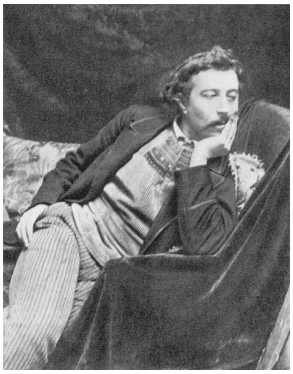
DNA Mixture Interpretation:
Where did we come from? What are we doing?
Where are we going?

Michael Coble, PhD
NIST

Official Disclaimer

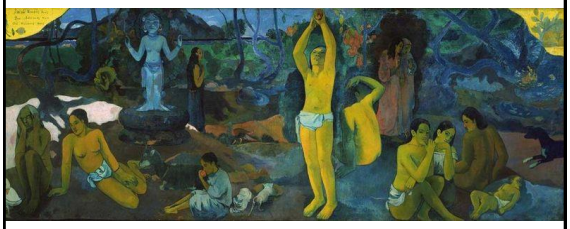
The opinions and assertions contained herein are solely those of the author and are not to be construed as official or as views of the U.S. Department of Commerce, U.S. Department of Justice, or the U.S. Department of Defense.

Commercial equipment, instruments, and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by the U.S. Department of Commerce, U.S. Department of Justice, or the U.S. Department of Defense nor does it imply that any of the materials, instruments or equipment identified are necessarily the best available for the purpose.



http://en.wikipedia.org/wiki/File:Paul_Gauguin_1891.png

Where Do We Come From? What Are We? Where Are We Going?



Paul Gauguin, 1897

http://en.wikipedia.org/wiki/File:Woher_kommen_wir_Wer_sind_wir_Wohin_gehen_wir.jpg

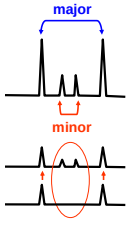
How did we get here?
(2000 – 2005)

DNA Mixtures

Most mixtures encountered in casework are 2-component mixtures
arising from a combination of victim and perpetrator DNA profiles

Torres *et al.* (2003) *Forensic Sci. Int.* 134:180-186 examined 1,547 cases from 1997-2000 containing **2,424 typed samples** of which **163 (6.7%) contained a mixed profile** with **only 8 (0.3%) coming from more than two contributors**

Ratios of the various mixture components stay fairly constant between multiple loci enabling deduction of the profiles for the major and minor components



Some mixture interpretation strategies involve using victim (or other reference) alleles to help isolate obligate alleles coming from the unknown portion of the mixture

Torres et al Spanish Case Summary Data

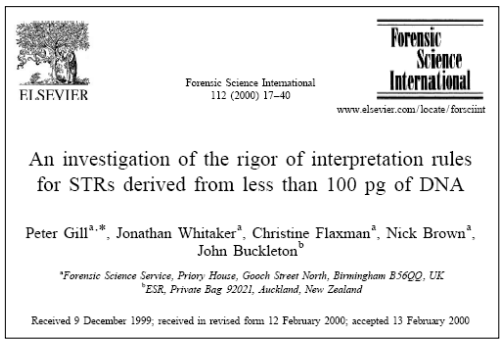
Case type	N = 163	Blood	Semen	Saliva
		Type of sample		
Victims	N = 60	23%	73%	---
Clothing/ bedding	N = 76	70%	30%	---
Weapons	N = 15	100%	---	---
Crime scene	N = 12	75%	---	25%

Torres et al. 4 year Spanish study

- Four year study (1/1997 to 12/2000)
- 2424 samples typed
  - 955 samples from sexual assaults
  - 1408 samples from other offenses
  - 49 samples from human remains identifications
- 163/2424 samples (6.7% showed mixed profile)

95.1% (155/163) were 2-component mixtures

Pushing the envelope…



ELSEVIER

Forensic Science International
112 (2000) 17–40

Forensic Science International

www.elsevier.com/locate/forsciint

An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA

Peter Gill[a,*], Jonathan Whitaker[a], Christine Flaxman[a], Nick Brown[a], John Buckleton[b]

[a]Forensic Science Service, Priory House, Gooch Street North, Birmingham B56QQ, UK
[b]ESR, Private Bag 92021, Auckland, New Zealand

Received 9 December 1999; received in revised form 12 February 2000; accepted 13 February 2000

