NJ CONFERENCE, 2012

What We Have Learned & Where We Need to Go

John M. Butler NIST

NIJ Conference Mixture Workshop June 20, 2012







My Presentation Outline

- Review recent mixture workshops
- Some responses from clicker questions
- Valuable mixture literature and how to obtain it
- Important lessons & common misunderstandings
- Thoughts on where we need to go as a community to improve mixture interpretation

Thanks to NIJ for Support of BU and NIST



- 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
- NIJ has an Interagency Agreement (IAA) with the NIST Office of Law Enforcement Standards (OLES)

BOSTON UNIVERSITY The NIJ grant to BU funded...

- Free ISHI 2010 and 2011 workshop registration for state and local forensic DNA analysts (~360 total)
- Visits to four regions to conduct four 8-hour workshops
 - FL (Apr 2011), TX (May 2011), MI (May 2011), AZ (June 2011)
 - About 50 attended each workshop (~200 total)
- <u>Creation of STR profiles</u> with 2, 3, and 4 person mixtures at various mixture ratios, DNA amounts, and CE injection
 - PP 16 HS, Identifiler, Yfiler, and MiniFiler (amplified in quadruplicate)
 - Over 2000 profiles available for download on BU website
- <u>Development of a web site for training</u> in mixture analysis
 STR mixtures profiles can be downloaded and used for training

Mixture Workshop Presenters





Robin Cotton Boston University

John Butler NIST



Catherine Grgicak Boston University

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Mike Coble



Charlotte Word Consultant

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Our presentations handouts are available on the NIST STRBase website http://www.cstl.nist.gov/strbase/mixture.htm

Use of Audience Response Systems (the TurningPoint Clickers)



Used in ISHI 2011 workshop 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

What is your role in the laboratory?

1. DNA analyst

- 2. DNA technician
- 3. Database analyst
- 4. DNA technical leader
- 5. QA Manager
- 6. Attorney
- 7. Other



BU Grant Supported Mixture Workshops

Meeting	Date	Location	# Attendees (approximate)	Clickers Used	
ISHI 2010	Oct 11, 2010	San Antonio, TX	200	Ν	
FL	Apr 2011	Palm Beach, FL	50	Y	
TX	May 2011	Houston, TX	50	Y	
MI	May 2011	Lansing, MI	50	Y	
AZ	June 2011	Mesa, AZ	50	Y	
ISHI 2011	Oct 3, 2011	Washington, DC	160	Y	

~600 people reached through these workshops

Additional NIST Mixture Workshops

Meeting/ Location	Date	#AttendedWho(approximate)Presented		Clickers Used	
AAFS	Feb 2011	220	JB & MC (and 6 others)	Ν	
NFTSC	Mar 2011	120	MC (and 5 others)	Y	
Indiana	Mar 2011	65	JB	Ν	
Maryland	Apr 2011	60	JB (and 2 others)	Ν	
Hawaii	Aug 2011	10	MC	Ν	
NYC OCME	Apr 2012	150	JB & MC	Y	
Canada (CSFS)	May 2012	40	MC	Y	
Taiwan	Jun 2012	60	JB	Ν	

>700 additional people reached with these workshops



Mixture Section of STRBase website

Information on DNA Mixture Interpretation

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

Workshops, Presentations, and Training Information

- <u>SWGDAM</u> Mixture Committee Resource Page: <u>http://www.cstl.nist.gov/biotech/strbase/mixture/SWGDAM-mixture-info.htm</u>
 - Training worked example by Bruce Heidebrecht: "Mixture 6" PowerPoint show (56 Mb)
 - Training worked example by Bruce Heidebrecht: "Mixture IQAS2904" PowerPoint show (35 Mb)
 - Mixture Interpretation: Using Scientific Analysis (ISHI 2011 Workshop: Butler, Coble, Cotton, Grgicak, Word)
- DNA Mixture Analysis: Principles and Practice of Mixture Interpretation and Statistical Analysis Using the SWGDAM <u>STR Interpretation Guidelines</u> (AAFS 2011 Workshop: Butler, Coble, Bille, Adamowicz, Sgueglia, Shutler, Gombos, Wickenheiser)
- Mixture Interpretation: Principles, Protocols, and Practice (Workshop: J.M. Butler, M.D. Coble, R.W. Cotton, C.M. Grgicak, C.J. Word, ISHI 2010)
- <u>SWGDAM Autosomal STR Interpretation Guidelines</u> (Talk: John Butler, ISHI 2010) [link to guidelines]
- <u>DNA Mixture Interpretation</u>: Principles and Practice in Component Deconvolution and Statistical Analysis (Workshop, AAFS 2008)

