Forensic DNA Mixture Interpretation

Mixture Fundamentals & Literature Review

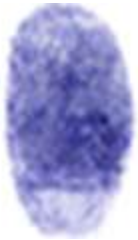
MAFS Workshop

Milwaukee, WI
September 25, 2012

Dr. John M. Butler
National Institute of Standards and Technology

john.butler@nist.gov

The Forensic Institute



Forensic e-Symposium™

The Forensic Institute

April 14, 2005

"If you show 10 colleagues a mixture, you will probably end up with 10 different answers."

- Dr. Peter Gill

Comments Regarding Mixture Training We Have Conducted the Past Several 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

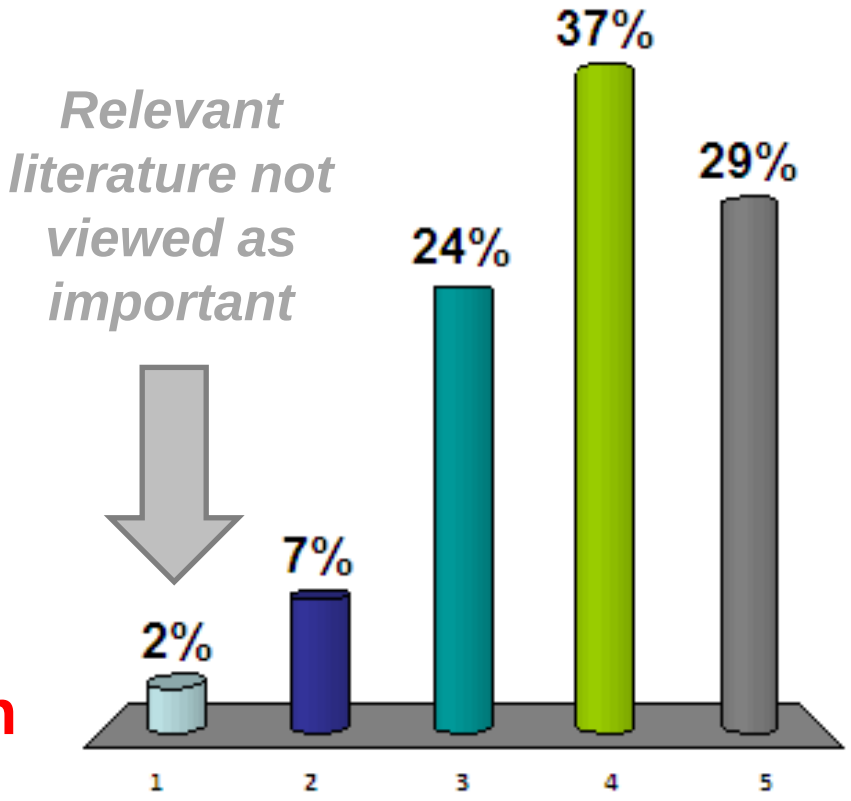
- **The information content in our workshops has continued to evolve to include the latest published articles…**

Feedback from a Previous Workshop

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

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

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

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

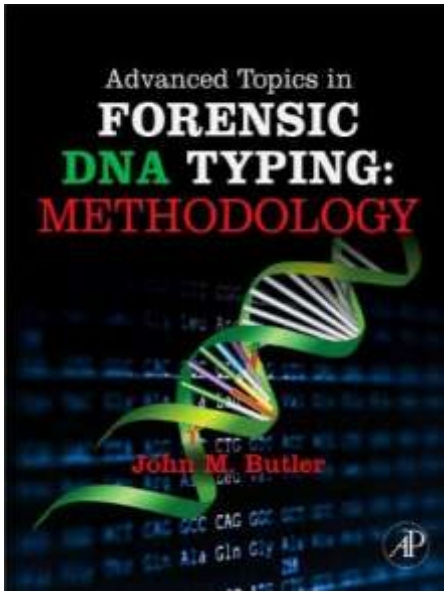
- If you want to be a technician, performing tests on requests, then just focus on the policies and procedures of your laboratory. If you want to be a scientist and a professional, learn the policies and procedures, but go much further and learn the philosophy of your profession. **Understand the importance 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.

Steps Involved in Process
of Forensic DNA Typing

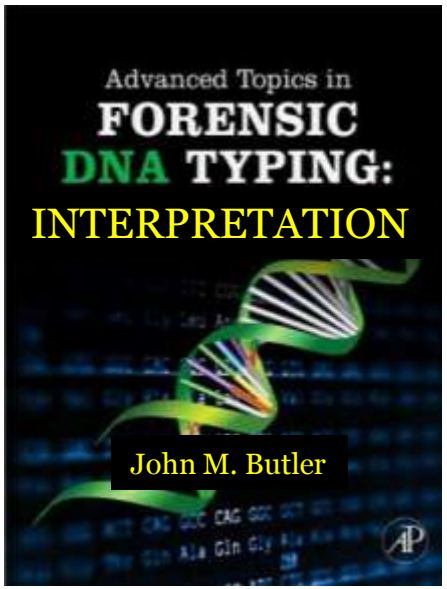
1) Data Interpretation
2) Statistical Interpretation

Gathering the Data **Understanding the Data**

Advanced Topics: Methodology



Advanced Topics: Interpretation



Advanced Topics in Forensic DNA Typing: INTERPRETATION

Chapter	Topic (current planned chapters)
	Introduction
1	Data interpretation overview
2	Thresholds
3	STR alleles & artifacts
4	STR genotypes & dropout
5	STR profiles
6	**Mixture interpretation**
7	Low-level DNA and complex mixtures
8	CE troubleshooting
9	Statistical interpretation overview
10	STR population data analysis
11	Profile frequency estimates
12	**Mixture statistics**
13	Coping with potential missing alleles
14	**Kinship and parentage analysis**
15	Lineage marker statistics
16	Drawing conclusions & **report writing**
	Glossary
App 1	U.S. Population Data (24 loci with N=938)
App 2	Revised Forensic DNA QAS (Sept 2011)
App 3	DAB Recommendations on Stats (Feb 2000)
App 4	NRC II Recommendations (1996)
App 5	SWGDAM STR Interp Guidelines (Jan 2010)

Features in New Book

(planned for Fall 2013 release)

- Explanations of SWGDAM interpretation guidelines
- Interviews on report writing from multiple perspectives
- Mixture interpretation
- Kinship analysis
- CE troubleshooting
- Standard U.S. pop data
- Numerous D.N.A. Boxes (**D**ata, **N**otes, & **A**pplications)
  - Worked examples to show relevance of equations
  - “Better know a statistician”

Purpose in Writing a Book on Interpretation

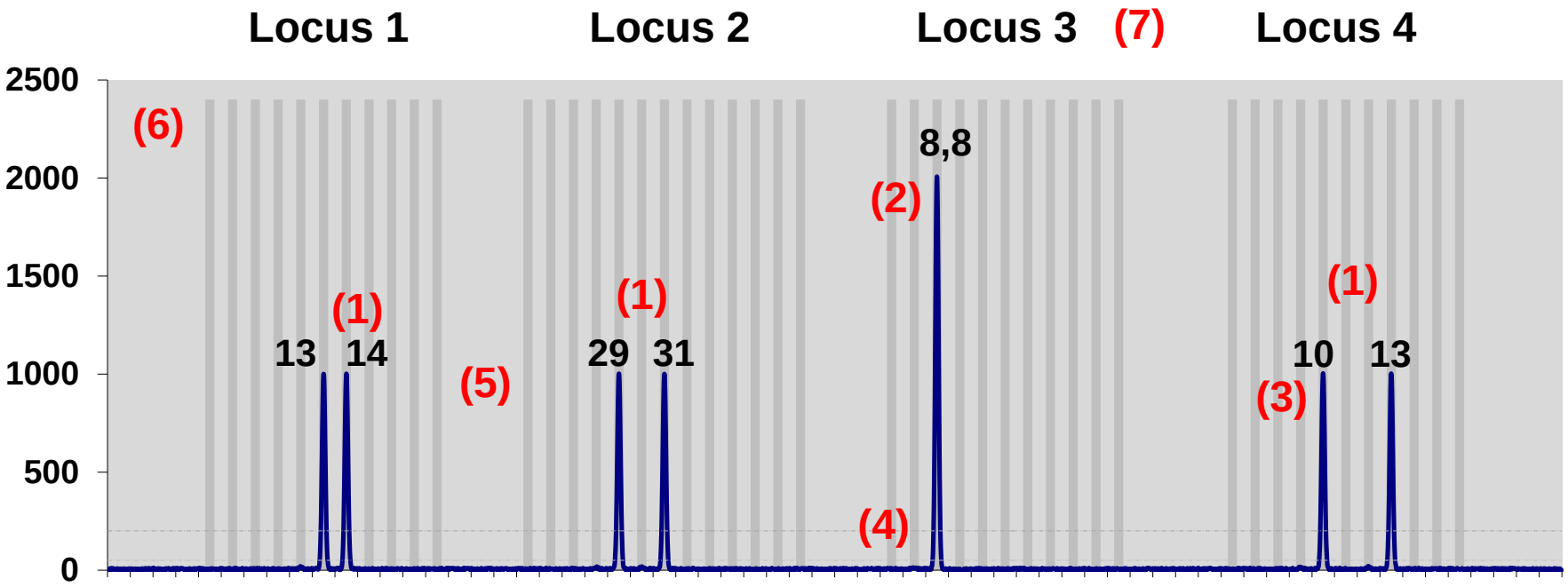
- Each of us thinks our own way is correct – but misinterpretations have given rise to a variety of approaches being undertaken today, some of which are not correct…

- I believe that **a better understanding of general principles will aid consistency and quality of work being performed**

D.N.A. Approach to Understanding

- **D**octrine or Dogma (why?)
  - A fundamental law of genetics, physics, or chemistry
    - Offspring receive one allele from each parent
    - Stochastic variation leads to uneven selection of alleles during PCR amplification from low amounts of DNA templates
    - Signal from fluorescent dyes is based on …
- **N**otable Principles (what?)
  - The amount of signal from heterozygous alleles should be similar
- **A**pplications (how?)
  - Peak height ratio measurements

