

# 2012 Mixture Interpretation Workshop:

Mixtures Using *SOUND* Statistics, Interpretation, & Conclusions



## Perspectives on the Future: What We Have Learned and Where We Need to Go

John M. Butler

October 15, 2012

Nashville, TN



# 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...**



# Greg Matheson on Forensic Science Philosophy

The CAC News – 2<sup>nd</sup> 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.

# My Goals in This Presentation

- 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

# Feedback from a Previous Workshop

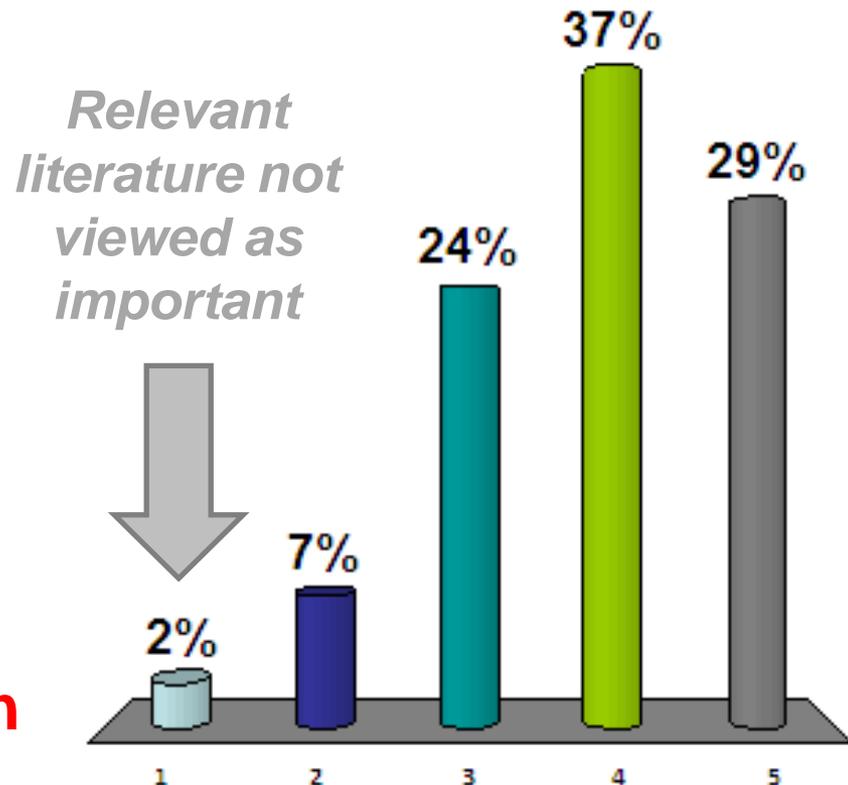
Which of the topics below would be your first choice for additional training?

