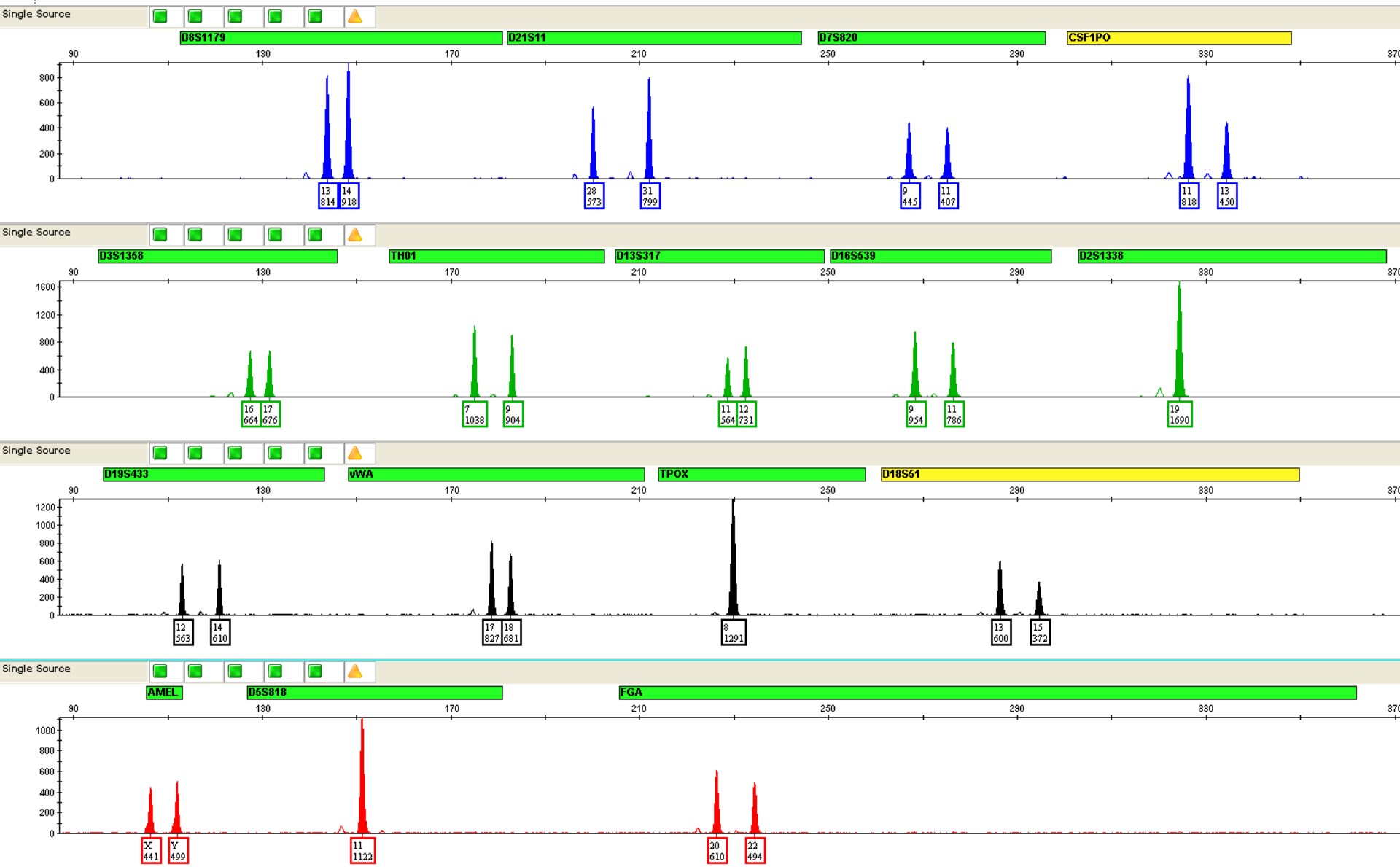
Genetics

Single-source Profile



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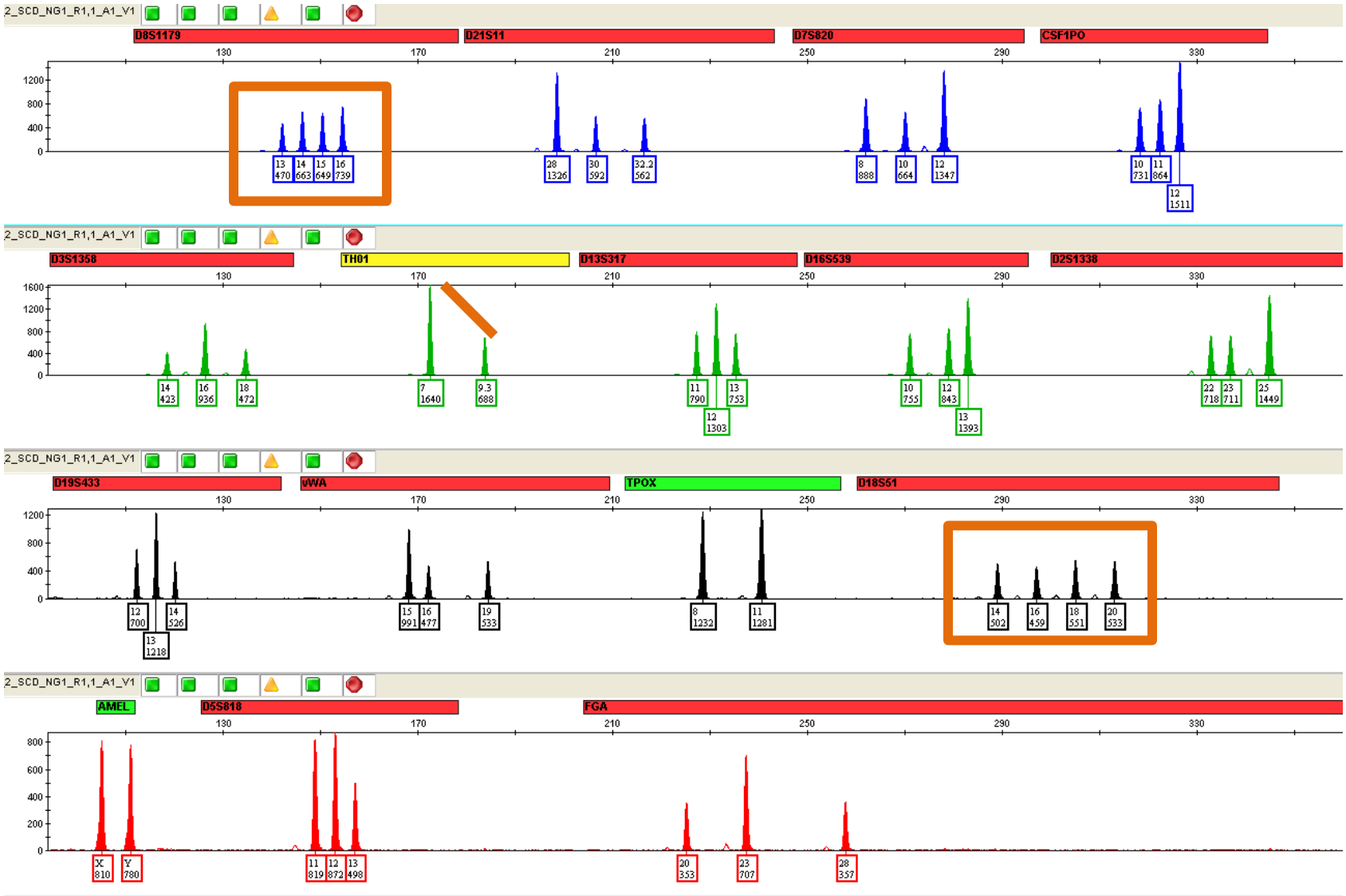
Mixture Profile



Mixture Case Summaries

	minimum # of contributors					
Crime Class	**1**	**2**	**3**	**4**	**>4**	**N**
Sexual Assault	884	787	145	11	0	1827
Major Crime	1261	519	182	32	0	1994
High Volume	344	220	140	11	5	720
Total	2489	1526	467	54	5	4541
Single source	**54.8%**	33.6%	10.3%	1.2%	0.1%	**mixtures**

http://www.cstl.nist.gov/biotech/strbase/pub_pres/Promega2008poster.pdf

Data Set from 14 Different Labs

U.S. Department of Justice
