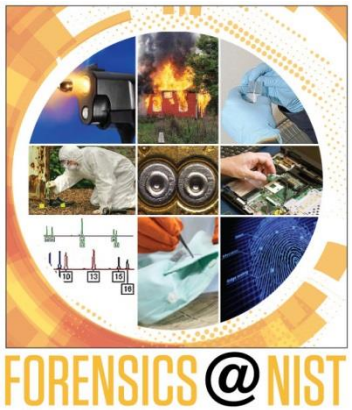


DNA Mixture Interpretation Webcast

April 12, 2013

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

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

Lessons Learned, Recent Literature, and Future Directions

John M. Butler

National Institute of Standards and Technology

Comments on Mixture Training We Have Conducted The Past Three 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 – 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.

My Goals in This Presentation

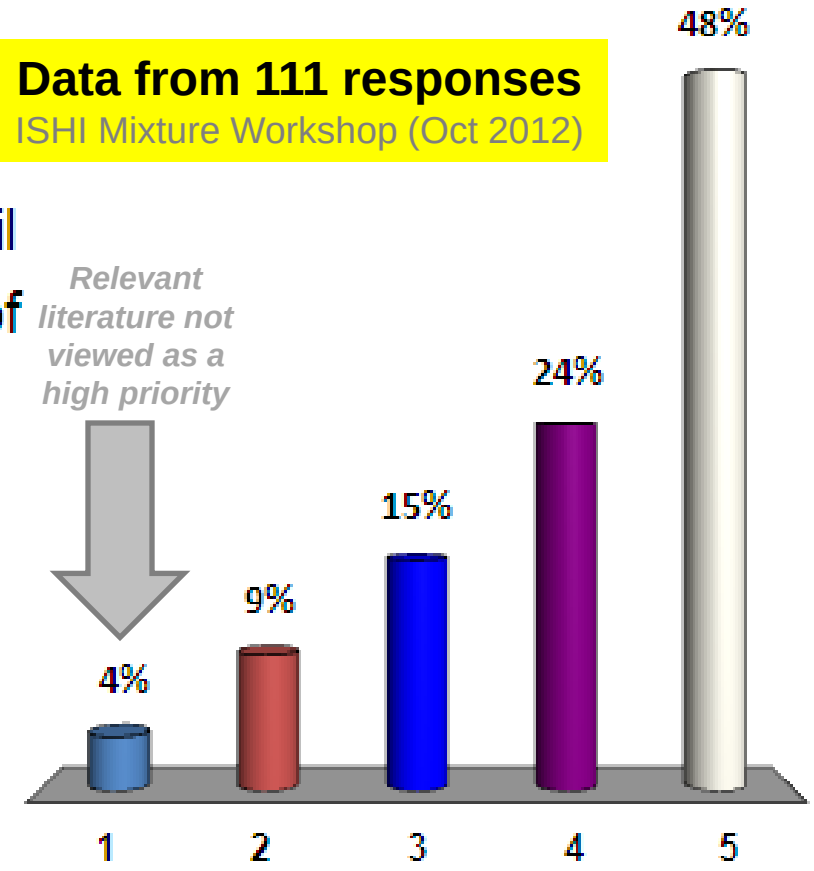
- Valuable mixture literature and how to obtain it
- Important lessons & common misunderstandings
- Thoughts on where we need to go as a community to improve mixture interpretation

2012 Response at ISHI Workshop

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)