Links to software programs or information

 Talk given by Mike Coble at the International Society of Forensic Genetics (ISFG) meeting (Vienna, Austria), September 3, 2011, "Exploring the Capabilities of Mixture Interpretation Using TrueAllele Software" [.pdf]

Website also lists >100 helpful references on DNA mixture interpretation...

Mixture Workshop Handouts

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

ISHI 2010 Mixture Workshop



Focused more on basics

MIXTURE INTERPRETATION: Principles, Protocols, and Practice

21" International Symposium on Human Identification October 11, 2010 (San Antonio, TX)

Presenters

John M. Butler, PhD Michael D. Coble, PhD Robin W. Cotton, PhD Catherine M. Grgicak, PhD Charlotte J. Word, PhD NIST, Applied Genetics Group NIST, Applied Genetics Group Boston University, Biomedical Forensic Sciences Boston University, Biomedical Forensic Sciences Consultant

Supported by funding from the National Institute of Justice

NII Grant 2008-DN-6X-K158 to Boston University and Interagency Agreement 2008-DN-R-121 between NU and the NIST Office of Law Enforcement Standards funds the presenters. The Boston University grant also covers the registration for 175 US State and Local Crime Laboratory personnel.

198 pages (60 pages of literature)

ISHI 2011 Mixture Workshop



Supported by funding from the National Institute of Justice

NU Grant 2008-DN-BX-K158 to Botton University and Interagency Agreement 2008-DN-R-121 between NU and the NIST Office of Law Enforcement Standards funds the presenters. The Boston University grant also covers the registration for 175 US State and Local Crime Laboratory personnel.

154 pages (literature not included)



Handouts Available on STRBase

ISHI 2010 Mixture Workshop MIXTURE INTERPRETATION: Principles, Protocols, and Practice

Module	Торіс	Presenter
1	Introduction to the Workshop	Robin Cotton
2	Introduction to the SWGDAM Guidelines	John Butler
3	Setting Analytical Thresholds	Catherine Grgicak
4	Determining & Dealing with Stutter Products	Mike Coble
5	Amplification Variation & Stochastic Effects	Robin Cotton
6	Peak Height Ratios	Charlotte Word
7	Estimating the Number of Contributors	John Butler
8	Calculating & Using Mixture Ratios	John Butler
9	Statistical Approaches (RMP, CPI, LR)	Mike Coble
10	Mixture Principles & Reporting Basics	Charlotte Word
11	Case Example 1: indistinguishable mixture	Robin Cotton
12	Case Example 2: distinguishable mixture	Charlotte Word & Mike Coble
13	Case Example 3: complex mixture	John Butler
14	Wrap Up and Evaluation	Robin Cotton

Comments on Mixture Training We Have Conducted These Past Two Years

- Trying to help analysts better understand the SWGDAM 2010 Interpretation Guidelines
 - It is important to note that the 2010 SWGDAM Guidelines were written primarily for 2-person mixtures situations
- However, many labs are doing or attempting more complex mixtures often without appropriate underlying validation support or consideration of complicating factors
- The information content in our workshops has continued to evolve to include the latest published articles...

Some of the Data Collected Using Audience Response "Clickers"

What is your role in the laboratory?

1. DNA analyst

- 2. DNA technician
- 3. Database analyst

4. DNA technical leader

- 5. QA Manager
- 6. Attorney
- 7. Other

Data from 152 responses ISHI Mixture Workshop (Oct 2011) 76% **9% 9%** 3% 1% 0% 1% 3 5 6 7 2 4 1

Your Experience Level as a DNA Analyst



How much DNA court testimony experience do you have?