Office of Justice Programs
National Institute of Justice

NIJ

IN SHORT

TOWARD CRIMINAL JUSTICE SOLUTIONS

NOV. 04

DNA in "Minor" Crimes Yields Major Benefits in Public Safety

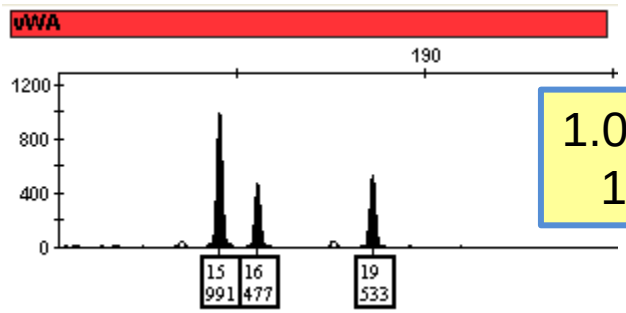
2010 SWGDAM Guidelines

- In 2010, the Scientific Working Group on DNA Analysis Methods (SWGDAM) released new guidelines for STR interpretation.

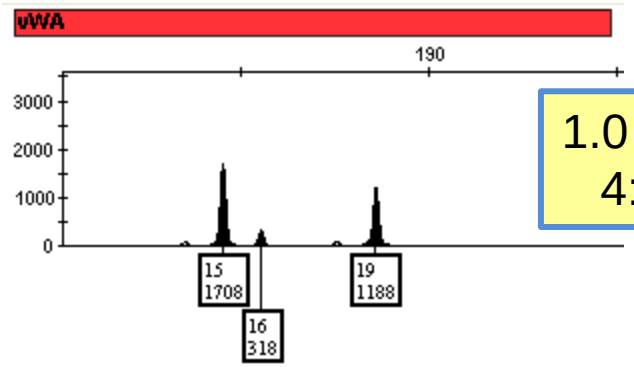
- The guidelines stressed the need for applying ***thresholds*** for data interpretation and including ***statistical support*** for any inclusion.

Why are DNA mixtures difficult to interpret?