1. Relevant literature
2. How to validate thresholds
3. How to develop relevant SOPs

4. Interpretation of low level mixtures
5. Statistics

**2/3 want more information on these topics**

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



# Mixture Literature

*you should be reading...*

See DNA Mixtures  
Reference List provided  
with workshop materials

# Quality Assurance Standard Requirement for Literature Review

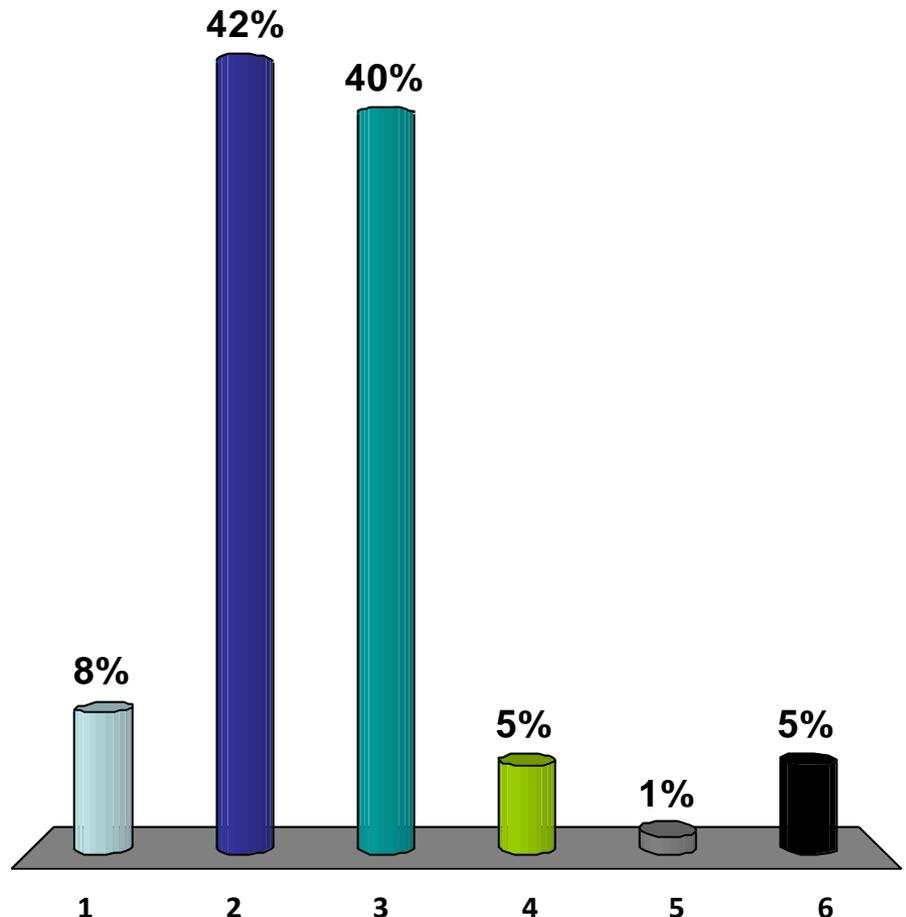
Quality Assurance Standards for Forensic DNA Testing Laboratories  
(effective September 1, 2011)

**5.1.3.2.** The laboratory shall have a program approved by the technical leader for the **annual review of scientific literature** that documents the analysts' ongoing reading of scientific literature. **The laboratory shall maintain or have physical or electronic access to a collection of current books, reviewed journals, or other literature applicable to DNA analysis.**

# 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!

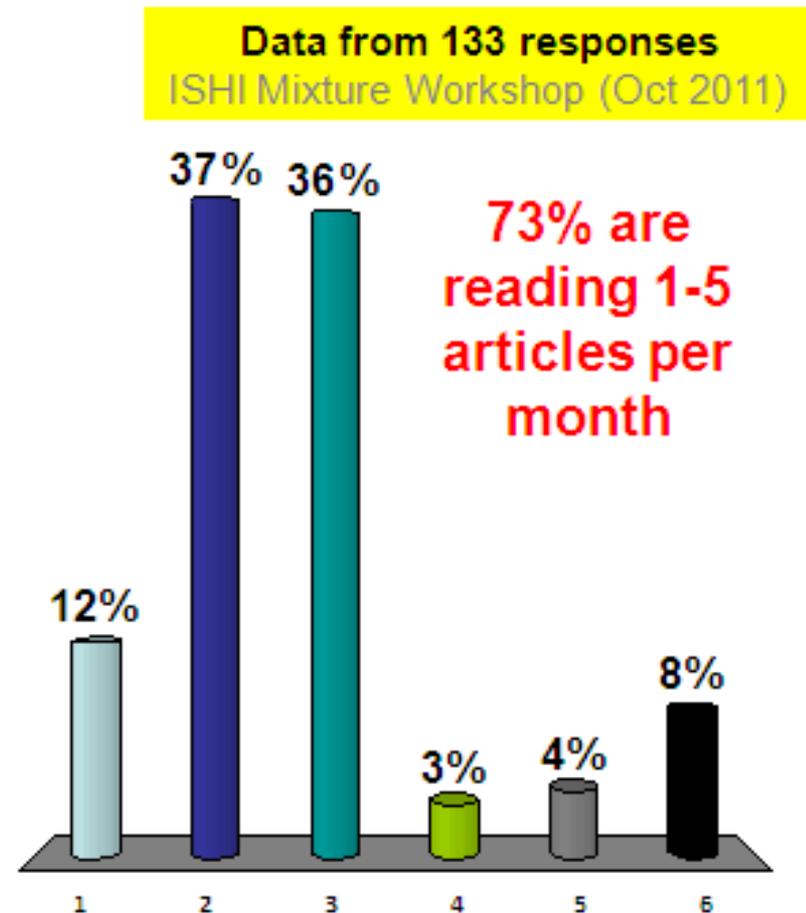
**Data from 106 responses**  
ISHI Mixture Workshop (Oct 2012)



# Last Year's Response

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!



# Importance of Reading the Literature

How can you keep up and improve?