~75% want more information on these topics

Mixture Literature

you should be reading…

See DNA Mixtures
Reference List on
STRBase mixture section

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

Quality Assurance Standard Requirement for Literature Review

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

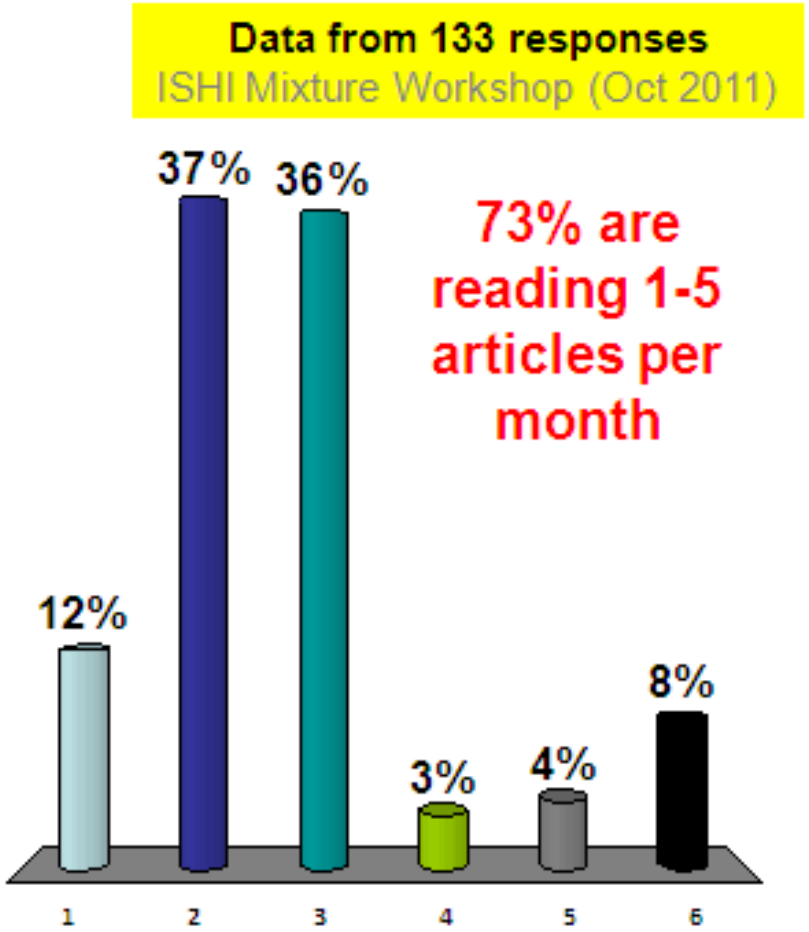
5.1.3.2. The laboratory shall have a program approved by the technical leader for the **annual review of scientific literature** that documents **the analysts' ongoing reading of scientific literature**. **The laboratory shall maintain or have physical or electronic access to a collection of current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

http://www.fbi.gov/about-us/lab/codis/qas-standards-for-forensic-dna-testing-laboratories-effective-9-1-2011

2011 Response at ISHI Workshop

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!

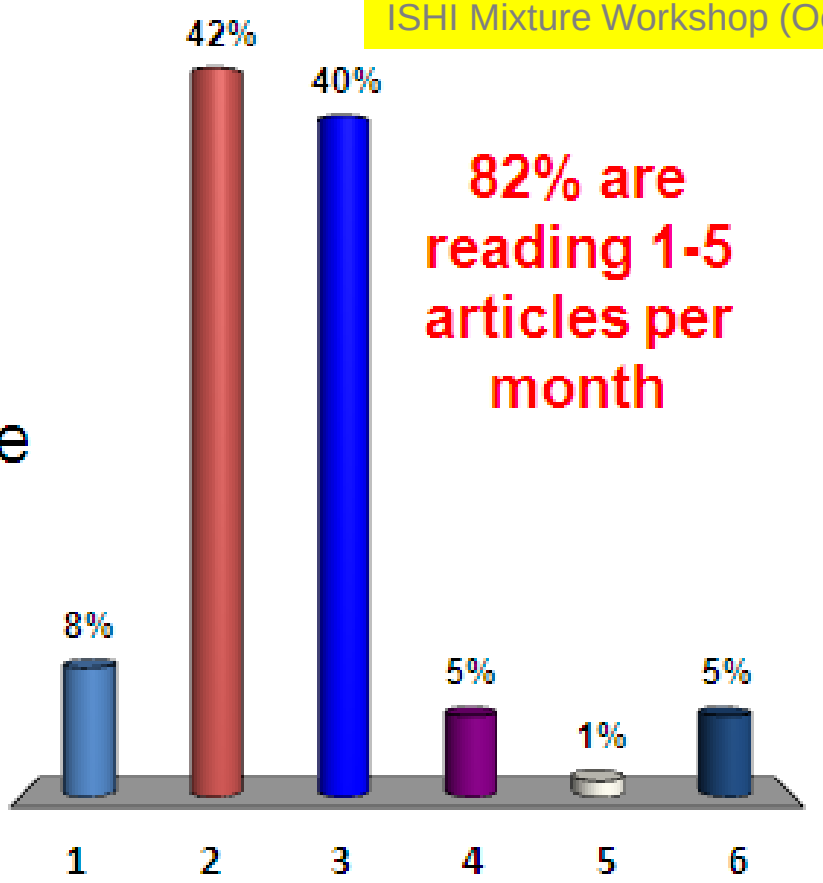


2012 Response at ISHI Workshop

How many DNA-related articles would you estimate that you read in a typical month?