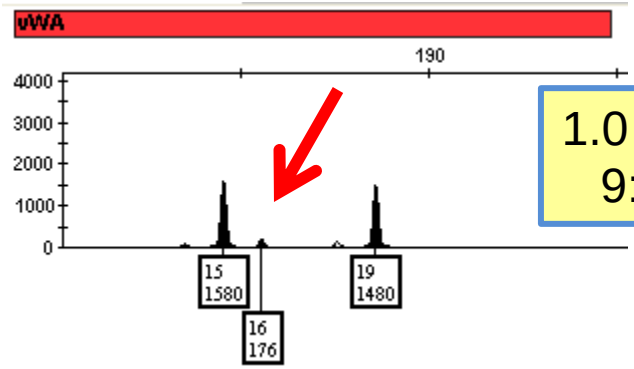
Data from Boston University Training Grant NIJ # 2008-DN-BX-K158



1.0 ng input
1:1 ratio



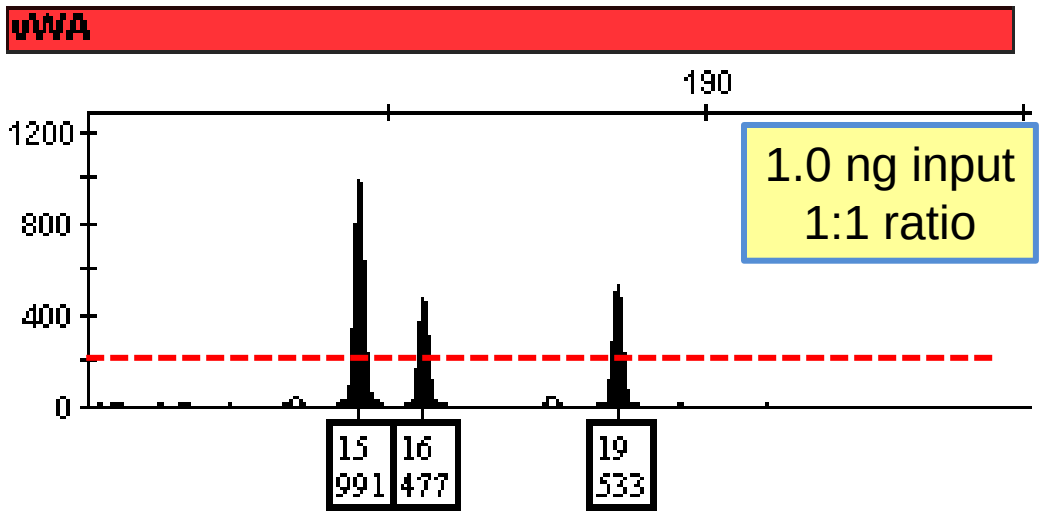
1.0 ng input
4:1 ratio



1.0 ng input
9:1 ratio

1. We don't know *a priori* the quantity of each component in the mixture (mixture ratio).

2. This can lead to uncertainty in determining if all the alleles are present.

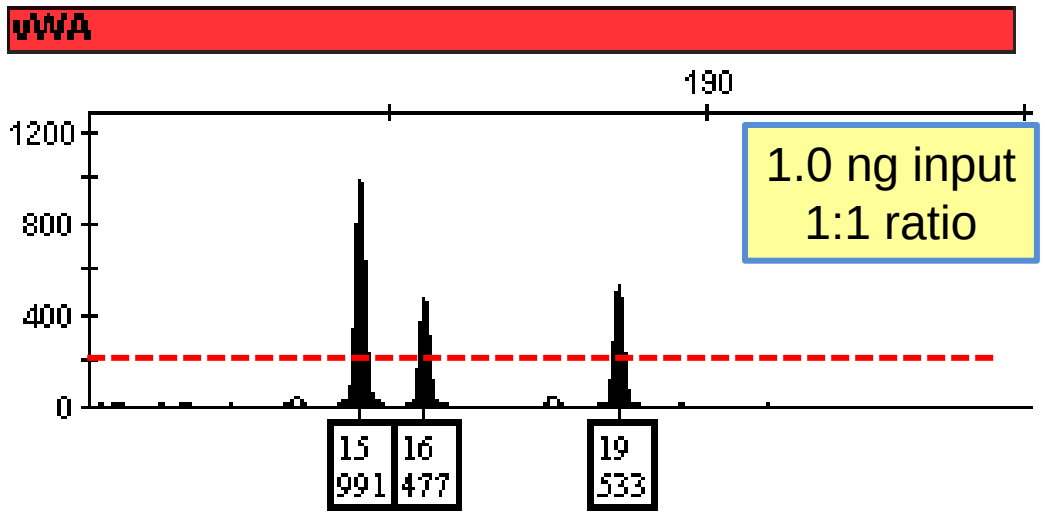


The laboratory established "Stochastic" threshold is set at 200 RFU. Since all peaks are above this value, we can be confident that all of the alleles are present at this marker.

Suspect – 16,18

Would you include him in this mixture?