Have you read the 2010 SWGDAM Interpretation Guidelines? Yes = 56%

N = 170 from 4 regional groups (16 different labs)



From ISHI 2011 poster "Impact of the SWGDAM Mixture Interpretation Guidelines: Successes, Issues and Suggested Future Directions"

Have you read the 2010 SWGDAM STR Interpretation Guidelines? Yes = 79%



If you asked 10 analysts in your laboratory to interpret a complex mixture you would get:

- 1. 100% consensus
- 2. 100% non-consensus
- 3. Mostly consensus
- 4. A large range of answers
- 5. We don't interpret complex mixtures

There is a recognition of the variation that exists with how analysts interpret complex mixtures. N=174 Regional mixture workshops (Apr – June 2011)



From ISHI 2011 poster "Impact of the SWGDAM Mixture Interpretation Guidelines: Successes, Issues and Suggested Future Directions"

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
- 5. Not applicable (I do not work in a forensic lab)

84% have undergone 61% recent changes or are in the midst of changing SOPs for mixture interpretation 23% 7% 5% 4% 2 3 1 5 4

Data from 150 responses ISHI Mixture Workshop (Oct 2011) Has your lab implemented changes to your SOPs based on the new guidelines?

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

N=147 Regional mixture workshops (Apr – June 2011)



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

89%

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

94% have undergone recent changes or are in the midst of changing SOPs for mixture interpretation



NYC Apr 2012

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 new guidelines – section 4.1)

Data from 138 responses ISHI Mixture Workshop (Oct 2011)



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

- 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!



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 singlesource samples

Therefore, there is greater uncertainty with associating genotypes of contributors in mixtures



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

- 1. Relevant literature
- 2. How to validate thresholds
- 3. How to develop relevant SOPs
- 4. Interpretation of low level mixtures
- 5. Statistics

From one of the regional mixture workshops (Apr – June 2011)



Mixture Literature you should be reading... How many DNA-related articles would you estimate that you read in a typical month?

- 1. None
- 2. 1 article
- 3. 2 to 5 articles
- 4. More than 5 articles
- 5. None, I only read the abstracts
- 6. I don't make time to read!

Data from 133 responses ISHI Mixture Workshop (Oct 2011)



Useful Articles on DNA Mixture Interpretation

- Buckleton, J.S. and Curran, J.M. (2008) A discussion of the merits of random man not excluded and likelihood ratios. *Forensic Sci. Int. Genet.* 2: 343-348.
- Budowle, B., *et al.* (2009) Mixture interpretation: defining the relevant features for guidelines for the assessment of mixed DNA profiles in forensic casework. *J. Forensic Sci.* 54: 810-821.
- Clayton, T.M., *et al.* (1998) Analysis and interpretation of mixed forensic stains using DNA STR profiling. *Forensic Sci. Int.* 91: 55-70.
- Gill, P., *et al.* (2006) DNA commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Sci. Int.* 160: 90-101.
- Gill, P., *et al.* (2008) National recommendations of the technical UK DNA working group on mixture interpretation for the NDNAD and for court going purposes. *FSI Genetics* 2(1): 76–82.
- Schneider, P.M., *et al.* (2009) The German Stain Commission: recommendations for the interpretation of mixed stains. *Int. J. Legal Med.* 123: 1-5.

Read to Maintain a Big Picture View!

If you are not following the recent literature, you would have missed:

- Software applications & implementation
- Impact of allele dropout on stats
- Studies on number of contributors
- The literature is changing very fast

- Read more than Journal of Forensic Sciences to stay caught up

 Make time in your schedule to read and ask critical questions

STRBase Mixture Reference List

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

Topic category	# References		
Mixture Principles & Recommendations	12		
Setting Thresholds	10		
Stutter Products & Peak Height Ratios	18		
Stochastic Effects & Allele Dropout	14		
Estimating the Number of Contributors	14		
Mixture Ratios	7		
Statistical Approaches	20		
Separating Cells to Avoid Mixtures	3		
Software	4		
Probabilistic Genotyping Approach	6		
General Information on Mixtures	6		
TOTAL	114		

Recent articles on mixtures not found in JFS...