Using **Ideal Data** to Discuss Principles



(1) 100% PHR between heterozygous alleles
(2) Homozygotes are exactly twice heterozygotes due to allele sharing
(3) No peak height differences exist due to size spread in alleles (any combination of resolvable alleles produces 100% PHR)
(4) No stutter artifacts enabling mixture detection at low contributor amounts
(5) Perfect inter-locus balance
(6) Completely repeatable peak heights from injection to injection on the same or other CE instruments in the lab or other labs
(7) Genetic markers that are so polymorphic all profiles are fully heterozygous with distinguishable alleles enabling better mixture detection and interpretation

Challenges in real-world data

- **Stochastic (random) variation** in sampling each allele during the PCR amplification process
  - This is highly affected by DNA quantity and quality
  - Imbalance in allele sampling gets worse with low amounts of DNA template and higher numbers of contributors
- **Degraded DNA** template may make some allele targets unavailable
- **PCR inhibitors** present in the sample may reduce PCR amplification efficiency for some alleles and/or loci
- **Overlap of alleles** from contributors **in DNA mixtures**
  - Stutter products can mask true alleles from a minor contributor
  - Allele stacking may not be fully proportional contributor contribution

Uncertainty and Probability

- "Contrary to what many people think, **uncertainty is present throughout any scientific procedure**."
  - Dennis V. Lindley, in his foreword to Aitken & Taroni (2004) *Statistics and the Evaluation of Evidence for Forensic Scientists, Second Edition*

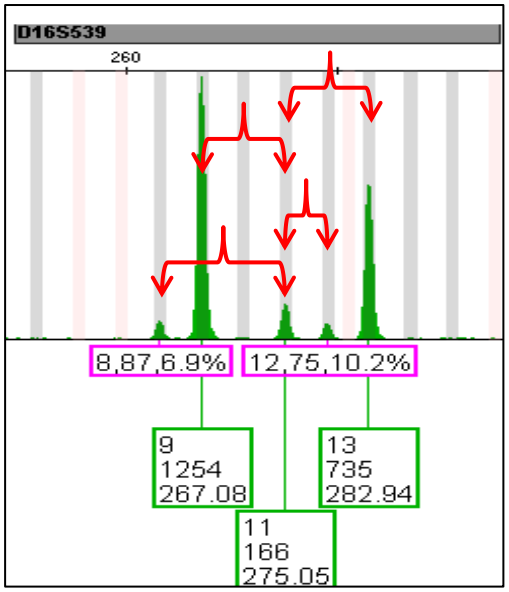
- "It is now recognized that **the only tool for handling uncertainty is probability**."
  - Dennis V. Lindley, in his foreword to Aitken & Taroni (2004) *Statistics and the Evaluation of Evidence for Forensic Scientists, Second Edition*

Do You Have Uncertainty in Your Data?

- **If allele dropout is a possibility** (e.g., in a partial profile), then there is uncertainty in whether or not an allele is present in the sample…and therefore what genotype combinations are possible

- **If different allele combinations are possible** in a mixture, then there is uncertainty in the genotype combinations that are possible…

Possible allele pairing with the 11



It is the Uncertainty that Matters…

http://www.thedailybeast.com/articles/2012/07/09/james-carville-on-his-new-book-it-s-the-middle-class-stupid.html

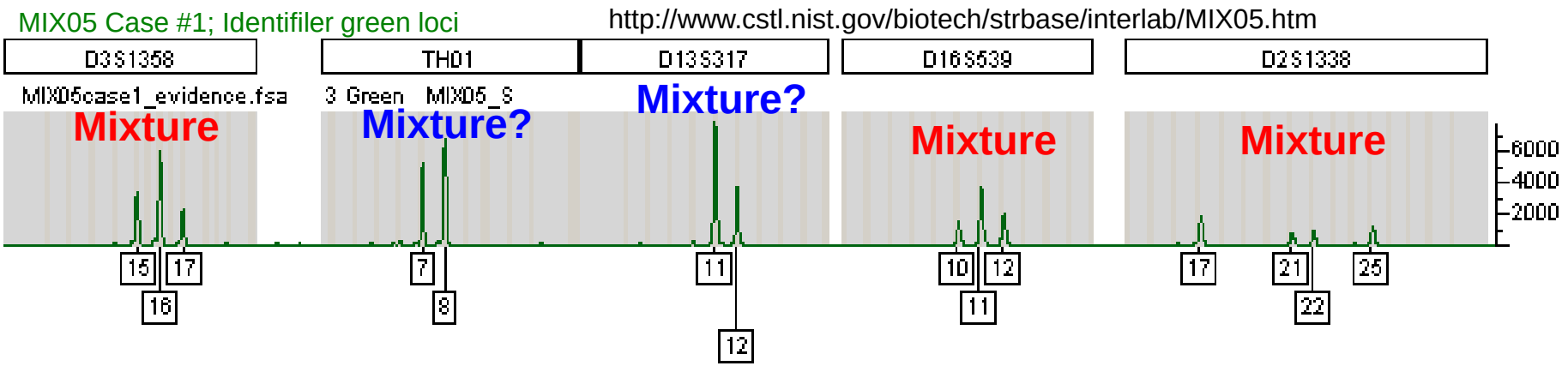
Mixtures: Issues and Challenges

From J.M. Butler (2005) *Forensic DNA Typing, 2nd Edition*, p. 155

- The probability that a mixture will be detected improves with the use of more loci and genetic markers that have a high incidence of heterozygotes.

- The detectability of multiple DNA sources in a single sample relates to the ratio of DNA present from each source, the specific combinations of genotypes, and the total amount of DNA amplified.

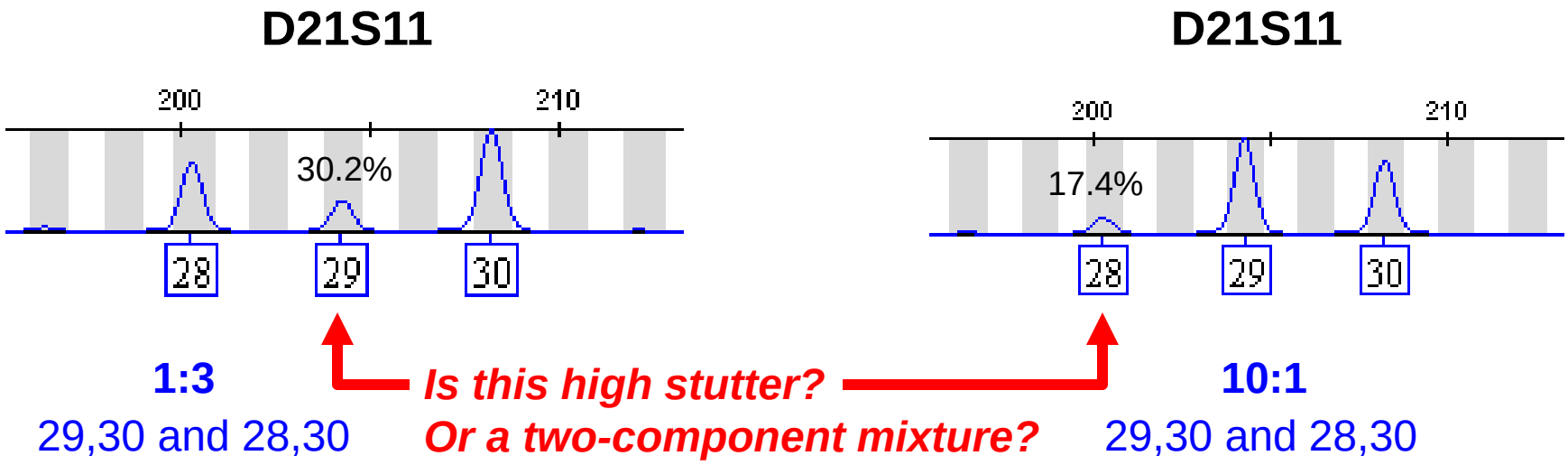
- Some mixtures will not be as easily detectable as other mixtures.



Mixtures: Issues and Challenges

- Artifacts of PCR amplification such as stutter products and heterozygote peak imbalance complicate mixture interpretation

- Thus, only a limited range of mixture component ratios can be solved routinely

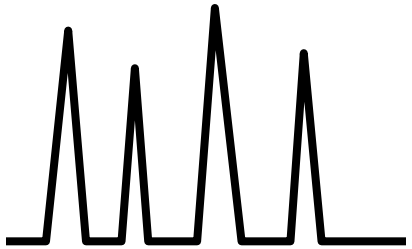


German Mixture Classification Scheme

Schneider *et al*. (2009) *Int. J. Legal Med*. 123: 1-5

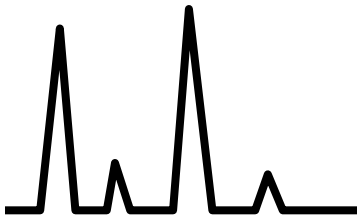
(German Stain Commission, 2006):

- **Type A**: no obvious major contributor, no evidence of stochastic effects
- **Type B**: clearly distinguishable major and minor contributors; consistent peak height ratios of ***approximately 4:1*** (major to minor component) for all heterozygous systems, no stochastic effects
- **Type C**: mixtures without major contributor(s), evidence for stochastic effects



Type A

"Indistinguishable"



Type B

"Distinguishable"

Type C

"Uninterpretable"

SWGDAM

Available for download from the ISFG Website:
http://www.isfg.org/Publication;Gill2006

ELSEVIER

Available online at www.sciencedirect.com

SCIENCE DIRECT®

Forensic Science International 160 (2006) 90–101

Forensic Science International

www.elsevier.com/locate/forsciint

DNA commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures

P. Gill [a,*], C.H. Brenner [b], J.S. Buckleton [c], A. Carracedo [d], M. Krawczak [e], W.R. Mayr [f], N. Morling [g], M. Prinz [h], P.M. Schneider [i], B.S. Weir [j]

[a] *Forensic Science Service, Trident Court, 2960 Solihull Parkway, Birmingham, UK*
[b] *Forensic Science Group, School of Public Health, University of California, Berkeley, CA 510-339-1911, USA*
[c] *ESR, Private Bag 92021, Auckland, New Zealand*

Our discussions have highlighted a significant need for continuing education and research into this area.