Data from Boston University Training Grant NIJ # 2008-DN-BX-K158



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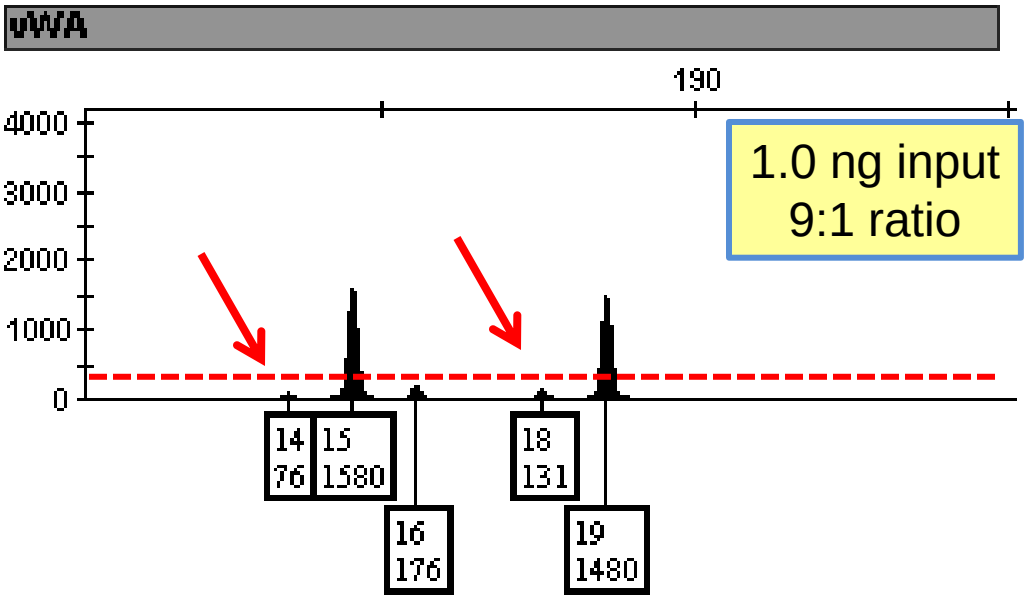


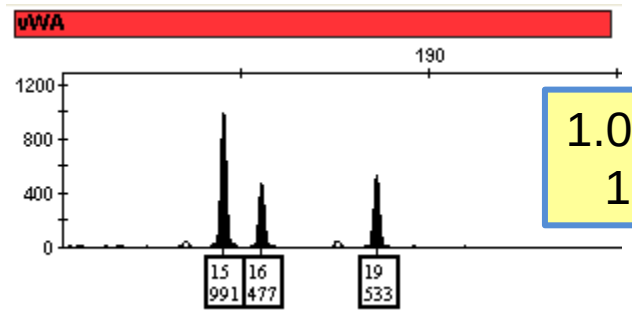
Now the 16 allele is below the 200 RFU threshold. We are not as confident that all of the alleles are present in the mixture.

The "14" and "18" peaks are typical PCR artifacts called "Stutter." However, the 18 allele is roughly the same height as the 16 allele.

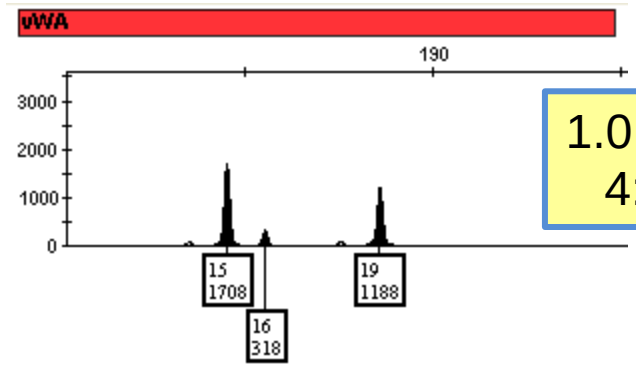
Suspect – 16,18

Would you include him in this mixture?

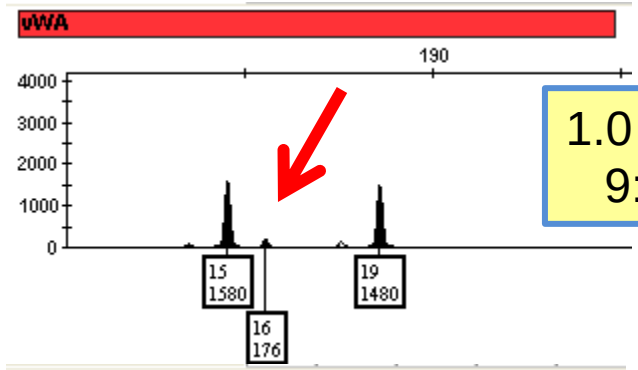
Data from Boston University Training Grant NIJ # 2008-DN-BX-K158