Contents lists available at ScienceDirect

Foremaic Sciencel Immutational: Genetics 6 (2012) 102-107



Forensic Science International: Genetics

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

Extended PCR conditions to reduce drop-out frequencies in low template STR typing including unequal mixtures

Natalie E.C. Weiler¹, Anuska S. Matai¹, Titia Sijen^{*} Netheritanh Foremic Institute, Lauss van Ypenburg 6, The Hague 2497CB, The Netherlands



A comparison of stochastic variation in mixed and unmixed casework and synthetic samples

Jo-Anne Bright **, Kurt McManus *, SallyAnn Harbison *, Peter Gill br, John Buckleton *

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Centre for Formuk Science, University of Strathclyde, Giagow, OK

Contents lists available at SolVerse ScienceDirect

Forensic Science International: Genetics

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

Inference about the number of contributors to a DNA mixture: Comparative analyses of a Bayesian network approach and the maximum allele count method

A. Biedermann^{a,*}, S. Bozza^b, K. Konis^c, F. Taroni^a

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Automating a combined composite-consensus method to generate DNA profiles from low and high template mixture samples

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Steps in DNA Interpretation



Important Lessons

- People think they understand the basics of interpretation better than they actually do – this is what leads to observed variation in interpreting mixtures...
- Increased complexity of mixtures (with more allele sharing) leads to higher uncertainty which leads to lack of confidence in potential contributor genotypes
- Worked examples are beneficial in training (participants need to work through the examples themselves)
- There is value in using a profile interpretation worksheet to document assumptions and decisions made

Value of Using a Profile Interpretation Worksheet

PROFILE INTERPRETATION WORKSHEET

PROFILE NAME: Case Example #3

ANALYST: John Butler

DATE: 11 October 2010

MIXTURE: ∎ yes □ no □ unsure

Allele and Locus Assessments

Analytical threshold: 30 RFU

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

Stochastic threshold: 150 RFU

Peak height ratio: 60%

Comments: low level DNA (125 pg)

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	Ν	N	

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

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 work in combination with CPI statistics but may not apply for >2 person mixtures (due to potential allele shares)
- 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



Greg Matheson on Forensic Science Philosophy

The CAC News – 2nd Quarter 2012 – p. 6 "Generalist vs. Specialist: a Philosophical Approach" http://www.cacnews.org/news/2ndq12.pdf

 If you want to be a technician, performing tests on requests, then just focus on the policies and procedures of your laboratory. If you want to be a scientist and a professional, learn the policies and procedures, but go much further and learn the philosophy of your profession. Understand the **importance of why things are done** the way they are done, the scientific method, the viewpoint of the critiques, the issues of bias and the importance of ethics.

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

ISHI 2010 Mixture Workshop October 11, 2010

got stutter?

got stochastic effects?

Catherine Grgicak Boston U.

got allele

Mike Coble NIST

Robin Cotton Boston U. John Butler NIST

got mixtures?

> Charlotte Word Consultant

got

estricted

enotypes?

Promega ISHI 2012 Mixture Workshop



Mixtures Using Sound statistical analysis Interpretation & Conclusions John Butler, Ph.D., NIST, Gaithersburg, MD
Michael Coble, Ph.D., NIST, Gaithersburg, MD
Robin Cotton, Ph.D., Boston University, Boston, MA
Catherine Grgicak, Ph.D., Boston University, Boston, MA
Charlotte J. Word, Ph.D., Gaithersburg, MD

This workshop is for analysts, technical reviewers and technical leaders performing and interpreting validation studies and/or interpreting and reviewing STR data, particularly more difficult mixtures. Various DNA profiles will be analyzed and interpreted using selected analytical thresholds and stochastic thresholds to demonstrate the impact of those values on the profiles amplified with low-template DNA vs. higher amounts of DNA. Different statistical approaches and conclusions suitable for the profiles will be presented.