[j] *University of Washington, Department of Biostatistics, Box 357232, Seattle, WA 98195, USA*

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Available online 5 June 2006

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

Budowle et al. (2009) Article from the FBI Mixture Committee

J Forensic Sci, May 2009, Vol. 54, No. 3
doi: 10.1111/j.1556-4029.2009.01046.x
Available online at: www.blackwell-synergy.com

Bruce Budowle,[1] Ph.D.; Anthony J. Onorato,[1] M.S.F.S., M.C.I.M.; Thomas F. Callaghan,[1] Ph.D.; Angelo Della Manna,[2] M.S.; Ann M. Gross,[3] M.S.; Richard A. Guerrieri,[1] M.S.; Jennifer C. Luttman,[1] M.F.S.; and David Lee McClure,[4] B.S.

Mixture Interpretation: Defining the Relevant Features for Guidelines for the Assessment of Mixed DNA Profiles in Forensic Casework*

In general we agree with the recommendations of Gill et al. that are: (i) when possible peak height/area should be included in mixture interpretation; (ii) stutter position peaks at similar peak height/area as that of obligate minor contributor alleles should be considered as potential alleles in the interpretation and statistics calculation; and (iii) a stochastic threshold (termed ‘‘dropout threshold’’) should be defined.

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.

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

ELSEVIER

Forensic Science International
91 (1998) 55–70

Forensic Science International

Analysis and interpretation of mixed forensic stains using DNA STR profiling

T.M. Clayton[a,*], J.P. Whitaker[a], R. Sparkes[b], P. Gill[b]

[a]*Forensic Science Service, Wetherby Laboratory, Sandbeck Way, Audby Lane, Wetherby, West Yorkshire LS22 4DN, UK*

[b]*Forensic Science Service, Priory House, Gooch Street North, Birmingham B56QQ, UK*

Received 13 May 1997; received in revised form 9 October 1997; accepted 27 October 1997

Steps in the interpretation of mixtures

(Clayton *et al.* *Forensic Sci. Int.* 1998; 91:55-70)

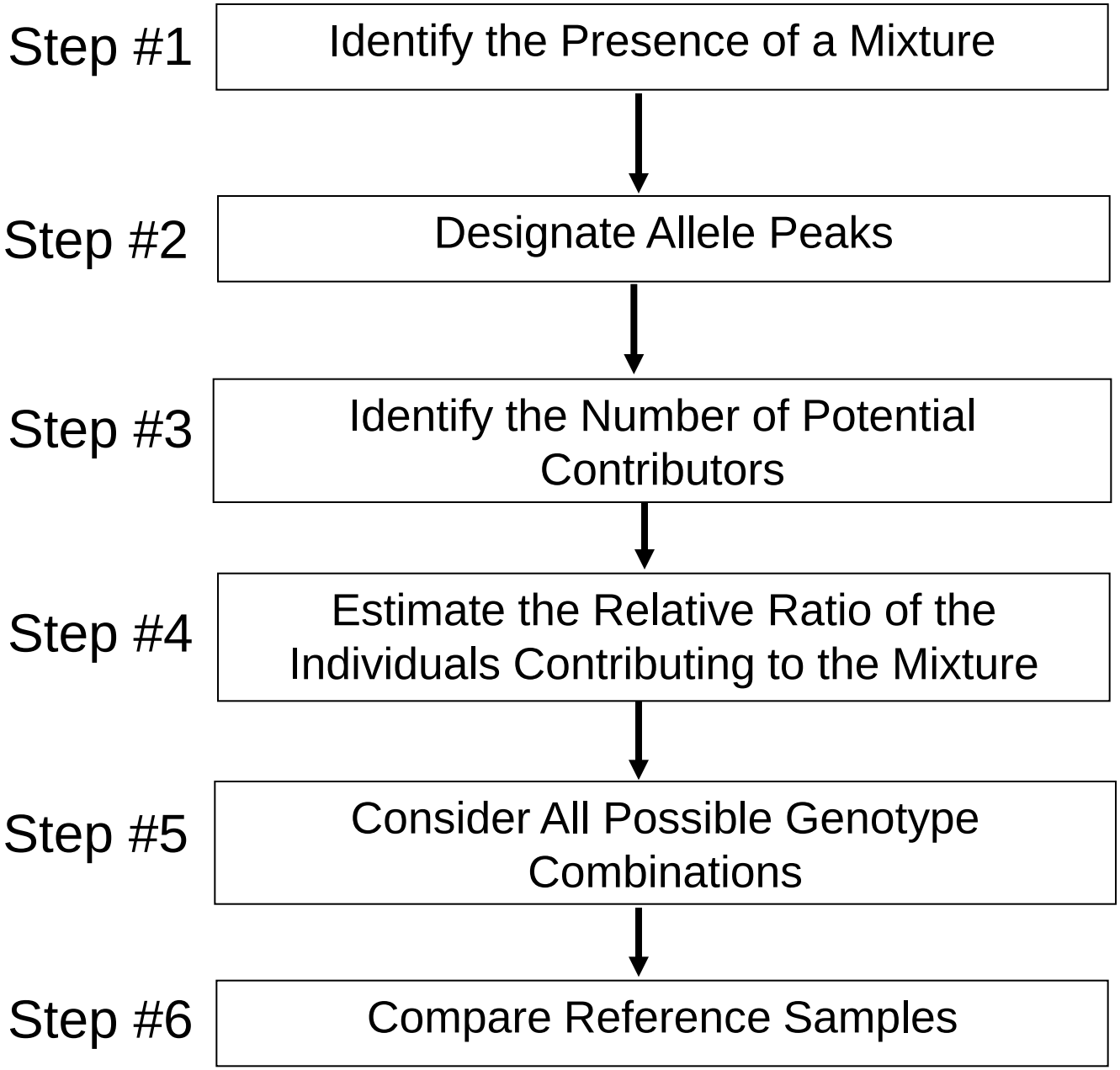
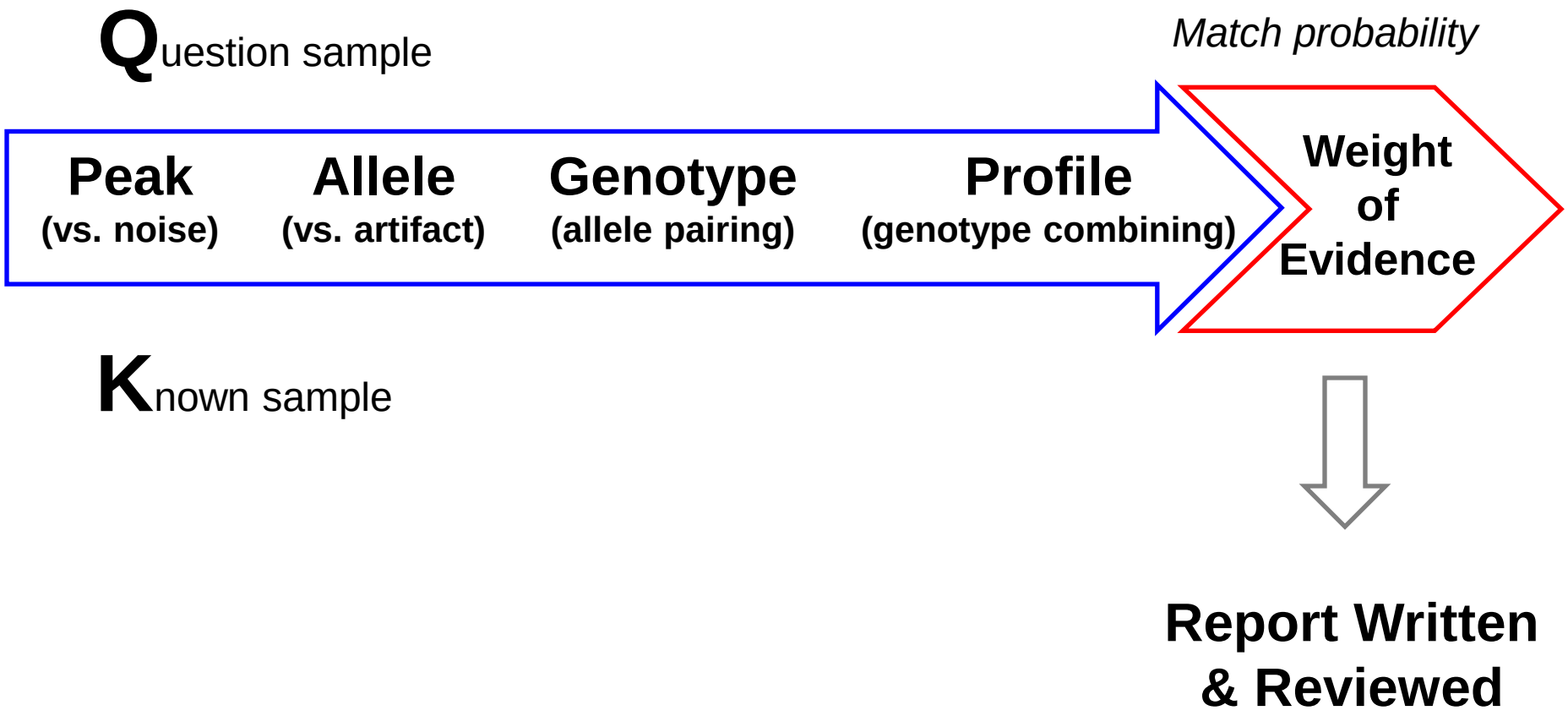