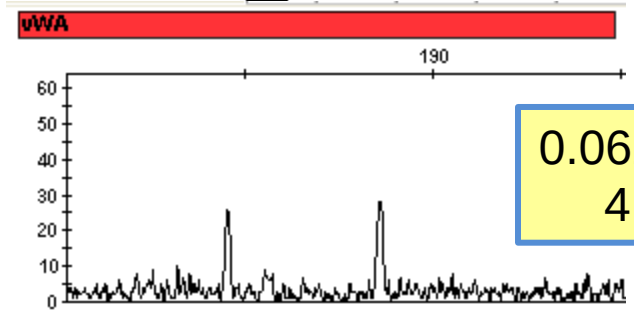
1.0 ng input
1:1 ratio



1.0 ng input
4:1 ratio



1.0 ng input
9:1 ratio



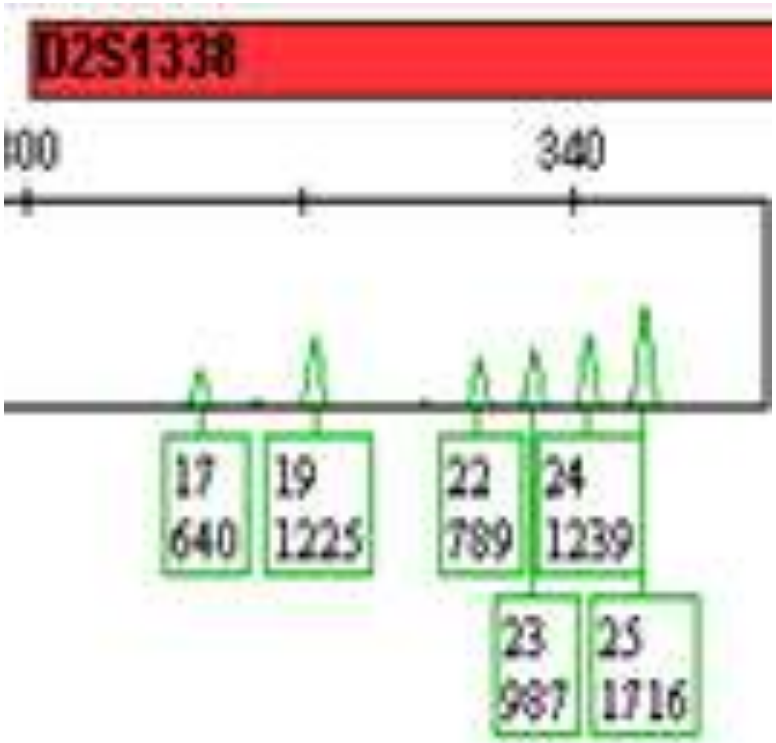
0.062 ng input
4:1 ratio

1. We don't know *a priori* the quantity of each component in the mixture (mixture ratio).

2. This can lead to uncertainty in determining if all the alleles are present.

3. This is exacerbated by very low level DNA evidence.

This difficulty is enhanced with 3+ person mixtures



Six alleles at this marker suggests a 3-person mixture.

Determining the number of contributors to a mixture is one of the first steps in interpretation.

Data from Boston University Training Grant NIJ # 2008-DN-BX-K158

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NIST's Role in Mixture Interpretation

Study	Dates	Labs	References
Mixed Stain Studies #1 and #2	(Apr.–Nov 1997 and Jan–May 1999)	45	Duewer et al. (2001)
			Kline et al. (2003)
Mixed Stain Study #3	(Oct 2000-May 2001)	74	Duewer et al. (2004)
Mixture Interpretation Study	(Jan-June 2005)	69	

NIST Mixture Interpretation Interlaboratory Study 2005 (MIX05)

John M. Butler and Margaret C. Kline

Biotechnology Division, National Institute of Standards and Technology, 100 Bureau Drive MS 8311, Gaithersburg, MD 20899-8311

"Some of the primary benefits we hope to gain from this study include **recommendations for a more uniform approach to mixture interpretation** and training tools to help educate the community."

Recent **NIJ** Training Support of BU

- NIJ Forensic Science Training Development and Delivery Program Grant # 2008-DN-BX-K158, awarded to Biomedical Forensic Science Program at Boston University School of Medicine