Data from 106 responses
ISHI Mixture Workshop (Oct 2012)

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!



82% are reading 1-5 articles per month

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

- **Analysts need time 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
Forensic Sci. Int. / FSI Genetics	1484	68	15
J. Forensic Sci.	1196	45	2
Int. J. Legal Med.	659	39	5
Croatian Med. J.	155	12	4
Science & Justice	73	5	0

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

STRBase 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
TOTAL	144

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

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

Forensic Science International: Genetics 6 (2012) 102–107

Contents lists available at ScienceDirect

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. Weiler [1], Anuska S. Matai [1], Titia Sijen [*]

Netherlands Forensic Institute, Laan van Ypenburg 6, The Hague 2497GB, The Netherlands

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

Contents lists available at ScienceDirect

Forensic Science International: Genetics

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

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

Jo-Anne Bright [a,*], Kurt McManus [a], SallyAnn Harbison [a], Peter Gill [b,c], John Buckleton [a]

[a] ESR, Private Bag 92021, Auckland, New Zealand
[b] Institute of Forensic Medicine, Oslo University, Norway
[c] 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

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 [*]

Department of Human Biological Traces, Netherlands Forensic Institute, P.O. Box 24044, 2490 AA The Hague, The Netherlands

Contents lists available at SciVerse 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]

[a] University of Lausanne, School of Criminal Justice, Lausanne, Switzerland
[b] University 'Ca' Foscari' of Venice, Department of Economics, Venice, Italy
[c] École Polytechnique Fédérale de Lausanne, Chair of Mathematical Statistics, Lausanne, Switzerland

Contents lists available at SciVerse ScienceDirect

Forensic Science International: Genetics

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

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

Bram Bekaert [a,1,*], Anneleen Van Geystelen [b,c,1], Nancy Vanderheyden [a], Maarten H.D. Larmuseau [a,d,e], Ronny Decorte [a,e]

[a] UZ Leuven, Laboratory of Forensic Genetics and Molecular Archaeology, UZ Leuven, Leuven, Belgium
[b] Applied Molecular Genomics Group, Department of Molecular Genetics, Flanders Institute for Biotechnology (VIB), Flanders, Belgium
[c] University of Antwerp (UA), Antwerp, Belgium
[d] K.U. Leuven, Laboratory of Animal Diversity and Systematics, Leuven, Belgium
[e] K.U. Leuven, Department of Human Genetics, Campus Gasthuisberg, Leuven, Belgium

December 2012 Issue of *FSI Genetics* is on **DNA Interpretation Challenges and Solutions**