Figure 7.4, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

Steps in DNA Interpretation



Overview of the SWGDAM 2010 Interp Guidelines

1. Preliminary evaluation of data – **is something a peak and is the analysis method working properly?**
2. Allele designation – **calling peaks as alleles**
3. Interpretation of DNA typing results – **using the allele information to make a determination about the sample**
    1. Non-allelic peaks
    2. Application of peak height thresholds to allelic peaks
    3. Peak height ratio
    4. Number of contributors to a DNA profile
    5. Interpretation of DNA typing results for mixed samples
    6. Comparison of DNA typing results
4. Statistical analysis of DNA typing results – **assessing the meaning (rarity) of a match**

Other supportive material: statistical formulae, references, and glossary

DNA Interpretation Process

Sample

Extraction

Quantitation

PCR

Amplification

CE

Separation/ Detection

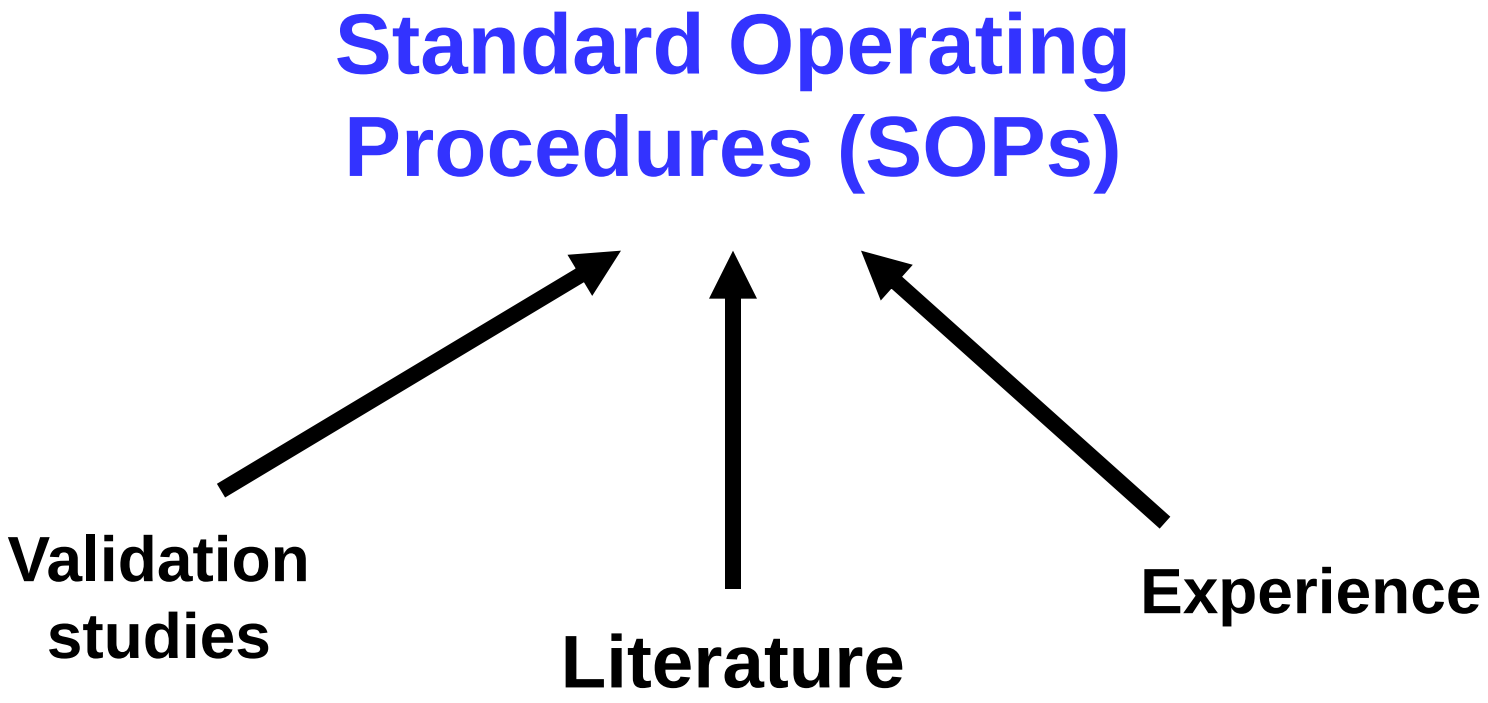
Locus specific

Stats

Peak (vs. noise)	**Allele** (vs. artifact)	**Any Missing Alleles?**	**Genotype** (allele pairing)	**Profile** (genotype combining)
	2.1, 3.1	**Amp variation** (potential allele dropout?)		
	3.1.1.1	**3.2**	**3.3**	**3.4**
1.1 Analytical threshold	Stutter threshold	Stochastic threshold	Peak height ratio threshold	Number of contributors
3.1.1.2 Sensitivity	Off-scale data threshold	**3.2.1**	Mixture ratio	
	3.1.1.3			

SWGDAM Guidelines (2010)

Your Laboratory Interpretation Protocols



SWGDAM Guidelines (2010) Introduction: *…the laboratory should utilize written procedures for interpretation of analytical results with the understanding that specificity in the standard operating protocols will enable greater consistency and accuracy among analysts within a laboratory. It is recommended that standard operating procedures for the interpretation of DNA typing results be sufficiently detailed that other forensic DNA analysts can review, understand in full, and assess the laboratory's policies and practices. The laboratory's interpretation guidelines should be based upon validation studies, scientific literature, and experience.*

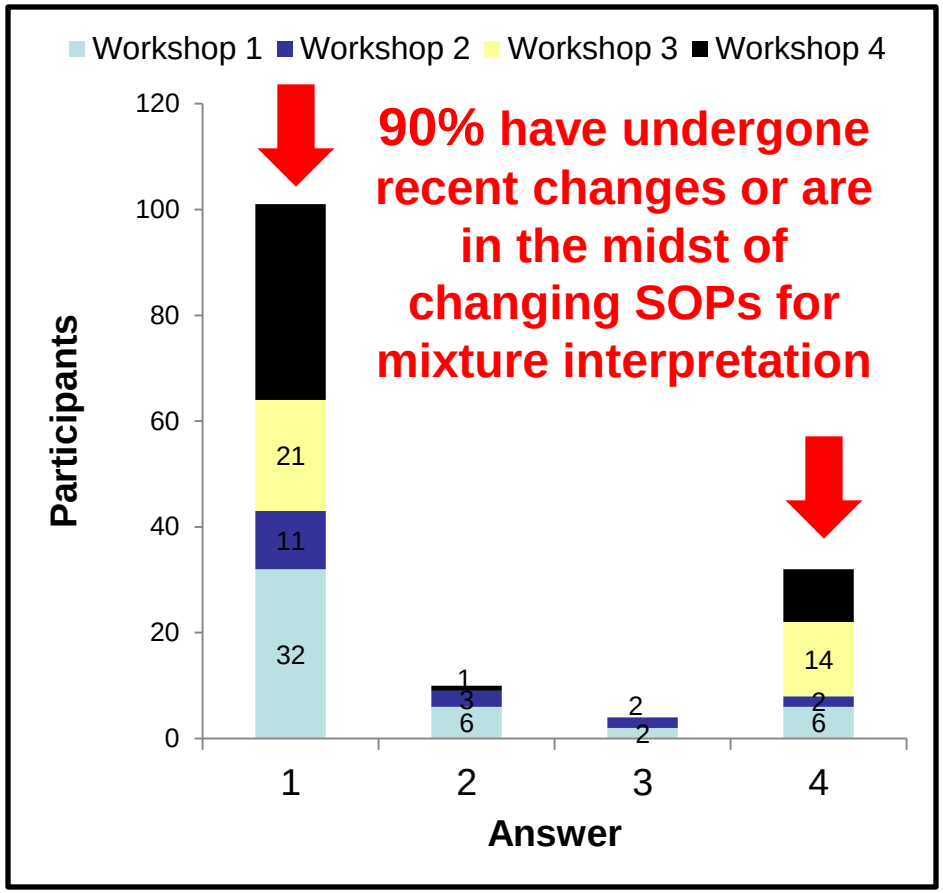
Is your lab in the process of changing your protocols?

Perhaps lowering your expected PHR 70% down to 55%?

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

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



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

Interpretation of Evidence Completed before Comparison to Known(s)

- "3.6.1. The laboratory **must establish guidelines** to ensure that, to the extent possible, **DNA typing results from evidentiary samples are interpreted before comparison with any known samples**, other than those of assumed contributors."

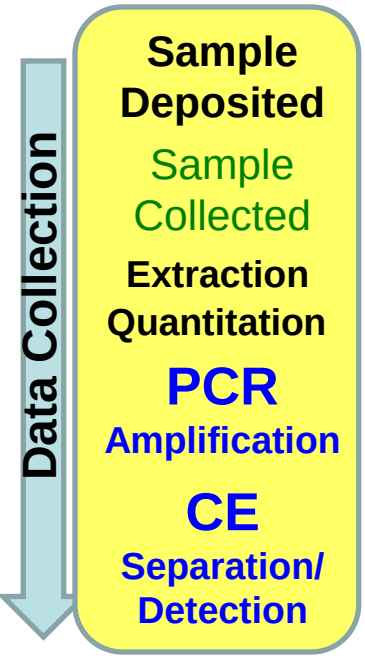
Q (question) before K (known)

  - While the FBI QAS do not address this issue, this is an example of an issue felt by the committee members to be of such importance that it warranted a "must."