- Develop a culture in your laboratory to read the literature and share information with one another
- Obtain access to appropriate journals
  - Join AAFS and/or ISFG
  - Develop a relationship with a local university in order to get access to the latest journal articles
- Read, Think, and Implement Improvements!

# 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**

# Number of Articles Published on DNA and DNA Mixtures

<http://www.ncbi.nlm.nih.gov/pubmed>

Journal Name	“DNA”	“DNA mixtures”	“DNA mixtures” in 2012
<i>Forensic Sci. Int. / FSI Genetics</i>	1484	<b>68</b>	<b>15</b>
<i>J. Forensic Sci.</i>	1196	45	2
<i>Int. J. Legal Med.</i>	659	39	5
<i>Croatian Med. J.</i>	155	12	4
<i>Science &amp; Justice</i>	73	5	0

**PubMed.gov search conducted September 14, 2012** using “DNA” or “DNA mixtures” and journal name with and without “and 2012”

# Workshop *DNA Mixtures Reference List*

Topic category	# References
Mixture Principles & Recommendations	13
Setting Thresholds	11
Stutter Products & Peak Height Ratios	19
Stochastic Effects & Allele Dropout	18
Estimating the Number of Contributors	15
Mixture Ratios	9
Statistical Approaches	23
Low Template DNA Mixtures	8
Separating Cells to Avoid Mixtures	3
Software (plus 12 websites)	7
Probabilistic Genotyping Approach	11
General Information on Mixtures	7
<b>TOTAL</b>	<b>144</b>

**7/8 in the past year;**  
mostly in *FSI Genetics*

Will be regularly updated on <http://www.cstl.nist.gov/strbase/mixture.htm>

# Recent articles on mixtures not found in JFS...

Forensic Science International: Genetics 6 (2012) 191–197

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: [www.elsevier.com/locate/fsig](http://www.elsevier.com/locate/fsig)



## The interpretation of low level DNA mixtures

Hannah Kelly<sup>a,\*</sup>, Jo-Anne Bright<sup>a</sup>, James Curran<sup>b</sup>, John Buckleton<sup>a</sup>

<sup>a</sup>ESR, PB 92021 Auckland, New Zealand

<sup>b</sup>Department of Statistics, University of Auckland, PB 92019 Auckland, New Zealand

Contents lists available at SciVerse ScienceDirect

Forensic Science International: Genetics

journal homepage: [www.elsevier.com/locate/fsig](http://www.elsevier.com/locate/fsig)



## Assessment of mock cases involving complex low template DNA mixtures: A descriptive study

Corina C.G. Benschop, Hinda Haned, Tanja J.P. de Blaeij, Alexander J. Meulenbroek, Titia Sijen<sup>\*</sup>

Department of Human Biological Traces, Netherlands Forensic Institute, P.O. Box 24044, 2400 AA The Hague, The Netherlands

Forensic Science International: Genetics 8 (2012) 102–107

Contents lists available at ScienceDirect

Forensic Science International: Genetics

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

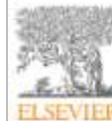
Natalie E.C. Weiler<sup>1</sup>, Anuska S. Matai<sup>1</sup>, Titia Sijen<sup>2</sup>

<sup>1</sup>Netherlands Forensic Institute, Laan van Spynburg 6, The Hague 2517CA, The Netherlands

Contents lists available at SciVerse ScienceDirect

Forensic Science International: Genetics

journal homepage: [www.elsevier.com/locate/fsig](http://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<sup>a,\*</sup>, S. Bozza<sup>b</sup>, K. Konis<sup>c</sup>, F. Taroni<sup>a</sup>

<sup>a</sup>University of Lausanne, School of Criminal Justice, Lausanne, Switzerland

<sup>b</sup>University Ca' Foscari of Venice, Department of Economics, Venice, Italy

<sup>c</sup>École Polytechnique Fédérale de Lausanne, Chair of Mathematical Statistics, Lausanne, Switzerland

Forensic Science International: Genetics 6 (2012) 180–184

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: [www.elsevier.com/locate/fsig](http://www.elsevier.com/locate/fsig)



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

Jo-Anne Bright<sup>a,\*</sup>, Kurt McManus<sup>a</sup>, SallyAnn Harbison<sup>a</sup>, Peter Gill<sup>b,c</sup>, John Buckleton<sup>a</sup>