Contents lists available at SciVerse ScienceDirect

Forensic Science International: Genetics

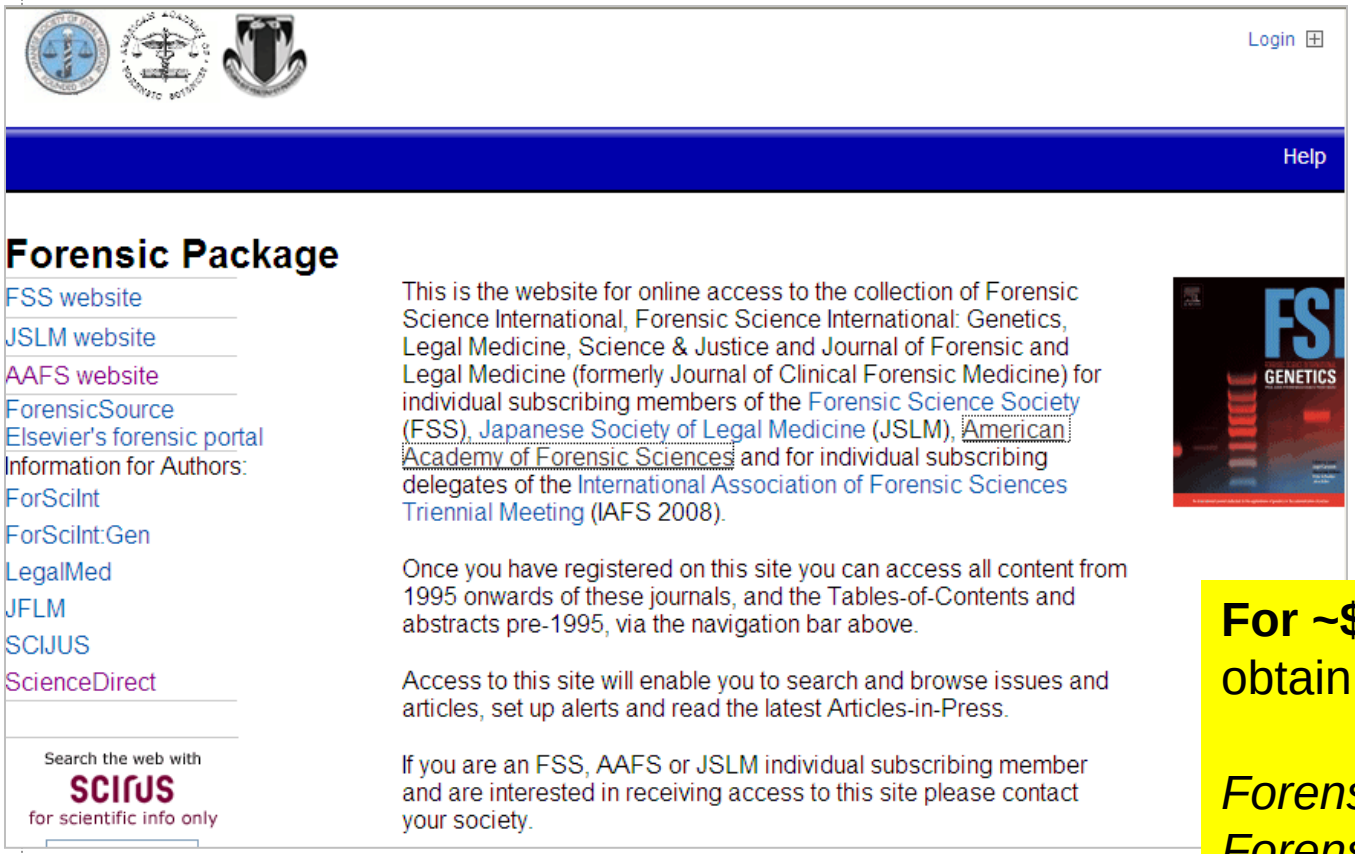
journal homepage: www.elsevier.com/locate/fsig

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]

[a] *Norwegian Institute of Public Health, Oslo, Norway*
[b] *University of Oslo, Oslo, Norway*
[c] *IPATIMUP, Institute of Molecular Pathology and Immunology of the University of Porto, Portugal*
[d] *Netherlands Forensic Institute, Department of Human Biological Traces, The Hague, The Netherlands*
[e] *Department of Blood Group Serology and Transfusion Medicine, Medical University of Vienna, Austria*
[f] *Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark*
[g] *Institute of Legal Medicine, Innsbruck Medical University, Innsbruck, Austria*
[h] *Comisaría General de Policía Científica, University Institute of Research in Forensic Sciences (IUICP), Madrid, Spain*
[i] *Office of the Chief Medical Examiner, Department of Forensic Biology, New York, USA*
[j] *Hessisches Landeskriminalamt, Wiesbaden, Germany*
[k] *Institute of Legal Medicine, Faculty of Medicine, University of Cologne, Germany*
[l] *University of Washington, Department of Biostatistics, Seattle, USA*

Elsevier Journal Package Available with AAFS Membership



http://www.sciencedirect.com/forpac

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Abstracts are Freely Available on Website

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On the Cover

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Supplement Series

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

Articles in Press | Most Read | Most Cited

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Journal Access

Full-text articles are available from 2007 to the present. Access to abstracts is complimentary. Access to full text is limited to ISFG members as well as personal subscribers.