Results Depend on Assumptions

- "Although courts expect one simple answer, statisticians know that **the result depends on how questions are framed and on assumptions tucked into the analysis**."
  - Mark Buchanan, Conviction by numbers. *Nature* (18 Jan 2007) 445: 254-255

Steps in DNA Interpretation



Signal observed

Data Interpretation

Analytical Threshold — Peak

Stutter Threshold — Allele

Stochastic Threshold — ***All Alleles Detected?***

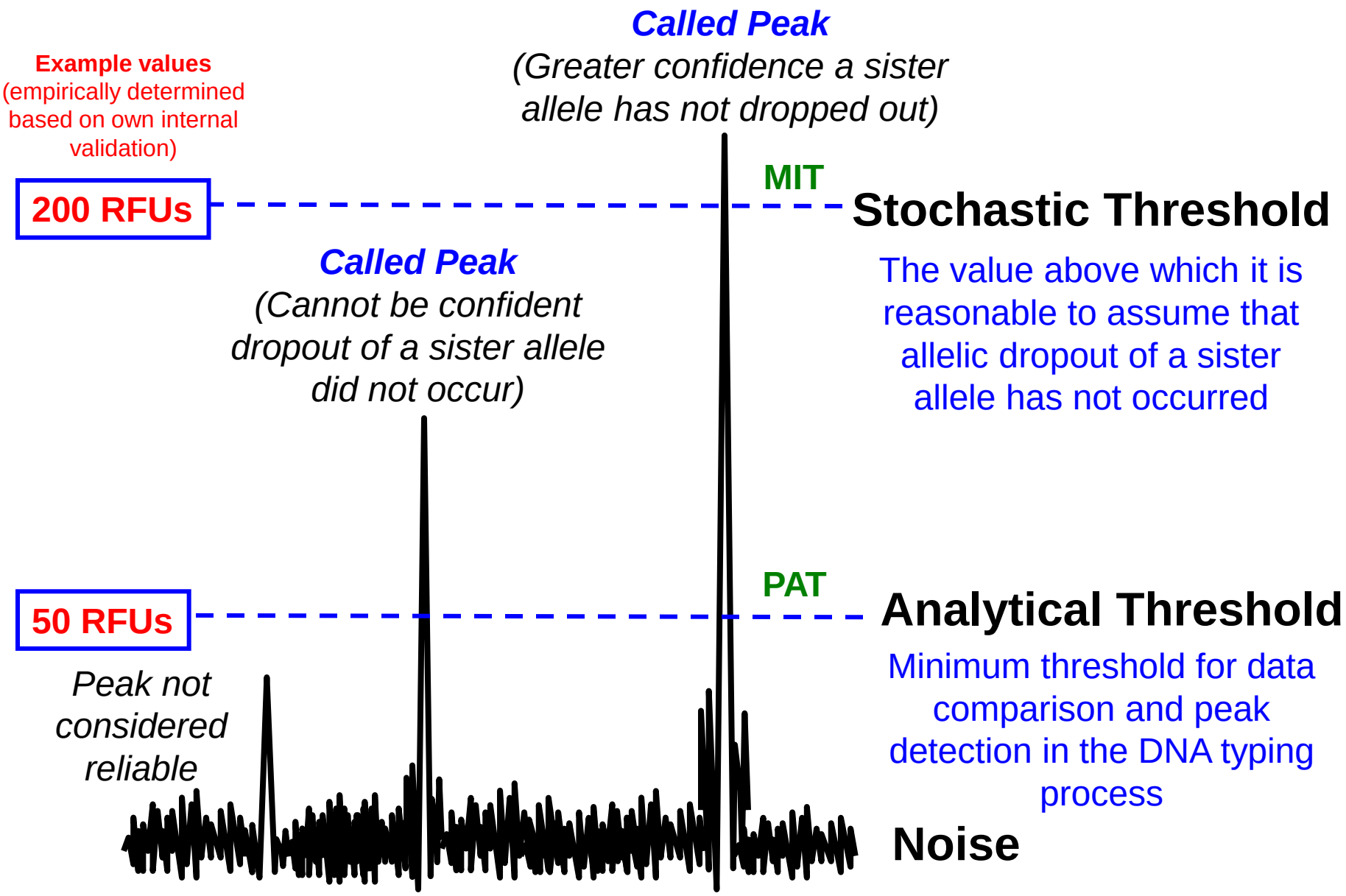
Peak Height Ratio — Genotype(s)

Mixture Ratio — Contributor profile(s)

Comparison to Known(s)

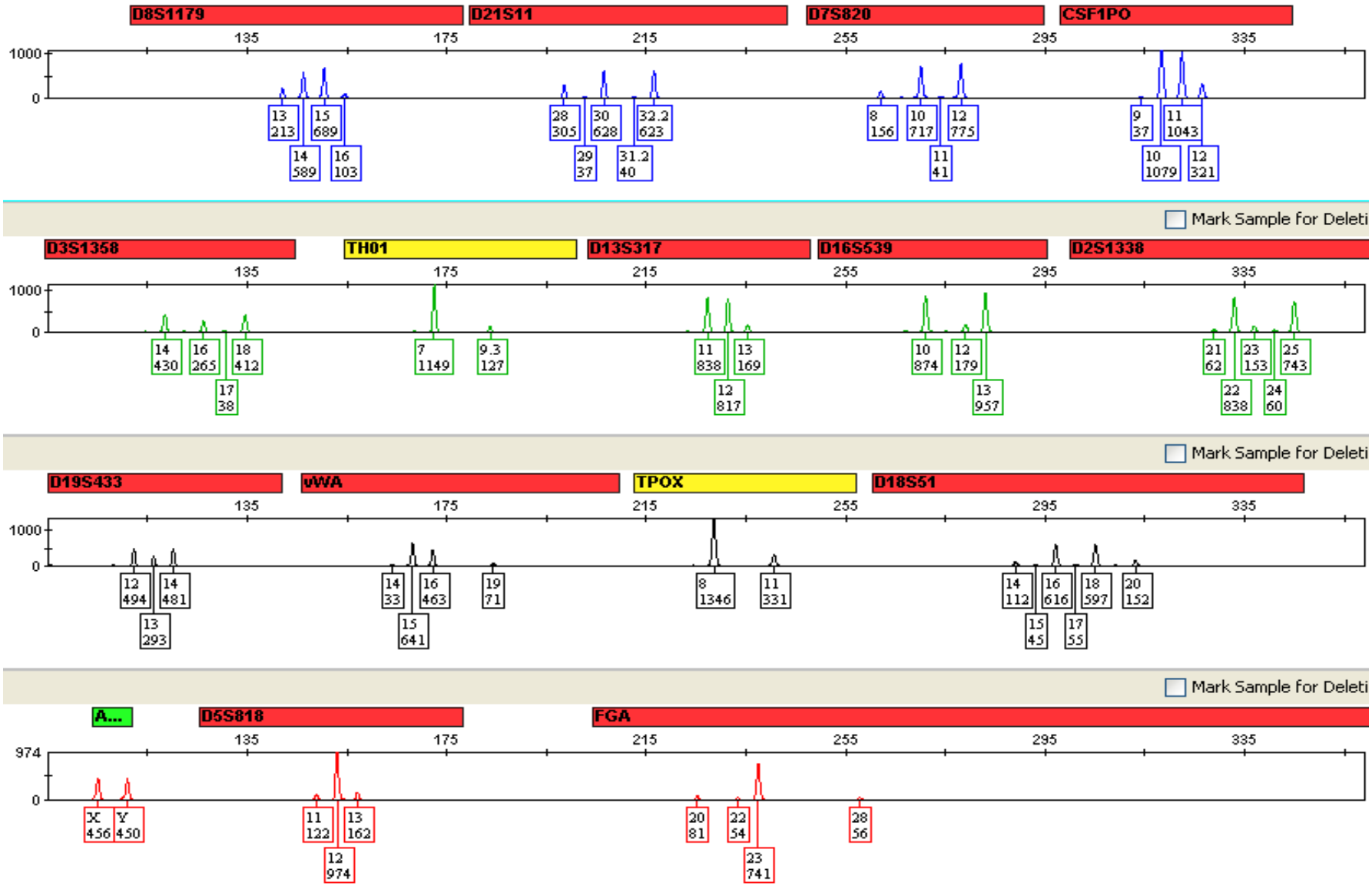
Weight of Evidence (Stats)

Overview of Two Thresholds

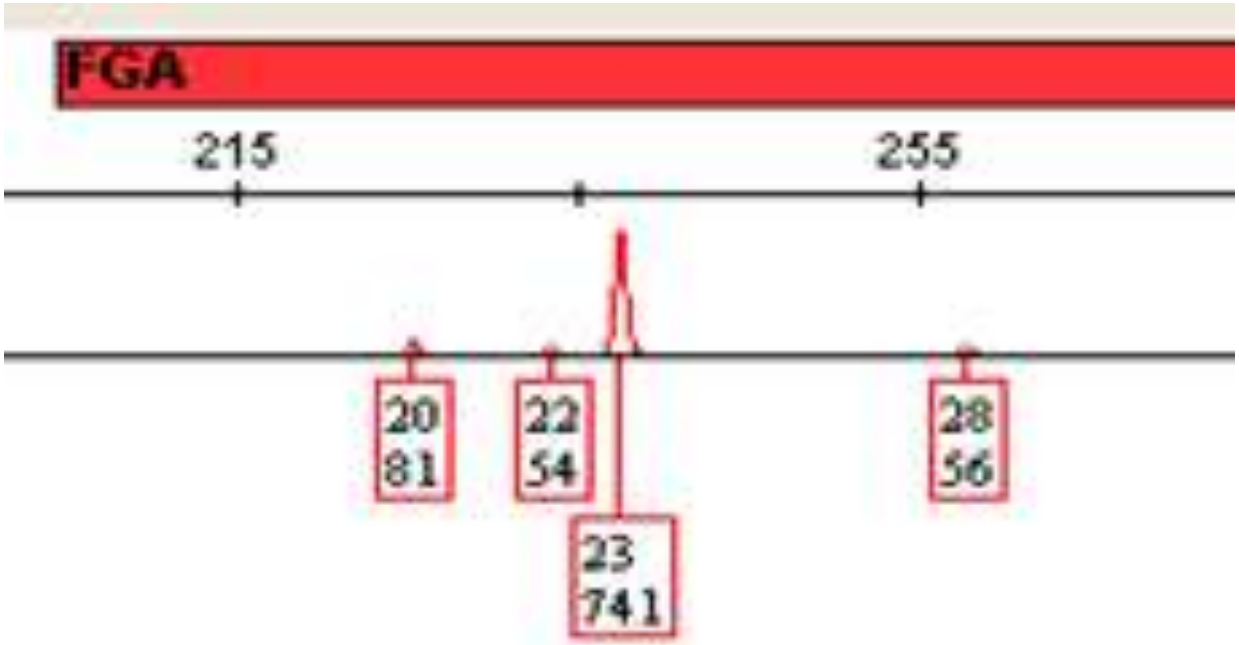


Butler, J.M. (2010) *Fundamentals of Forensic DNA Typing*. Elsevier Academic Press: San Diego.