<sup>a</sup>ESR, Private Bag 92021, Auckland, New Zealand

<sup>b</sup>Institute of Forensic Medicine, Oslo University, Norway

<sup>c</sup>Centre for Forensic Science, University of Strathclyde, Glasgow, UK

Contents lists available at SciVerse ScienceDirect

Forensic Science International: Genetics

journal homepage: [www.elsevier.com/locate/fsig](http://www.elsevier.com/locate/fsig)



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

Bram Bekaert<sup>a,1,\*</sup>, Anneleen Van Geystelen<sup>b,c,1</sup>, Nancy Vanderheyden<sup>a</sup>, Maarten H.D. Larmuseau<sup>a,d,e</sup>, Ronny Decorte<sup>a,b</sup>

<sup>a</sup>UZ Leuven, Laboratory of Forensic Genetics and Molecular Anthropology, UZ Leuven, Leuven, Belgium

<sup>b</sup>Applied Molecular Genetics Group, Department of Molecular Genetics, Flanders Institute for Biotechnology (VIB), Flanders, Belgium

<sup>c</sup>University of Antwerp (UAntwerp), Antwerp, Belgium

<sup>d</sup>RUG Leuven, Laboratory of Animal Diversity and Systematics, Leuven, Belgium

<sup>e</sup>RUG Leuven, Department of Human Genetics, Campus Gasthuisberg, Leuven, Belgium

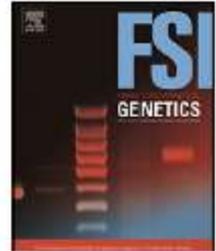
# The Latest Issue of *FSI Genetics* is on DNA Interpretation and Mixture Challenges



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Forensic Science International: Genetics

journal homepage: [www.elsevier.com/locate/fsig](http://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<sup>a,b,\*</sup>, L. Gusmão<sup>c</sup>, H. Haned<sup>d</sup>, W.R. Mayr<sup>e</sup>, N. Morling<sup>f</sup>, W. Parson<sup>g</sup>, L. Prieto<sup>h</sup>,  
M. Prinz<sup>i</sup>, H. Schneider<sup>j</sup>, P.M. Schneider<sup>k</sup>, B.S. Weir<sup>l</sup>

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<sup>f</sup> Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

<sup>g</sup> Institute of Legal Medicine, Innsbruck Medical University, Innsbruck, Austria

<sup>h</sup> Comisaría General de Policía Científica, University Institute of Research in Forensic Sciences (IUICP), Madrid, Spain

<sup>i</sup> Office of the Chief Medical Examiner, Department of Forensic Biology, New York, USA

<sup>j</sup> Hessisches Landeskriminalamt, Wiesbaden, Germany

<sup>k</sup> Institute of Legal Medicine, Faculty of Medicine, University of Cologne, Germany

<sup>l</sup> University of Washington, Department of Biostatistics, Seattle, USA

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Please note that you will receive the confirmation of your membership by email. Together with this mail, you will receive information about the payment of membership fees (at present EUR 60.00 per year). The membership fee includes access to the congress proceedings [Progress in Forensic Genetics](#), published online every other year after the ISFG conference.

In addition, all ISFG members receive a complimentary subscription (print and online version) of the scientific journal [Forensic Science International: Genetics](#) which is published in affiliation with our society.



# Abstracts are Freely Available on Website

<http://www.fsigenetics.com/>

The screenshot shows the homepage of the FSIGENETICS website. At the top left is the logo for FSIGENETICS. To the right, there is a navigation bar with links for "Welcome, Dr. John BUTLER", "Claim", "My Account", and "Logout". Below this is a secondary navigation bar with links for "Articles & Issues", "For Authors", "Journal Info", "Subscribe", "ISFG", and "More Periodicals". A search bar is located below the navigation, with a dropdown menu set to "All Fields" and a "Go" button. The main content area is divided into three columns. The left column features a "New Issue Alert" button and a "Free Trial Issue" button, both highlighted with a red arrow. The middle column displays the "Current Issue" for September 2012, Vol. 6, No. 5, with a section for "Issue Highlights" listing three articles: "Competition for DNA binding sites using Promega DNA IQ™ paramagnetic beads", "Performance of two 17 locus forensic identification STR kits—Applied Biosystems's AmpFℓSTR® NGMSelect™ and Promega's PowerPlex® ESI17 kits", and "An investigation of admixture in an Australian Aboriginal Y-chromosome STR database". The right column contains links for "Access this journal on SciVerse ScienceDirect", "Print or Share This Page", "Journal Access" information, "ISFG Member Access", "Activate Online Access", and "About Forensic Science International: Genetics".