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About *Forensic Science International: Genetics*

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

FSI Genetics Supplement Series Articles are Freely Available

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

On the Cover

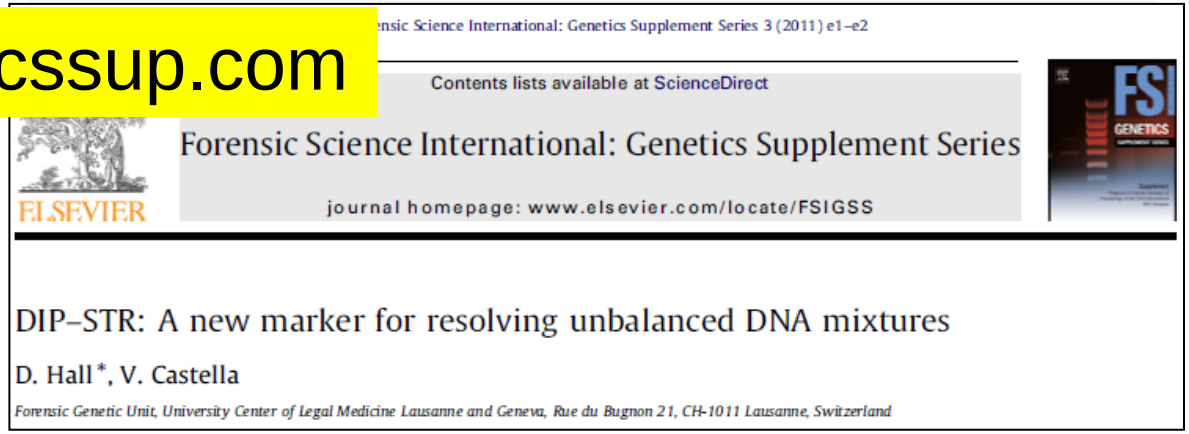
Current Issue | December 2011, Vol. 3, No. 1

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


nsic 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

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)

Mixtures **U**sing *SOUND* **S**tatistics, **I**nterpretation, & **C**onclusions

2012

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, which is typically due to using different subsets of the data and/or different assumptions