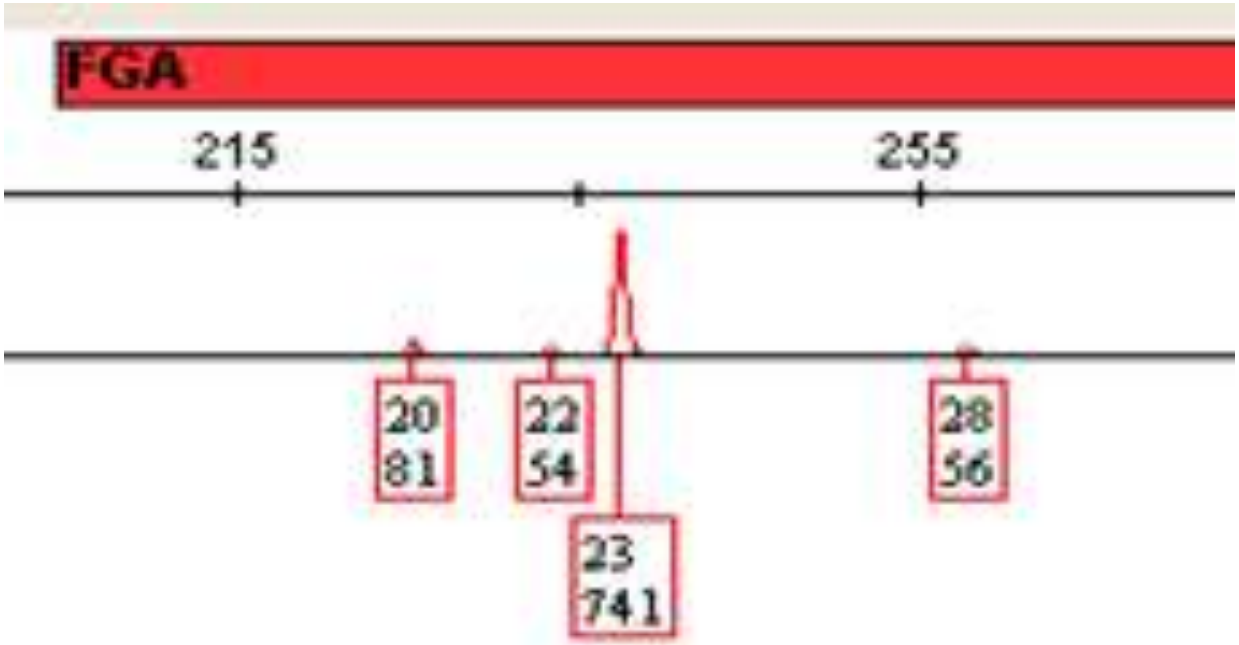
Profile 1 (stutter filter off)



Analytical Threshold (Peaks vs. Noise)



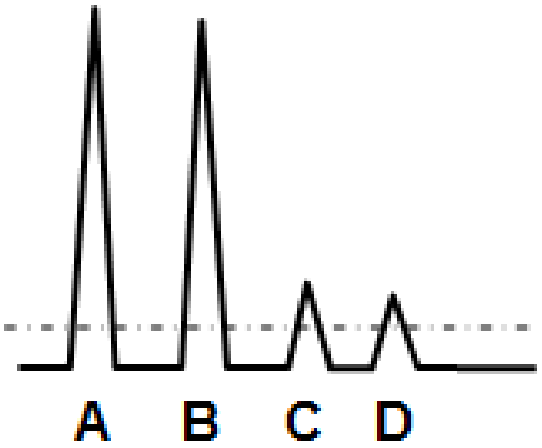
Stutter Threshold (Alleles vs. Artifacts)



Assumptions based upon # of contributors

Unrestricted vs. Restricted

Use of peak height information to select only certain combinations



Unrestricted

All combinations of alleles are deemed possible (relative peak height differences are not utilized)

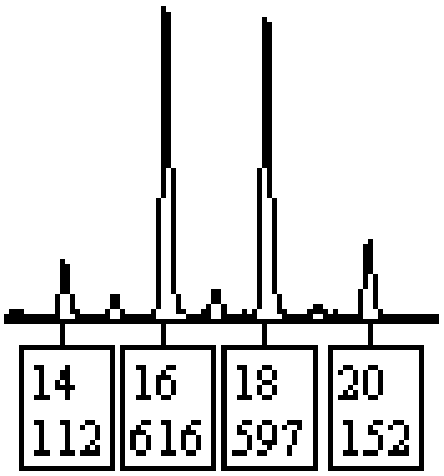
AB + AC + AD + BC + BD + CD

Restricted

Based on relative peak heights, alleles are paired only where specific combinations of alleles are deemed possible

AB + ~~AC~~ + ~~AD~~ + ~~BC~~ + ~~BD~~ + CD

Determination of Genotypes (PHR)



D18S51

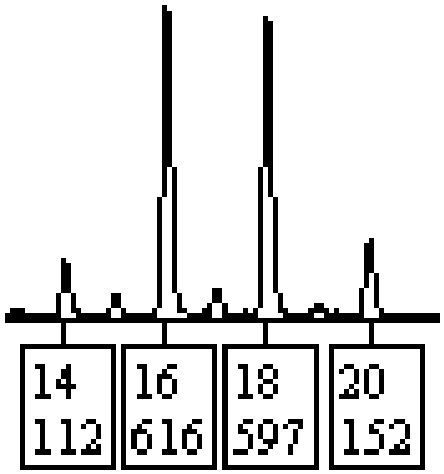
Possible Combinations

14, 16 and 18, 20
(18%) (25%)

14, 18 and 16, 20
(19%) (25%)

14, 20 and 16, 18
(74%) (97%)

Determination of Mixture Ratio



Major: 16,18
Minor: 14,20

D18S51

Total of all peak heights
= 112 + 616 + 597 + 152
= 1477 RFUs

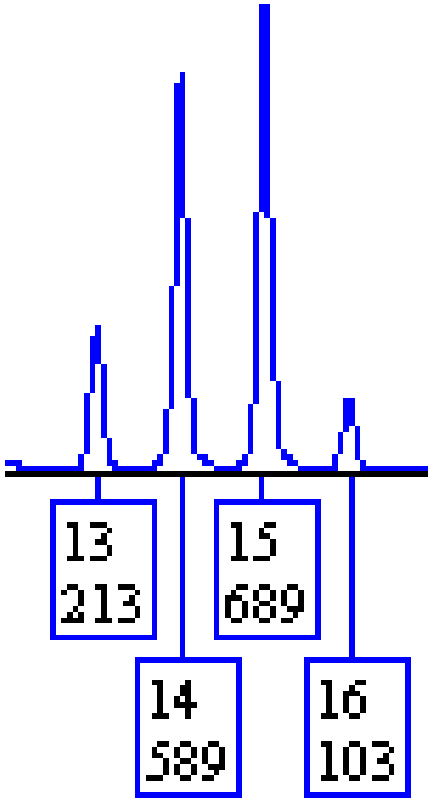
Minor component:
("14"+"20")/total = (112+152)/1477 = **0.179**

Major component:
("16"+"18")/ total = (616+597)/1477 = **0.821**

≈ 4.6 : 1

Four Peaks **(4 allele loci)**
heterozygote + heterozygote, no overlapping alleles (genotypes are unique)

Determination of Genotypes (PHR)



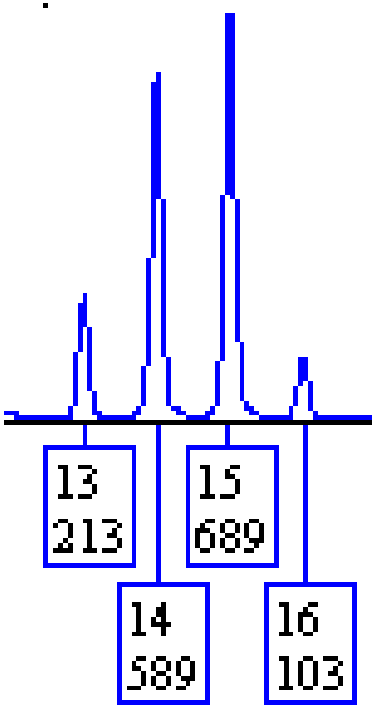
D8S1179

Possible Combinations

13, 14 (36%)	and	15, 16 (15%)
13, 15 (31%)	and	14, 16 (17%)
13, 16 (48%)	and	14, 15 (85%)

Includes “stutter” from the 14 allele

Determination of Mixture Ratio



Major: 14,15
Minor: 13,16

D8S1179

Total of all peak heights
= 213 + 589 + 689 + 103
= 1594 RFUs

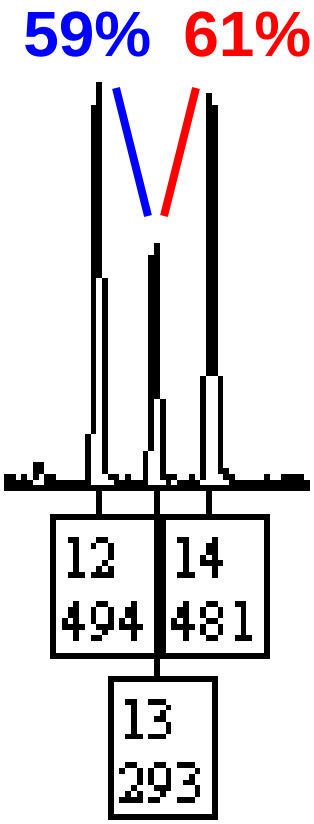
Minor component:
("13"+"16")/total = (213+103)/1594 = **0.198**

Major component:
("14"+"15")/ total = (589+689)/1594 = **0.802**

≈ 4 : 1

Four Peaks **(4 allele loci)**
heterozygote + heterozygote, no overlapping alleles (genotypes are unique)

Application of the Mixture Ratio



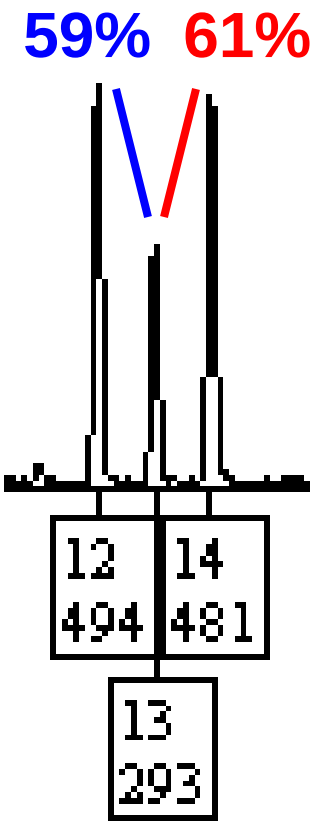
D19S433

Using peak height ratio, all genotypes possible:

12,12	**12,13**
13,13	**12,14**
14,14	**13,14**

Is there a major:minor here?