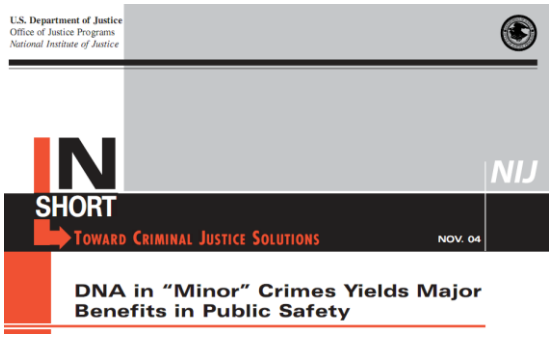
Low Template DNA situations exist in many samples

- In a 1:1 mixture, each DNA source is LT when the total amount of DNA in the amplification reaction is ~ 0.125 ng.
- In a 1:9 mixture, the minor component could be LT **even when the total amount of DNA in the amplification is 1 ng**.

Two different amplifications would be useful with a 1:9 mixture situation:
Normal level of total DNA (e.g., 1 ng) so that major component is on-scale
High level of total DNA (e.g., 5 ng) so that minor (e.g., ~500 pg) is out of LT realm – yes, the major component will be off-scale…

Robin Cotton, AAFS 2003 LCN Workshop
"Are we already doing low copy number (LCN) DNA analysis?"

Mixture Case Summaries



U.S. Survey - Spreadsheet Information Requested

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

Labs requested to also provide info on kit, PCR volume used, etc.

- **Case#**
- **Item#**

This information retained by lab and not returned…

- **Type of sample (biological material if ID'd)**
- **Type of substrate**
- **Quantity amp'd**
- **Minimum # of contributors (1, 2, 3, 4, or >4)**
- **Predominant type (major profile) determined?**
- **Stats reported**
- **Comments**

Data collected by Ann Marie Gross (2007-2008)

MN BCA Case Summary Data #2

Case type	N = 373	1	2	3	4	>4
			# contributors			
Sexual Assault	N = 144	57%	39%	4%	--	--
Major Crime	N = 98	70%	21%	8%	1%	--
High Volume	N = 131	33%	47%	18%	2%	--
		Single source	Mixtures			

Mixture Case Summaries

Collection organized by Ann Gross (July 2007 – Feb 2008)

14 Labs	State	# Samples
MN BCA	Minnesota	334
CA DOJ	California	285
GBI	Georgia	19
Kern Co	California	31
CT	Connecticut	610
USACIL	US Army	119
RCMP	CANADA	1555
NJSP	New Jersey	101
MSP	Michigan	225
WSP	Washington	419
IL	Illinois	76
MT	Montana	408
AA Co MD	Maryland	322
CFS-Toronto	CANADA	276
	Total =	4780

	minimum # of contributors						
Sample Type	1	2	3	4	>4	N	
Blood	1207	296	72	1	0	1576	35.9%
Bone	4	0	0	0	0	4	0.1%
e-cells	215	165	94	13	2	489	11.2%
Hair	62	5	1	0	0	68	1.6%
PBM	183	127	45	7	0	362	8.3%
Rectal swab	0	16	1	0	0	17	0.4%
Saliva	114	81	23	0	0	218	5.0%
Semen/sperm	536	546	92	9	0	1183	27.0%
Sweat	3	3	1	0	0	7	0.2%
Touch	85	143	77	9	0	314	7.2%
Vaginal swab	3	62	4	0	0	69	1.6%
Wearer	17	36	22	2	0	77	1.8%
Total	2429	1480	432	41	2	4384	
	55.4%	33.8%	9.9%	0.9%	0.05%		

	minimum # of contributors						
Crime Class	1	2	3	4	>4	N	
Sexual Assault	884	787	145	11	0	1827	40.2%
Major Crime	1261	519	182	32	0	1994	43.9%
High Volume	344	220	140	11	5	720	15.9%
Total	2489	1526	467	54	5	4541	
	54.8%	33.6%	10.3%	1.2%	0.1%		

	minimum # of contributors						
Crime Class	1	2	3	4	>4	N	
Sexual Assault	884	787	145	11	0	1827	40.2%
Major Crime	1261	519	182	32	0	1994	43.9%
High Volume	344	220	140	11	5	720	15.9%
Total	2489	1526	467	54	5	4541	
	54.8%	33.6%	10.3%	1.2%	0.1%		