Mixture Training Workshops


John Butler


Mike Coble


Robin Cotton


Catherine Grgicak

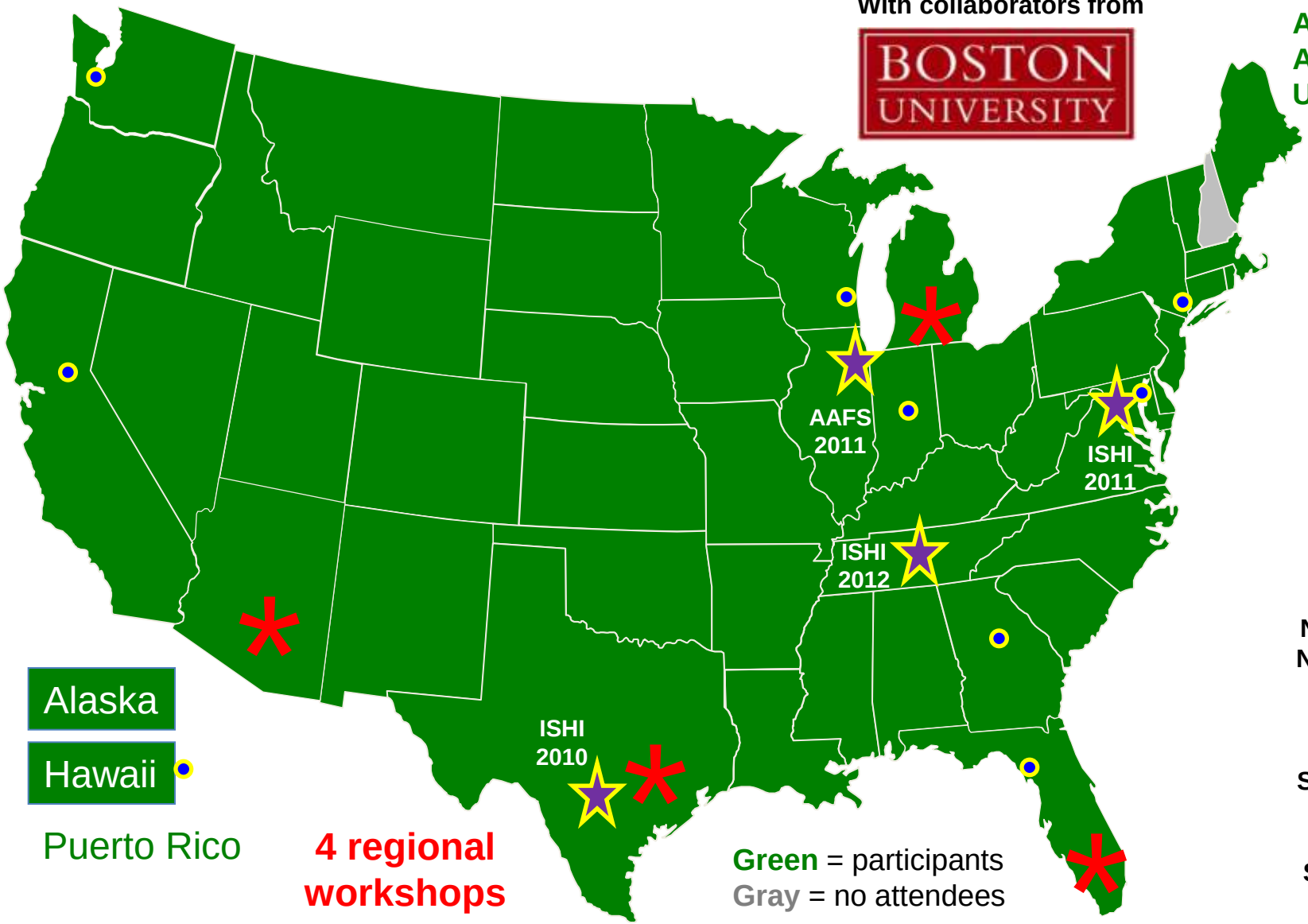

Charlotte Word

- Collaborators from Boston University (formerly Cellmark)
- ISHI 2012 workshop covered issues with thresholds, statistics, probabilistic genotyping, complex mixtures, court testimony, and assumptions made
  - Audience response systems (clickers) used to gather data from participants
- **Slides are available on STRBase**

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

DNA Mixture Workshop Attendees

49 states and 25 other countries, so far:



Federal Labs

FBI
ATF
AFDIL
USACIL

Algeria
Argentina
Bahamas
Belgium
Brazil
Canada
Croatia
Finland
France
Israel
Italy
Jamaica
Japan
Korea
Mexico
Netherlands
New Zealand
Panama
Peru
Russia
Saudi Arabia
Singapore
Spain
Switzerland
UK

Training Workshops (Past 2 Years; selected)

Full listing available at **http://www.cstl.nist.gov/strbase/training.htm**

Training for DNA Analysts

(organized by National Forensic Science Technology Center)

March 2011

Training for Capital Litigators

(organized by National Clearinghouse on Science, Technology, and Law)

Aug 2012

Regional DNA Mixture Workshops Taught in Crime Laboratories

with Boston University Collaborators (NIJ-Funded Training)

April 2011

April 2011

May 2011

June 2011

Use of Audience Response Systems (the TurningPoint Clickers)

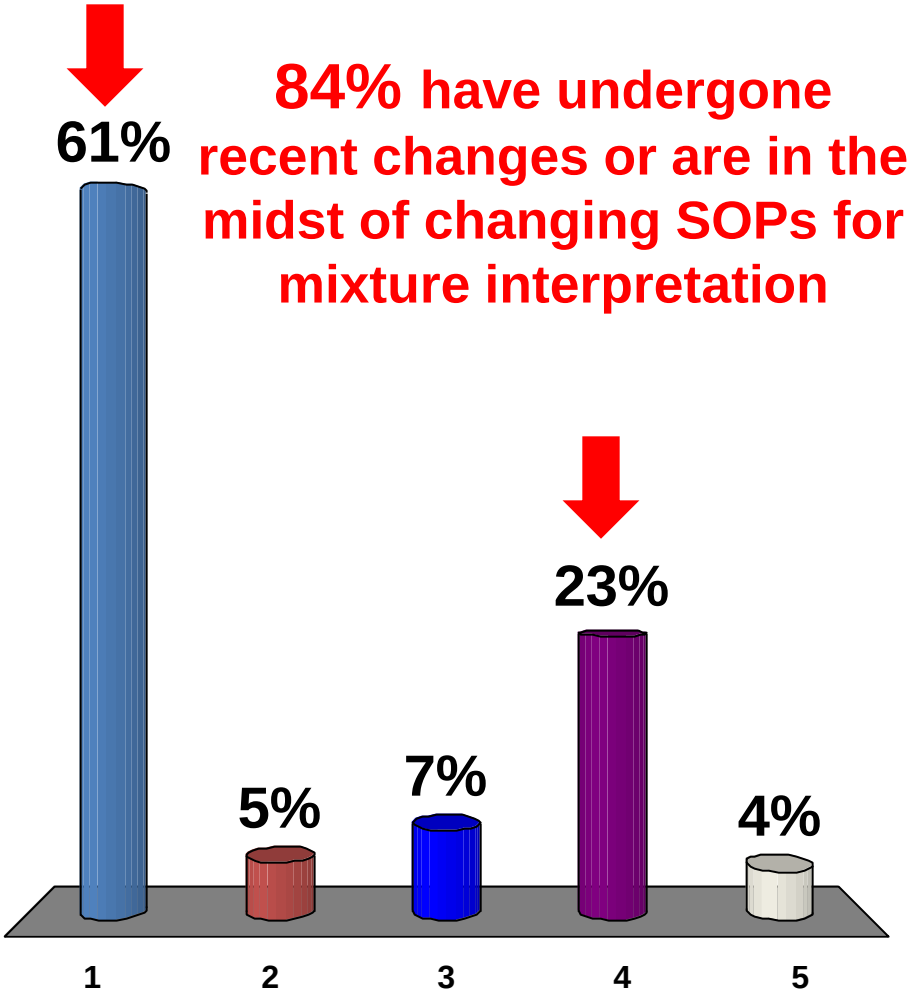
Used in ISHI 2011-12 workshops and FL, TX, MI, and AZ regional workshops

- **Kept the audience engaged** with the opportunity to participate and offer their opinions with anonymity
- **Provided real-time results** so the audience could enjoy learning how everyone responded to the question
- **Enabled us to gather information** from audience members
  - answers can be tracked across the questions to the specific clicker used

Has your lab implemented changes to your SOPs based on the [SWGDAM 2010] guidelines?