Application of the Mixture Ratio

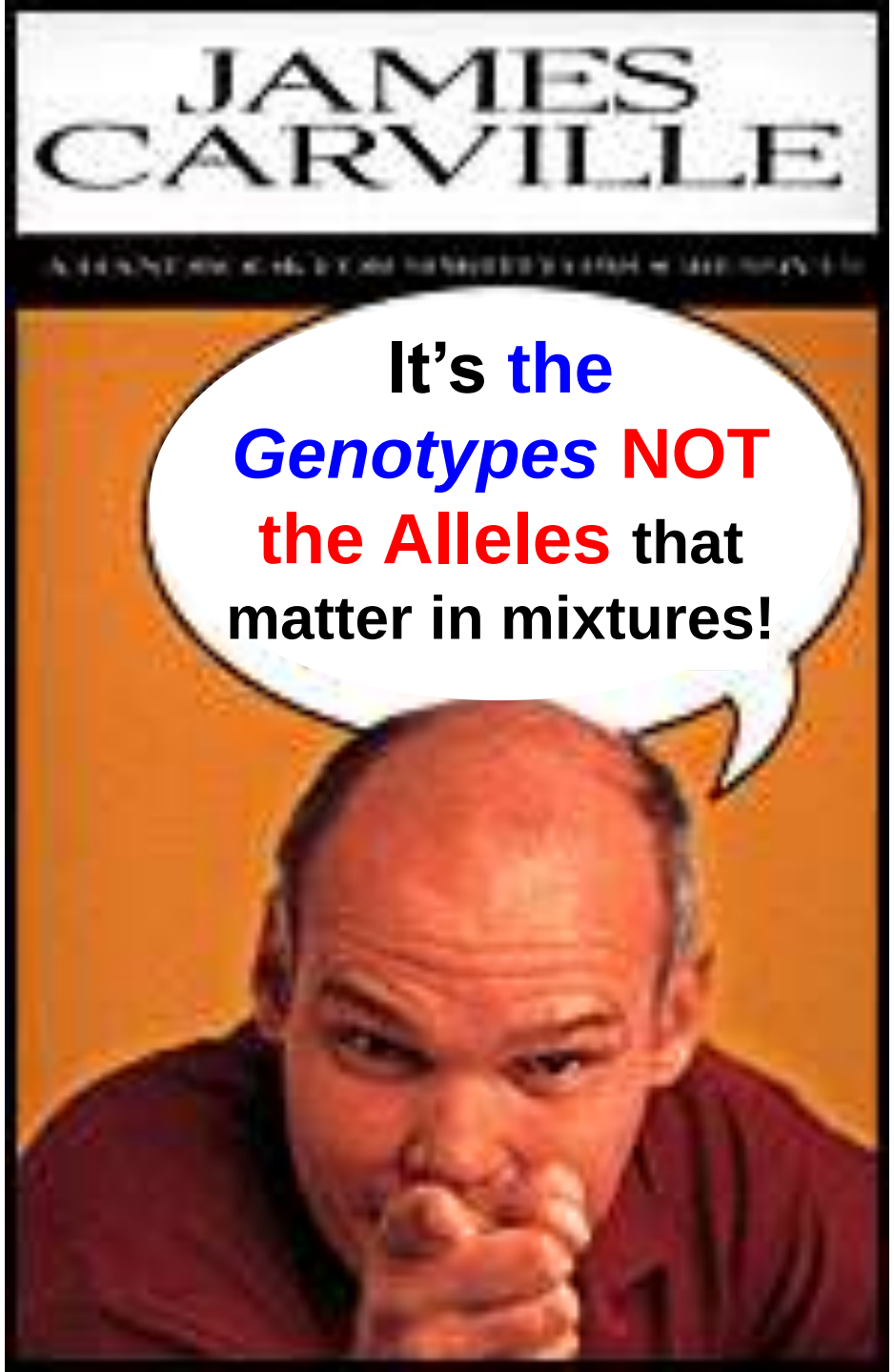


All possible genotype combinations:

12,12 + 13,14	**1:1.6**
13,13 + 12,14	**1:3.3**
14,14 + 12,13	**1:1.6**
12,13 + 12,14	**1:1.4**
~~**12,13 + 13,14**~~	~~**1:1**~~
12,14 + 13,14	**1:1.4**

Using MIXTURE RATIO calculations, can eliminate genotype pairs

Just Remember



Mixture Literature

you should be reading…

See DNA Mixtures Reference List provided with workshop materials

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.

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!

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
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"

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
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

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

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

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

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

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

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

Contents lists available at SciVerse ScienceDirect

Forensic Science International: Genetics

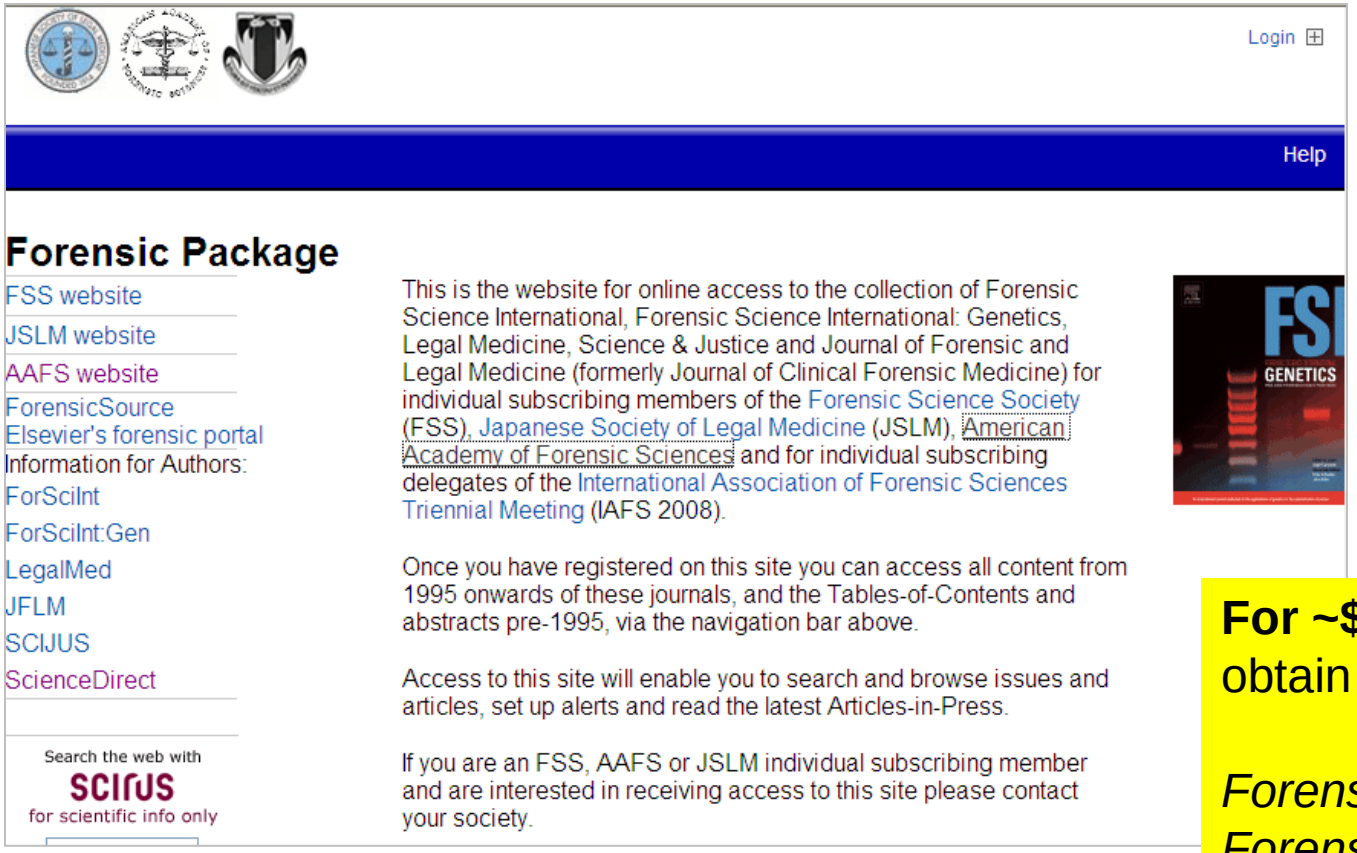
journal homepage: www.elsevier.com/locate/fsig

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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Forensic Sci Int
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Forensic & Legal Medicine