- 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

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%*

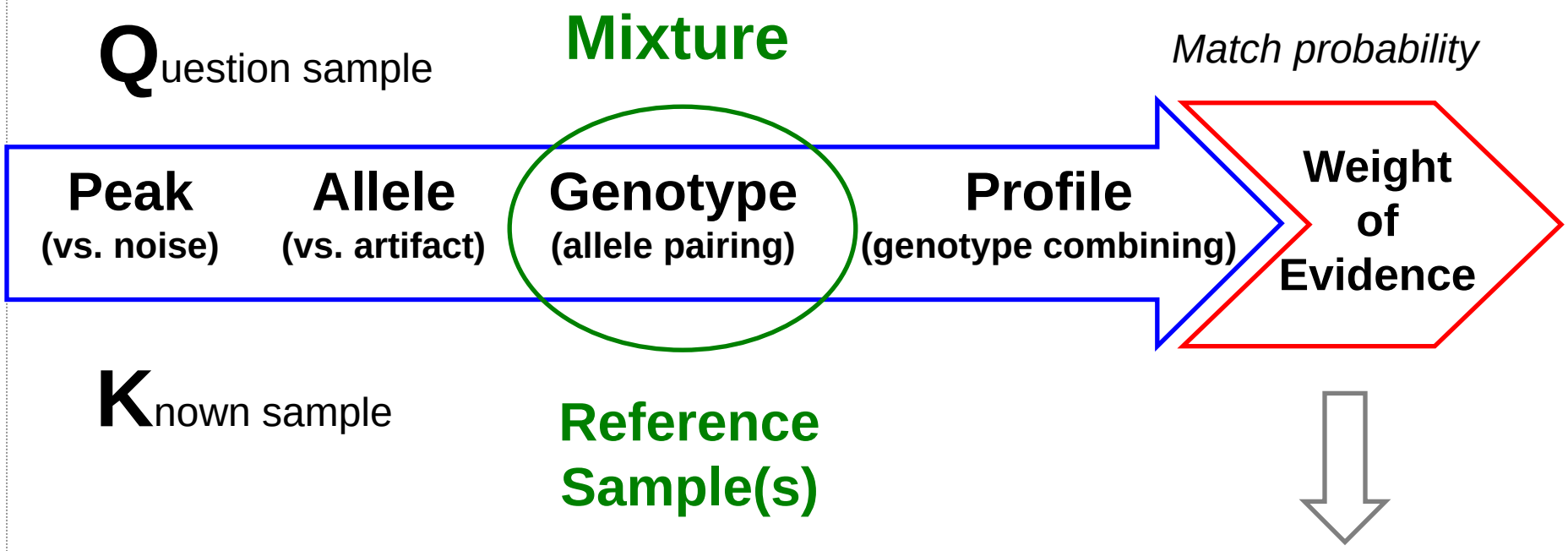
Comments: *low level DNA (125 pg)*

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

Steps in DNA Interpretation



It's the potential *Genotypes* NOT the Alleles that matter in mixtures!

Report Written & Reviewed

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
  - With PopStats, a single peak is calculated as p^2 (not 2p)

- 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 a stochastic threshold may not hold much meaning 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 <u>sound</u> interpretation**.” (Rome, April 2012 meeting)

Thoughts on Where We Need to Go (1)

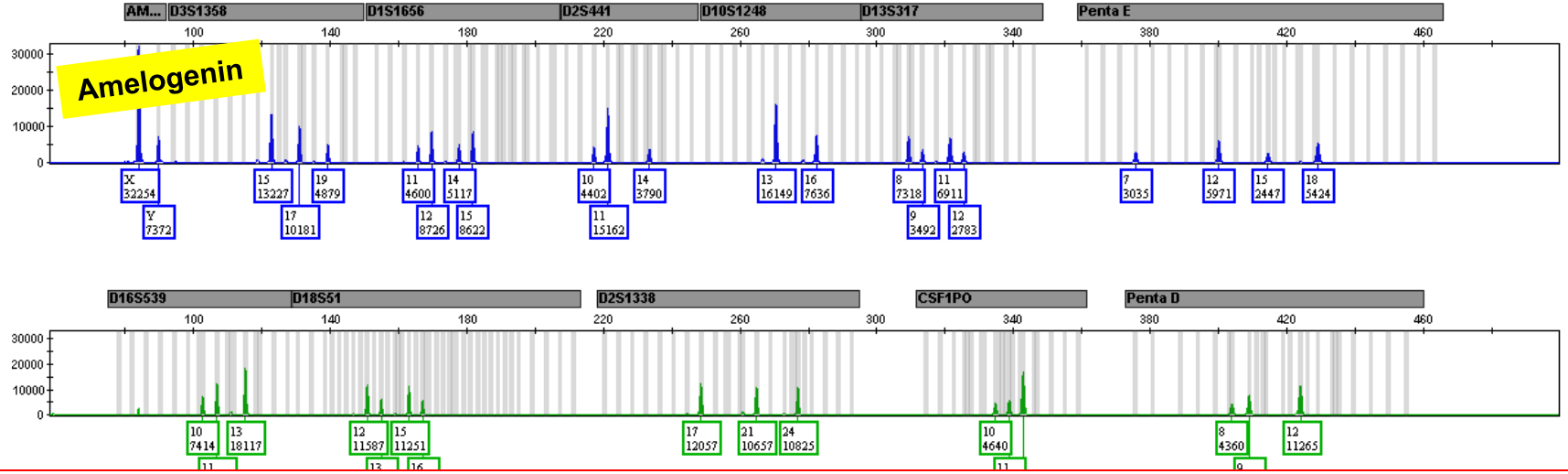
- Away from CPI and towards likelihood ratio approaches
  - As noted in the Gill et al. (2006) ISFG DNA Commission recommendation #2
- This will require software to perform the calculations
  - This software will need to be validated
  - Peter Gill and others are pushing freeware solutions
- Still will require analysts to understand what is going on in the computer calculations!
  - Will require more significant engagement in mixture training

Thoughts on Where We Need to Go (2)