Laboratory	Crime Class	Minimum # of Contributors 2	3+
Minn.	Sexual Assault	59	5
	High Volume	43	24
Cal DOJ	Sexual Assault	62	15
	High Volume	5	0
Conn.	Sexual Assault	17	3
	High Volume	8	25
NJ	Sexual Assault	8	0
	High Volume	17	4
Michigan	Sexual Assault	63	14
	High Volume	32	21
Wash.	Sexual Assault	64	9
	High Volume	17	13
Illinois	Sexual Assault	122	23
	High Volume	25	35
Montana	Sexual Assault	77	11
	High Volume	22	16
AA (MD)	Sexual Assault	19	1
	High Volume	51	18
Tor-CFS	Sexual Assault	76	13
	High Volume	9	9
RCMP	Sexual Assault	243	64
	High Volume	0	0

Major ID'd	minimum # of contributors 2	3	4	>4	N	
yes	920	152	8	2	1082	60.4%
no	402	273	32	3	710	39.6%
Total	1322	425	40	5	1792	
	73.8%	23.7%	2.2%	0.3%		

~40% of mixtures are Indistinguishable or Uninterpretable

Overall Summary 2007-2008

- ~40-50% of samples from all types of cases are single source
- ~30-40% of samples from all types of cases are mixtures of at least two contributors
- ~5-15% of samples from all types of cases are mixtures of at least three contributors

2005 - 2010

- Major shift in the types of casework being submitted to the lab.
- Movement away from high-quantity DNA, 2-person sexual assault evidence to more "touch" DNA samples often with multiple numbers of contributors.

"The Quote"

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

ISFG DNA Commission on Mixture Interpretation

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

Who is the ISFG

and why do their recommendations matter?

International Society of Forensic Genetics

http://www.isfg.org/

- An international organization responsible for the promotion of scientific knowledge in the field of genetic markers analyzed with forensic purposes.
- Founded in 1968 and represents more than 1100 members from over 60 countries.
- **A DNA Commission regularly offers recommendations on forensic genetic analysis.**

DNA Commission of the ISFG

- DNA polymorphisms (1989)
- PCR based polymorphisms (1992)
- Naming variant alleles (1994)
- Repeat nomenclature (1997)
- Mitochondrial DNA (2000)
- Y-STR use in forensic analysis (2001)
- Additional Y-STRs - nomenclature (2006)
- **Mixture Interpretation (2006)**
- Disaster Victim Identification (2007)
- Biostatistics for Parentage Analysis (2007)
- Non-Human DNA testing (2011)

http://www.isfg.org/Publications/DNA+Commission

ISFG Executive Committee

President
Mecki Prinz
(New York City, USA)

Vice-President
Niels Morling
(Copenhagen, Denmark)

Working Party Representative
Walther Parson
(Innsbruck, Austria)

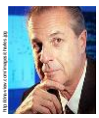
Treasurer
Leonor Gusmão
(Porto, Portugal)

Secretary
Peter Schneider
(Köln, Germany)

Authors of ISFG Mixture Article

Peter Gill
Pioneer of forensic DNA techniques and applications
UK's Forensic Science Service (1978-2008)
University of Strathclyde (Apr 2008 – present)

The Statisticians



Charles Brenner
DNA-View,
Berkeley, CA, USA

John Buckleton
ESR,
Auckland, New Zealand

Michael Krawczak
Christian-Albrechts-University,
Kiel, Germany

Bruce Weir
U. Washington,
Seattle, USA

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

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

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

Received 4 April 2006; accepted 10 April 2006
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

Available online at www.sciencedirect.com
SCIENCE DIRECT
Forensic Science International 160 (2006) 89
Forensic Science International
www.elsevier.com/locate/forsciint

Editorial

Editorial on the recommendations of the DNA commission of the ISFG on the interpretation of mixtures

"…**These recommendations have been written** to serve two purposes: to define a generally acceptable mathematical approach for typical mixture scenarios and to address open questions where practical and generally accepted solutions do not yet exist. This has been done **to stimulate the discussion among scientists in this field**. **The aim is to invite proposals and criticism in the form of comments and letters to the editors of this journal**…**We are hoping to continue the process to allow the DNA Commission to critically revise or extend these recommendations in due time…"**