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Abstracts are Freely Available on Website

http://www.fsigenetics.com/

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On the Cover

New Issue Alert

FREE Free Trial Issue

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

http://www.fsigeneticssup.com

2011: 281 articles
2009: 253 articles
2007: 272 articles

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%*

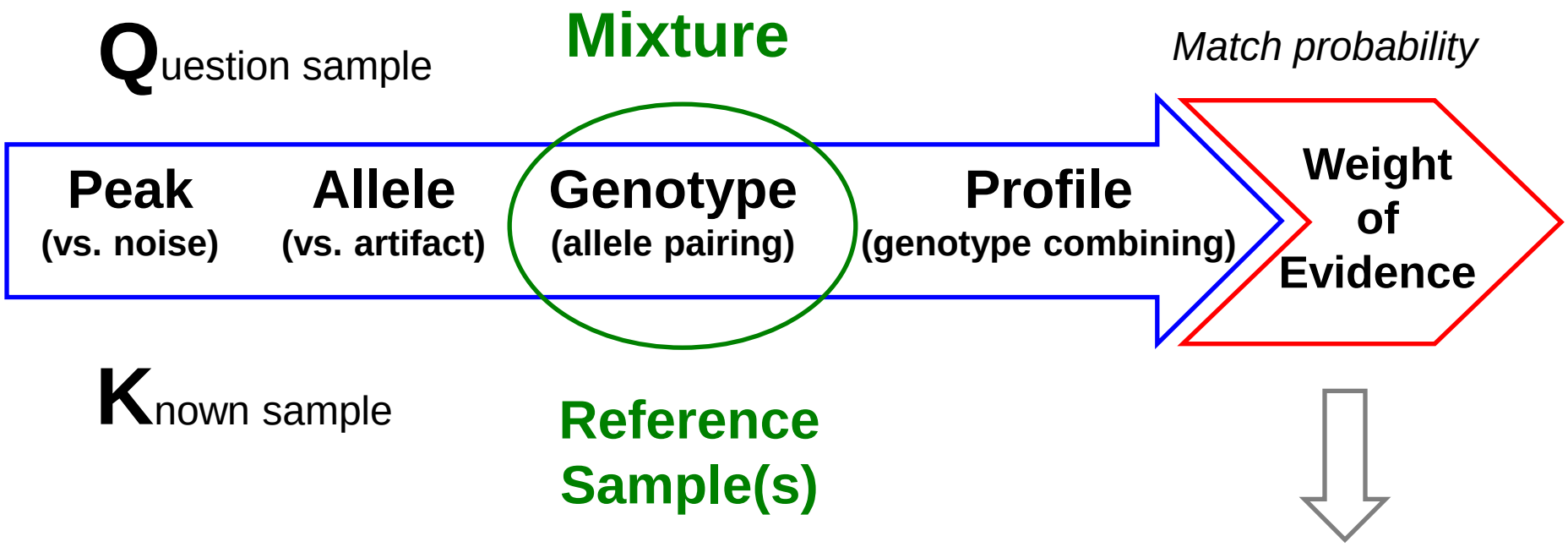
Comments: *low level DNA (125 pg)*

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



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

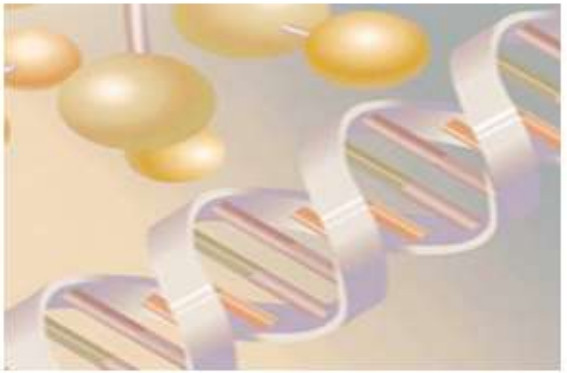
Report Written & Reviewed

Questions about any of the articles in the reference list?

Are there any other articles you have read recently that you would like to briefly discuss today as part of this workshop?

SWGDAM Website and Resources Available

http://www.swgdam.org/resources.html



Additional Resources

Beginning with the development or/and revision of its next draft guidance document(s), SWGDAM will make a "Draft for Comment" or other work product available for the purpose of receiving comments from the general public. This "Draft for Comment" solicitation will be open for a minimum of 60 days, usually through SWGDAM.org. SWGDAM will make all reasonable efforts to advise the forensic DNA community of the open comment period for a proposed guidance document or standard, guideline, best practice, study, or other recommendation and/or finding via as many avenues as possible to include posting notices through discipline-specific and related professional organizations. SWGDAM strongly encourages all interested parties to regularly monitor SWGDAM.org for the posting of such draft documents as well. All public comments received by SWGDAM will forwarded to the appropriate SWGDAM Committee for review and consideration as a part of its formal business practice for the development of the guidance documents or other work product.

*The following information resources have been produced and reviewed by members of the **Mixture Committee** of SWGDAM and are available at*
www.cstl.nist.gov/biotech/strbase/mixture/SWGDAM-mixture-info.htm

- Home
- ByLaws
- Members
- Committees
- Meetings
- Publications

Link to http://www.cstl.nist.gov/biotech/strbase/mixture/SWGDAM-mixture-info.htm

Mixture Training Materials

Reviewed by SWGDAM Mixture Committee

SWGDAM Mixture Committee Resource Page

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*The following information resources have been produced and reviewed by members of the **Mixture Committee** of the Scientific Working Group on DNA Analysis Methods (SWGDAM) -- see* http://www.swgdam.org/resources.html *for additional information.*

Mixture Training Examples

- Download "Mixture 6" PowerPoint show (56 Mb)

  - with voice-over by Bruce Heidebrecht (Maryland State Police); may work best if file is first saved to your computer

- Download "Mixture IQAS2904" PowerPoint show (35 Mb)

  - with voice-over by Bruce Heidebrecht (Maryland State Police); may work best if file is first saved to your computer

http://www.cstl.nist.gov/biotech/strbase/mixture/SWGDAM-mixture-info.htm

Recent Training Workshops


John Butler


Mike Coble

- AAFS (February 22, 2011)
  - **Mixture Interpretation (with 6 other speakers)**
- ISFG (August 30, 2011)
  - **CE Fundamentals and Troubleshooting**
- Int. Symp. Human Ident. (October 3, 2011)
  - **Mixture Interpretation (with Boston University)**
- Int. Symp. Human Ident. (October 6, 2011)
  - **Troubleshooting Laboratory Systems**

Slide handouts available at
http://www.cstl.nist.gov/strbase/training.htm

Mixture Workshop (Promega ISHI 2010)

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

Regional workshops presented in FL, TX, MI, and AZ (April – June 2011)
Updated mixture workshop presented at ISHI 2011 (October 3, 2011)

Handout >200 pages

Literature list of >100 articles

13 Modules Pres

Introductio
SWGD (John)
olds (Catherine)
astic effects (Robin)
eak height ratios (Charlotte)
Number of contributors (John)
Mixture ratios (John)
Mixture principles (Charlotte)
Statistics (Mike)

Case Example 1 (Robin)
Case Example 2 (Charlotte)
Case Example 3 (John)

Catherine Grgicak Boston U.
Mike Coble NIST
Robin Cotton Boston U.
John Butler NIST
Charlotte Word Consultant

NIJ Grant to Boston University
funded ~150 state & local lab analysts to attend

Promega ISHI 2012 Mixture Workshop

Mixtures Using *SOUND* Statistics, 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

Slides will be available after the workshop on STRBase **at http://www.cstl.nist.gov/strbase/mixture.htm**

Written summary of a recent interview…

The CAC News • 1st Quarter 2012 pp. 8-11

norah rudin & keith inman • the proceedings of lunch

www.forensicdna.com • norah@forensicdna.com • kinman@ix.netcom.com

The Discomfort of Thought
—a discussion with John Butler

Several years ago, we began to keep a list of topics that we thought would either be worthwhile topics for a Proceedings (POL in our vernacular, for Proceedings of Lunch, capitalization optional), or just fun to talk or write about. Recently we added *discussion with John Butler* to the list. Although one of us (NR) has had sporadic conversations with John over the years, we've never actually had the opportunity to share a meal. Fortuitously, all three of us attended the recent CAC meeting in Sacramento (We don't think we provided Mr. Houde with any photo ops, but there were reliable witnesses), and were able to huddle around the salad and other lunch offerings to at least begin this session. John has indicated that he routinely reads the *CACNews*, including this column. And he expressed some fascination with the process of how these Proceedings actually come about. What better way to find out than to participate in one? We agreed to present him with a list of questions to

What, we wonder, was the impetus for the SWGDAM 2010 Autosomal STR Interpretation Guidelines? What was wrong with the previous SWGDAM guidelines? Or what needed updating? John responds by saying that the Quality Assurance Standards (QAS) were, after a decade hiatus, revised in 2009. It was felt that the SWGDAM STR Interpretation Guidelines should also be updated to include more information and specifically to aid with mixture interpretation. The previous SWGDAM STR Interpretation Guidelines were released in 2000 and were very general. The 2010 guidelines expanded the text from 4 pages (1066 words) to 28 pages (9862 words) but followed the same general format. More information was needed on mixture interpretation and statistical approaches as the 2000 guidelines only had a few sentences on these topics without any real detail.

"For the greatest enemy of truth is very often not the lie – deliberate, contrived and dishonest – but the myth – persistent, persuasive, and unrealistic. Too often we hold fast to the clichés of our forebears. We subject all facts to a prefabricated set of interpretations. We enjoy the comfort of opinion without the discomfort of thought."

—John F. Kennedy

Available at **http://www.cacnews.org/news/1stq12.pdf**

"…**we should spend as much time developing our interpretation skills as we do our methodological skills.** Technological progress (more sensitivity in detecting DNA, for example), can be a double–edged sword; without equivalent progress in interpretation skill, we are just as likely to cut ourselves as we are the target."

"Your interpretation and statistical methods should have consistent assumptions and go together for each assumption being made (e.g., you may interpret a mixture under alternative sets of assumptions)…"

President John F. Kennedy

Yale University commencement address (June 11, 1962)

"For the greatest enemy of truth is very often not the lie – deliberate, contrived and dishonest – but the myth – persistent, persuasive, and unrealistic. Too often we hold fast to the clichés of our forebears. We subject all facts to a prefabricated set of interpretations. **We enjoy the comfort of opinion without the discomfort of thought**."

http://www.jfklibrary.org/Research/Ready-Reference/Kennedy-Library-Miscellaneous-Information/Yale-University-Commencement-Address.aspx