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**On the Cover**

**Current Issue** | [September 2012, Vol. 6, No. 5](#)

**Issue Highlights**

Competition for DNA binding sites using Promega DNA IQ™ paramagnetic beads  
September 2012(Vol. 6 | No. 5 | Pages 511-522)  
[Abstract](#) | [Full Text](#) | [PDF \(656 KB\)](#) | [Supplemental Materials](#)

Performance of two 17 locus forensic identification STR kits—Applied Biosystems's AmpFℓSTR® NGMSelect™ and Promega's PowerPlex® ESI17 kits  
September 2012(Vol. 6 | No. 5 | Pages 523-531)  
[Abstract](#) | [Full Text](#) | [PDF \(507 KB\)](#) | [Supplemental Materials](#)

An investigation of admixture in an Australian Aboriginal Y-chromosome STR database  
September 2012(Vol. 6 | No. 5 | Pages 532-538)  
[Abstract](#) | [Full Text](#) | [PDF \(366 KB\)](#) | [Supplemental Materials](#)

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Forensic Science International: Genetics is an international journal dedicated to the applications of genetics in the administration of justice.

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# *FSI Genetics Supplement Series*

## Articles are Freely Available

**Articles (2-3 pages each) covering presentations given at the ISFG meetings every two years**



Current Issue | December 2011, Vol. 3, No. 1

### Issue Highlights

DIP-STR: A new marker for resolving unbalanced DNA mixtures

December 2011 (Vol. 3 | No. 1 | Pages e1-e2)

D. Hall, V. Castella

[Abstract](#) | [Full Text](#) | [PDF \(156 KB\)](#)

<http://www.fsigeneticssup.com>

**2011: 281 articles**

**2009: 253 articles**

**2007: 272 articles**

Forensic Science International: Genetics Supplement Series 3 (2011) e1–e2

Contents lists available at ScienceDirect

 Forensic Science International: Genetics Supplement Series

journal homepage: [www.elsevier.com/locate/FSIGSS](http://www.elsevier.com/locate/FSIGSS)



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DIP-STR: A new marker for resolving unbalanced DNA mixtures

D. Hall\*, V. Castella

Forensic Genetic Unit, University Center of Legal Medicine Lausanne and Geneva, Rue du Bugnon 21, CH-1011 Lausanne, Switzerland

# Know the Literature

- Sometimes articles may not be all that they claim to be – evaluate them critically
- Stay informed in order to be a good scientist
- **M**ixtures **U**sing *SOUND* **S**tatistics, **I**nterpretation, and **C**onclusions involves knowing the literature (past and present)