Summary of ISFG Recommendations on Mixture Interpretation

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

Responses to ISFG DNA Commission Mixture Recommendations

- UK Response
  - Gill *et al.* (2008) *FSI Genetics* 2(1): 76–82

Responses to ISFG DNA Commission Mixture Recommendations

Available online at www.sciencedirect.com

ScienceDirect

Forensic Science International: Genetics 2 (2008) 76–82

www.elsevier.com/locate/fsig

Letter to the Editor

National recommendations of the Technical UK DNA working group on mixture interpretation for the NDNAD and for court going purposes

Responses to ISFG DNA Commission Mixture Recommendations

- UK Response
  - Gill *et al.* (2008) *FSI Genetics* 2(1): 76–82
- **German Stain Commission**
  - Schneider *et al.* (2006) *Rechtsmedizin* 16:401-404 (German version)
  - Schneider *et al.* (2009) *Int. J. Legal Med.* 123: 1-5 (English version)

Rechtsmedizin 2006 · 16:401–404
DOI 10.1007/s00194-006-0411-1
Online publiziert: 16. November 2006
© Springer Medizin Verlag 2006

P. M. Schneider[1] · R. Fimmers[4] · W. Keil[2] · G. Molsberger[5] · D. Patzelt[6] · W. Pflug[7] · T. Rothämel[8] · H. Schmitter[3] · H. Schneider[9] · B. Brinkmann[10]
[1] Institut für Rechtsmedizin, Universitätsklinikum, Köln
[2] Institut für Rechtsmedizin, Ludwig-Maximilians-Universität, München
[3] Bundeskriminalamt, Wiesbaden
[4] Institut für Medizinische Biometrie, Informatik und Epidemiologie, Bonn
[5] Landeskriminalamt Nordrhein-Westfalen, Düsseldorf
[6] Institut für Rechtsmedizin, Bayr. Julius-Maximilians-Universität, Würzburg
[7] Landeskriminalamt Baden-Württemberg, Stuttgart
[8] Institut für Rechtsmedizin der Medizinischen Hochschule, Hannover
[9] Hessisches Landeskriminalamt, Wiesbaden
[10] Vorsitzender der Spurenkommission, Institut für Rechtsmedizin, Münster

Allgemeine Empfehlungen der Spurenkommission zur Bewertung von DNA-Mischspuren

Int J Legal Med (2009) 123:1–5
DOI 10.1007/s00414-008-0244-4

REVIEW ARTICLE

The German Stain Commission: recommendations for the interpretation of mixed stains

P. M. Schneider · R. Fimmers · W. Keil · G. Molsberger · D. Patzelt · W. Pflug · T. Rothämel · H. Schmitter · H. Schneider · B. Brinkmann

Adapted from Peter Schneider slide (presented at EDNAP meeting in Krakow in April 2007)

Mixture Classification Scheme

Schneider *et al*. (2006) *Rechtsmedizin* 16:401-404

(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 **Type B** **Type C**

Responses to ISFG DNA Commission Mixture Recommendations

Forensic Science International: Genetics 3 (2009) 144–145
Contents lists available at ScienceDirect
Forensic Science International: Genetics
journal homepage: www.elsevier.com/locate/fsig

Letter to the Editor

Interpretation of DNA Mixtures—Australian and New Zealand consensus on principles

crime stain DNA profile is low level and some minor alleles are the same size as stutters of major alleles, and/or if drop-out is possible, then extra consideration needs to be given to the method of statistical interpretation.

- New Zealand/Australia Support Statement
  - Stringer *et al.* (2009) *FSI Genetics*
- **SWGDAM** – Mixture Interpretation subcommittee was started Jan 2007

SWGDAM Mixture Interpretation Subcommittee

- **John Butler** (NIST) – chair
- **Mike Adamowicz** (CT)
- **Terry Coons** (OR)
- Jeff Modler (RCMP)
- **Phil Kinsey** (MT)
- Todd Bille (ATF)
- Allison Eastman (NYSP)
- **Bruce Heidebrecht** (MD)
- **Tamyra Moretti** (FBI DNA Unit I)
- **George Carmody** (Carleton U)
- **Roger Frappier** (CFS-Toronto)
- **Jack Ballantyne** (UCF/NCFS)
- **Gary Sims** (CA DOJ) - co-chair
- **Joanne Sgueglia** (MA)
- **Gary Shutler** (WA)
- Cecelia Crouse (PBSO)
- **Hiron Poon** (RCMP)
- Steve Lambert (SC)
- **Steven Myers** (CA DOJ)
- **Ann Gross** (MN BCA)