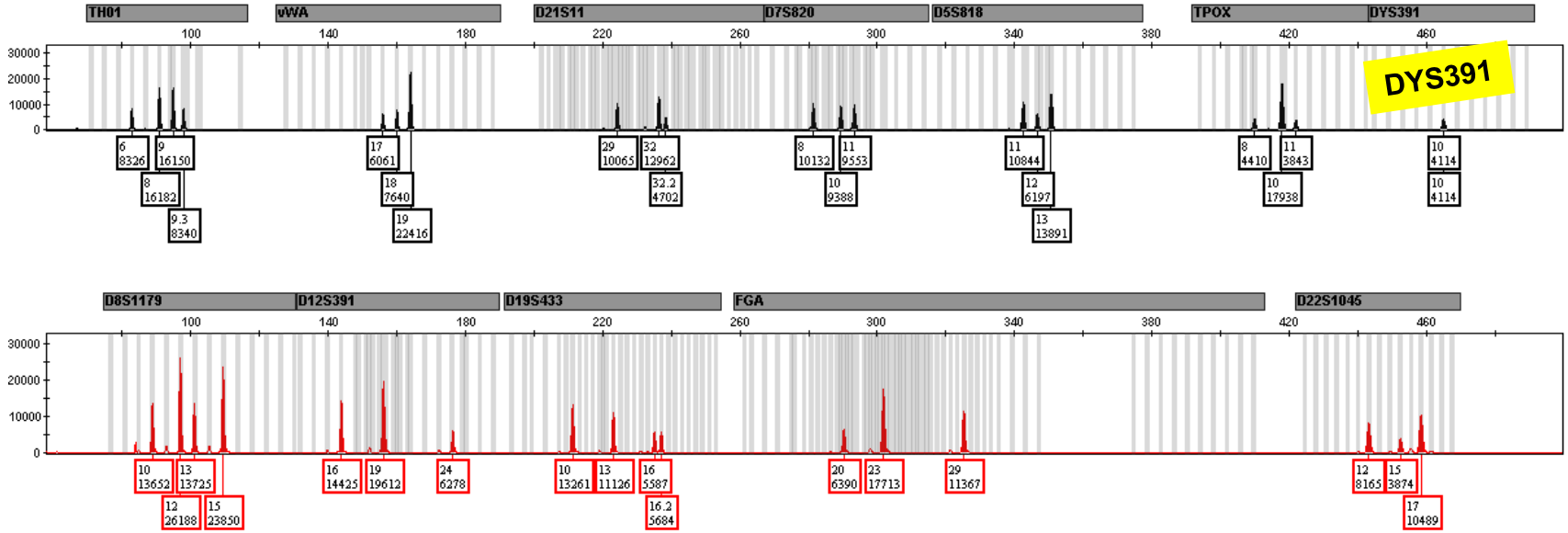
- Validation studies need to support interpretation SOPs and software packages

- The U.S. will be moving to more STR loci in the near future (from 13 to ~20 core STRs)
  - Using additional loci with better powers of discrimination will improve detection of mixtures
  - **But more loci means more interpretation time!**

DNA Mixture Detected with PowerPlex Fusion (24plex STR kit)



22 autosomal STR loci need to be interpreted…(+50% over current 15 STRs)



Size standard not shown

Data courtesy of Becky Hill (NIST)

Webcast Format for Training

- **With cuts in federal budgets, webcasts or webinars may become more appealing in the future to reduce costs in providing training**

- Please let us know about any technical difficulties that you may have faced so that we can improve future webcasts

- We welcome suggestions for additional content or topics to cover in future webcast training events

- Please contact John Paul Jones at 301-975-2782 or john.jones@nist.gov

Posting of Video from this Event

- Following transcription of this webcast (this process takes about a month), **we plan to post videos of each presentation on a publicly-available NIST website**
- All those who registered for the webcast (onsite or online) will receive email notification of this website URL
- A link to the webcast video website will also be available from the STRBase mixture website to enable future viewing or downloading of video or presentation materials
- Due to costs of maintaining large video files on NIST servers, **webcast videos may only be available for a limited time** (we are planning on at least six months)

Concern for Potential Misuse of Webcast Presentations

- We remind current and future viewers that presentations reflect the presenters' opinions at the time they were given on April 12, 2013
- Please do not take any specific comments of the webcast presenters out of context in order to advance either scientific or legal arguments
- Science advances with new discoveries and therefore scientific opinions may change over time given exposure to new ideas or techniques