# 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 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%*

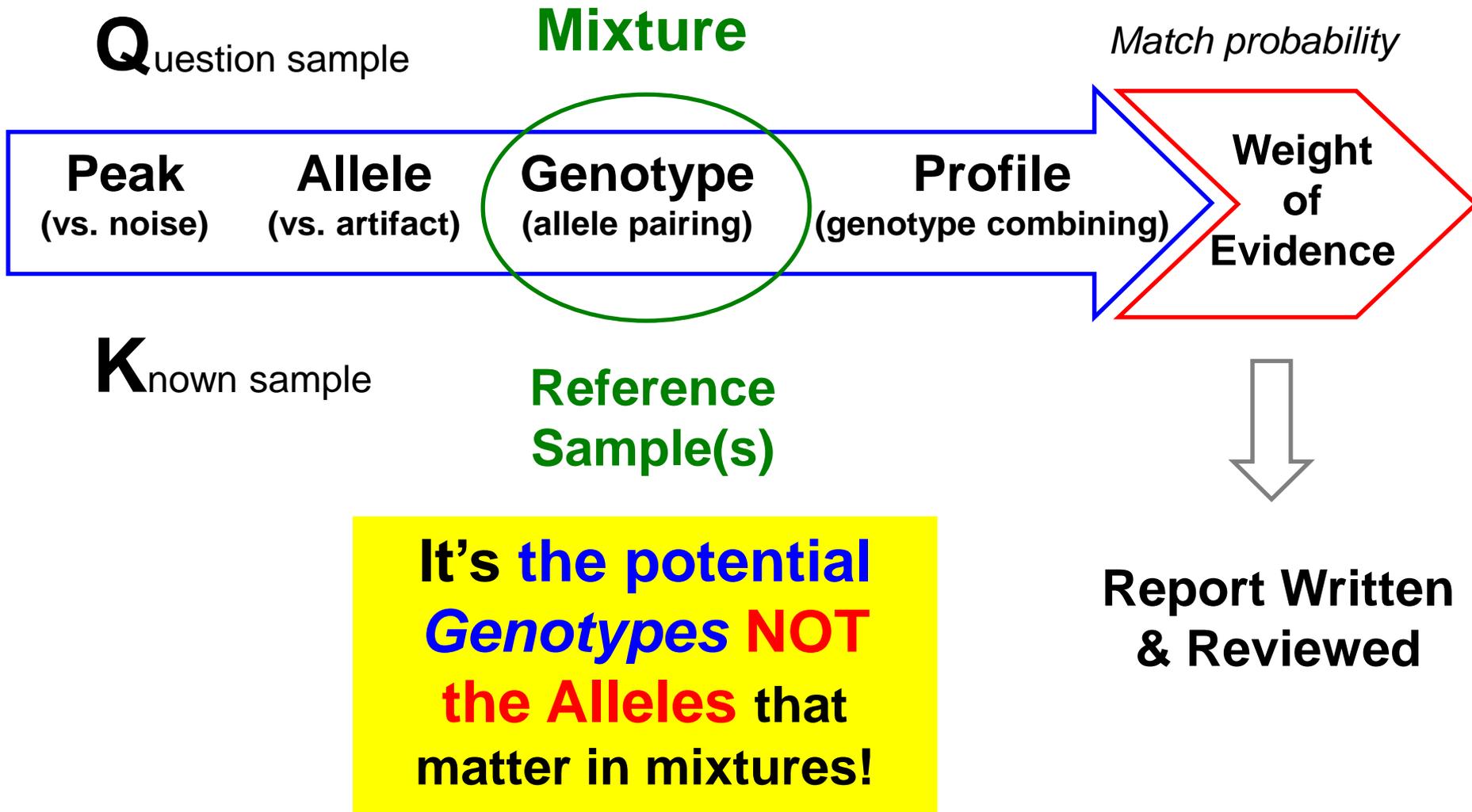
Comments: *low level DNA (125 pg)*

### 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**

# Steps in DNA Interpretation



# 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 sound 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

# 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**

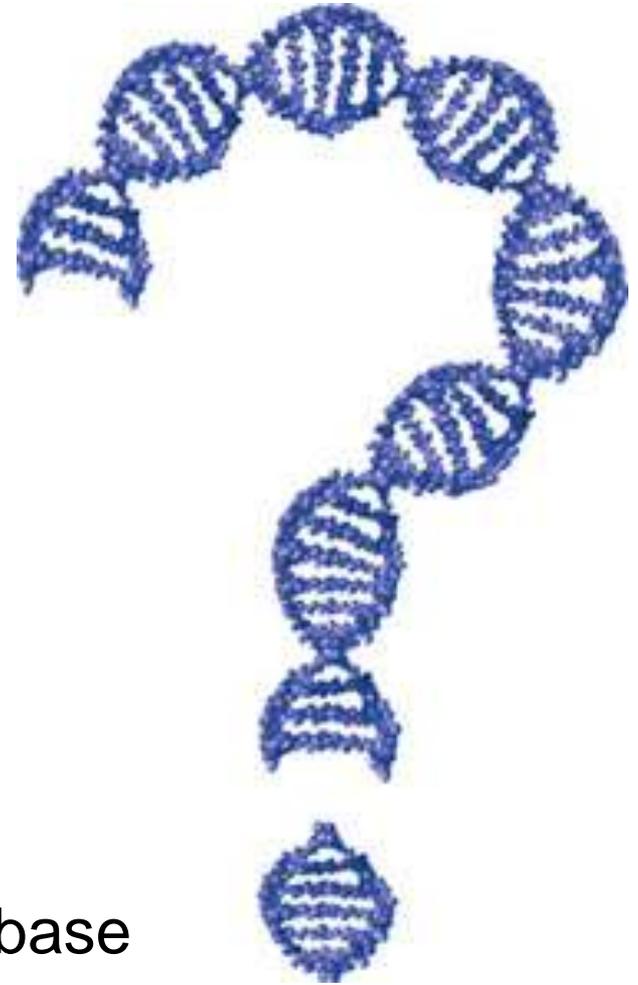
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<http://www.cstl.nist.gov/biotech/strbase>



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