1. Yes
2. No
3. Reviewed SOPs but no changes needed
4. Working on it
5. Not applicable (I do not work in a forensic lab)

Data from 150 responses
ISHI Mixture Workshop (Oct 2011)



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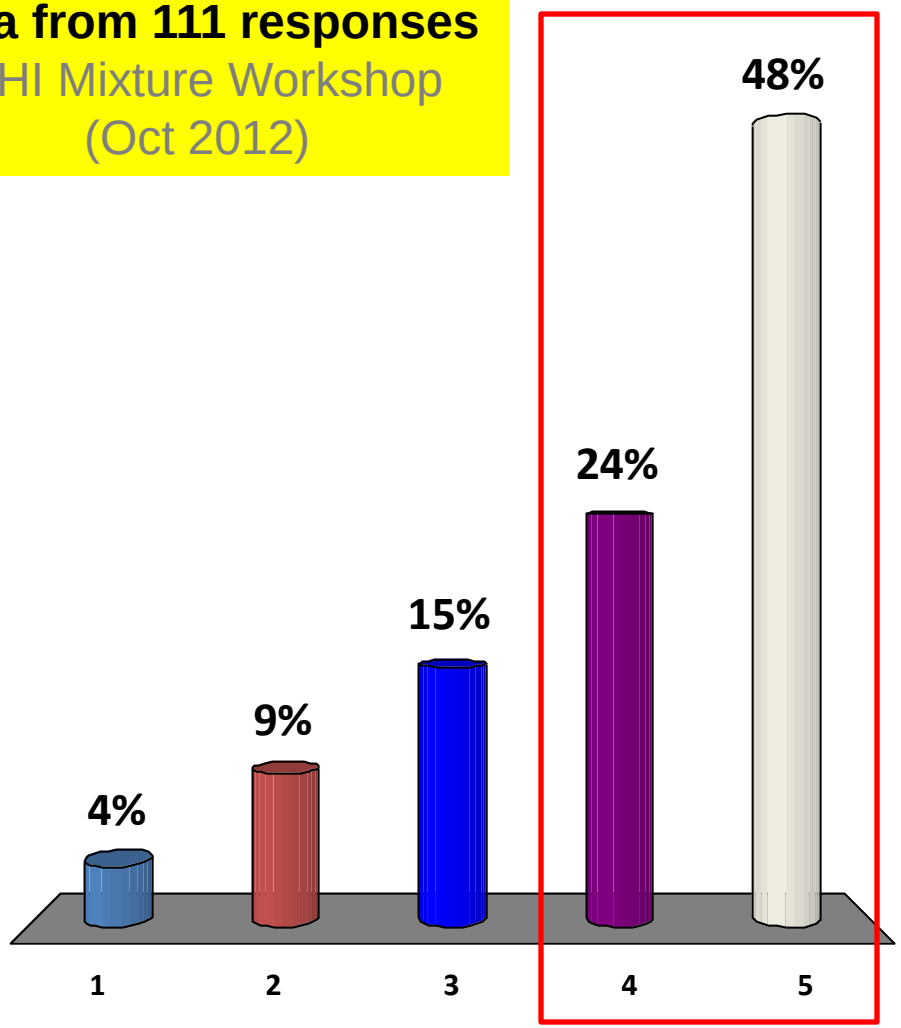
Our Own Evolution…

ISHI 2010 San Antonio, TX	ISHI 2011 Washington, DC	ISHI 2012 Nashville, TN
Back to the Basics, SWGDAM Guidelines	Focus on Examples, Validation, Stats	Challenging examples, Limitations of current strategies

Which of the topics below would be your first choice for additional training?

1. Relevant literature
2. How to validate thresholds in more detail
3. Reporting and the use of assumptions
4. Interpretation of low level mixtures
5. Likelihood ratios and other statistical approaches

Data from 111 responses
ISHI Mixture Workshop
(Oct 2012)