Acknowledgments

- **Fellow presenters: Mike, Robin, Bruce, and Charlotte**
- **NIST A/V and webcast staff**
- Past and present funding to the NIST Applied Genetics Group from the **National Institute of Justice** (NIJ) and the **NIST Law Enforcement Standards Office** (OLES)
- NIJ Forensic Science Training Development and Delivery Program Grant (2008-DN-BX-K158) to the Biomedical Forensic Science Program at **Boston University** School of Medicine
- **Catherine Grgicak** (BU Program) for mixture data
- **Maryland State Police Forensic Sciences Division** for permitting involvement of Bruce Heidebrecht
- **John Paul Jones** from NIST OLES for organizing and coordinating this event

Thank you for your attention

Contact Information

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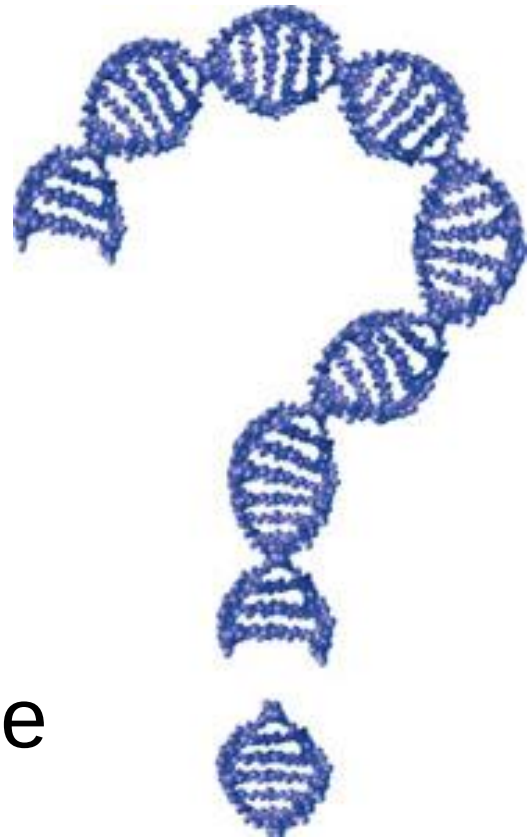
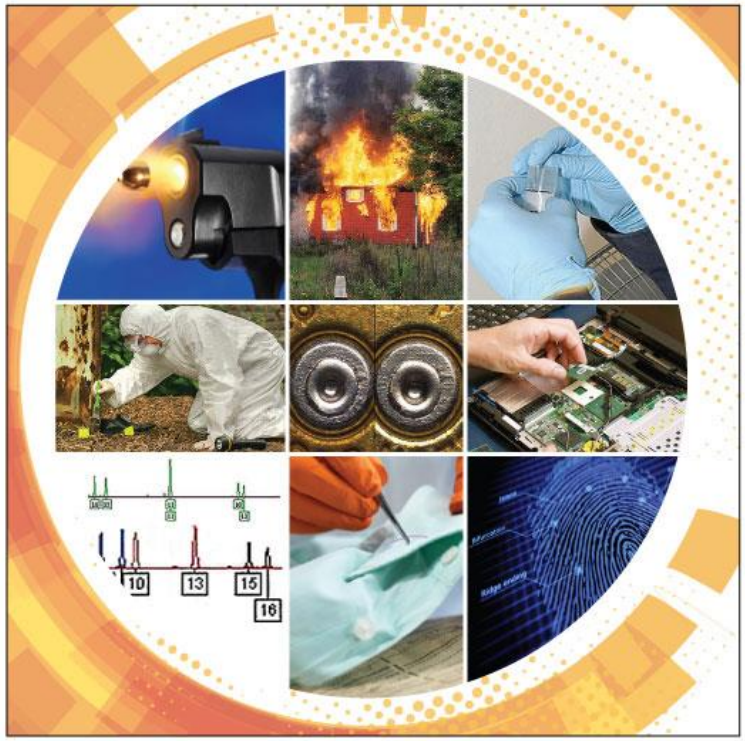
NIST Fellow
john.butler@nist.gov
301-975-4049

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



Additional DNA mixture information available at:
http://www.cstl.nist.gov/strbase/mixture.htm

NIST FORENSIC SCIENCES



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