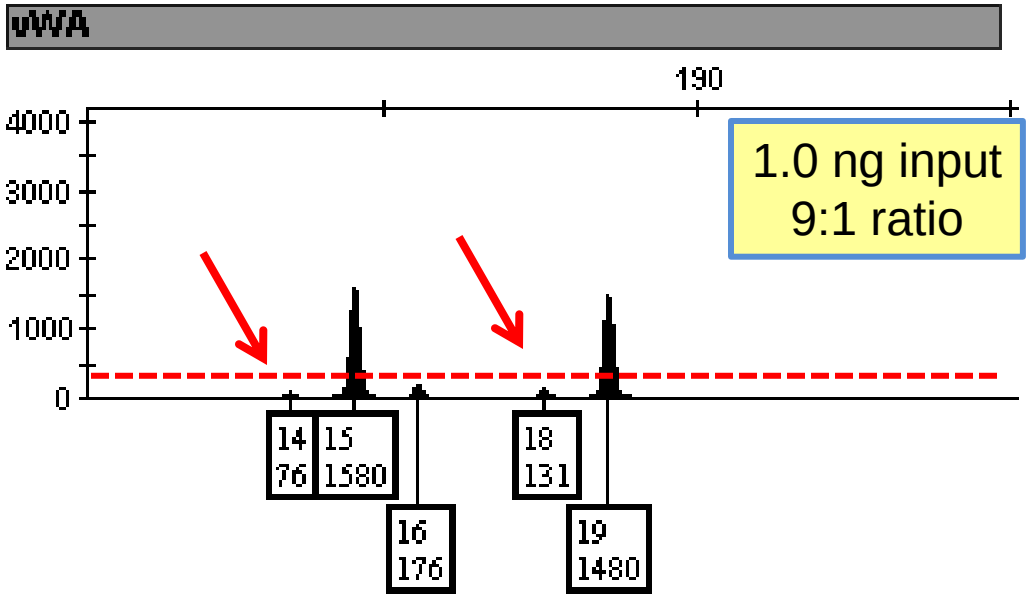
Two Parts to Mixture Interpretation

- Determination of alleles present in the evidence and **deconvolution of mixture components** where possible
  - Many times through comparison to victim and suspect profiles
- **Providing some kind of statistical answer** regarding the weight of the evidence
  - There are multiple approaches and philosophies

Software tools can help with one or both of these…

Expert Software for Mixture Analysis

Software Limitations…

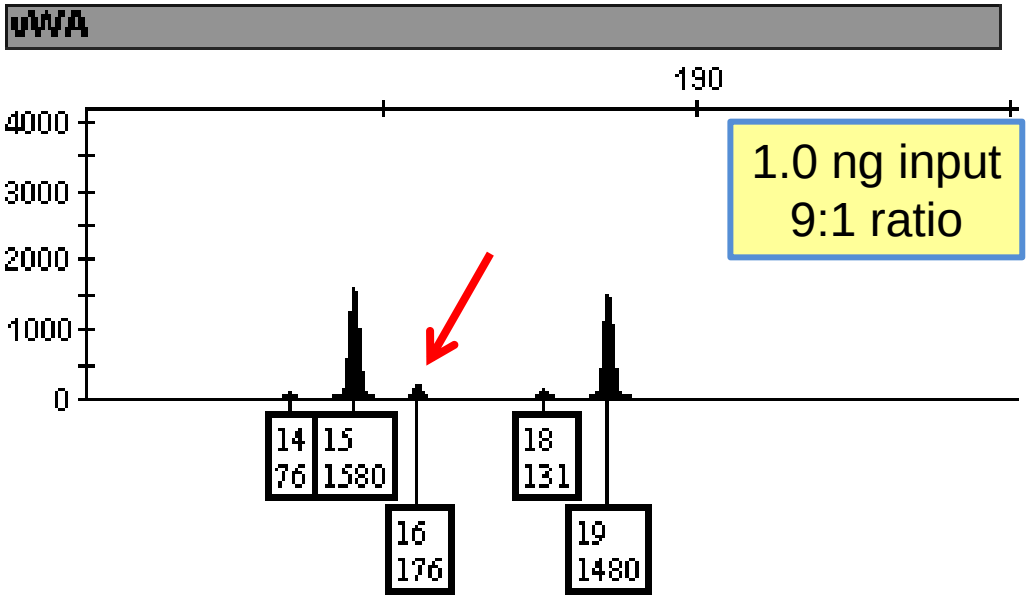


Software programs often use fixed values for mixture interpretation (e.g. “maximum” values for stutter peaks).

Thresholds are “all or none” propositions – using a threshold of 150 RFUs in the example above will change the analytical and statistical interpretation of the mixture.

“Probabilistic” approaches to mixture interpretation

Probabilistic Approaches

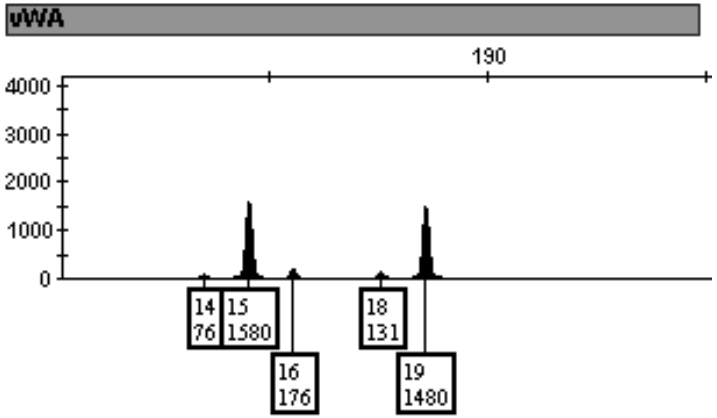


A probability that a sister allele has been lost (called “drop-out”) is determined from validation data.

For example, at 176 RFUs, there is a 3% chance that drop-out has occurred. This value can now be incorporated in the statistical interpretation of the data.

“Simulation” Approach

Mathematical Modeling of the Data



Peak Height Ratio,
Mix Ratio, Stutter etc…

50,000 - 100,000

Markov Chain
Monte Carlo
Simulations

Probable ***Genotypes*** to explain the mixture

Genotypes	Probability
15,16	77%
16,16	12%
16,19	10%
16,18	<1%
14,16	<1%

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

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Summary of the Issues

- Strategies to improve mixture interpretation protocols are progressing, but there is still a need for training – especially for statistical interpretation.
- The challenges of DNA mixture interpretation will only become worse as we move to higher sensitivity STR kits and instruments ***along with*** a focus on solving complex high volume crimes.
- Probabilistic methods will be necessary for resolving highly complex, low-level mixtures. We have generated data sets at NIST that can be useful for evaluating these new software programs.

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

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Acknowledgments

NIST

John Butler

Collaborators

Robin Cotton
Boston University

Catherine Grgicak
Boston University

Charlotte Word
Consultant

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