The 15 members in bold font were involved with most of the writing (July-Oct 2009)

Started in January 2007

SWGDAM STR Guidelines

- Guidelines were approved at the January 14, 2010 SWGDAM meeting. The guidelines were publically released on April 8, 2010 on the FBI website for the CODIS group:

  http://www.fbi.gov/hq/lab/html/codis1.htm

  (under "Quality Assurance" information)

http://www.fbi.gov/filelink.html?file=hq/lab/html/codis_swgdam.pdf (PDF)

http://www.fbi.gov/hq/lab/html/codis_swgdam.htm (HTML text))

SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories

- Guidelines
  - Not Standards
  - No lab should be audited against this document
- Autosomal STR Typing
  - This document does not address Y-STRs, mtDNA testing, or CODIS entries
- Forensic DNA Testing Laboratories
  - Databasing labs may have different issues since they are working with known single source samples

Needed Revisions After a Decade…

Quality Assurance Standards (1998/1999) → **Quality Assurance Standards (2009)**

http://www.fbi.gov/hq/lab/fsc/backissu/july2000/strig.htm

STR Interpretation Guidelines (2000) → http://www.fbi.gov/hq/lab/html/codis_swgdam.pdf **STR Interpretation Guidelines (2010)**

1066 words (4 pages)

9862 words (28 pages)

3. Interpretation of Results

3.1. The laboratory should define conditions in which the data would lead to the conclusion that the source of the DNA is either from a single person or more than one person. This may be accomplished by an examination of the number of alleles at each locus, peak height ratios, and/or band intensities.

3.1.1. *Single Contributor:* A sample may be considered to be from a single contributor when the observed number of alleles at each locus and the signal intensity ratios of alleles at a locus are consistent with a profile from a single contributor. All loci should be evaluated in making this determination.

3.1.2. *Mixtures With Major/Minor Contributors:* A sample may be considered to consist of a mixture of major and minor contributors if there is a distinct contrast in signal intensities among the alleles. The difference is evaluated on a case-by-case context. All loci should be evaluated in making this determination.

3.1.3. *Mixtures With a Known Contributor(s):* In some cases, when one of the contributors (e.g., the victim) is known, the genetic profile of the unknown contributor may be inferred. Depending on the profiles in the specific instance, this can be accomplished by subtracting the contribution of the known donor from the mixed profile.

3.1.4. *Mixtures With Indistinguishable Contributors:* When major or minor contributors cannot be distinguished because of similarity in signal intensities or the presence of shared or masked alleles, individuals may still be included or excluded as possible contributors.

Mixtures – 7 sentences

Purpose and Scope (1)

This document provides guidelines for the interpretation of DNA typing results from short tandem repeats (STR) and supersedes the Scientific Working Group on DNA Analysis Methods (SWGDAM) Short Tandem Repeat (STR) Interpretation Guidelines (2000). The revised guidelines are not intended to be applied retroactively.

Purpose and Scope (2)

Guidance is provided for forensic casework analyses on the identification and application of thresholds for allele detection and interpretation, and appropriate statistical approaches to the interpretation of autosomal STRs with further guidance on mixture interpretation.

Purpose and Scope (3)

Laboratories are encouraged to review their standard operating procedures and validation data in light of these guidelines and to update their procedures as needed. It is anticipated that these guidelines will evolve further as future technologies emerge. Some aspects of these guidelines may be applicable to low level DNA samples. However, this document is not intended to address the interpretation of analytical results from enhanced low template DNA techniques.

Purpose and Scope (4)

- Due to the multiplicity of forensic sample types and the potential complexity of DNA typing results, it is impractical and infeasible to cover every aspect of DNA interpretation by a preset rule. However, 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.***

Hold that thought!!!

"Must" (used 29 times) vs. **"Should"** (used 41 times)

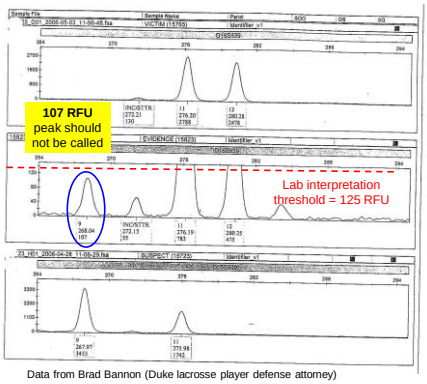
"Must" used when the FBI revised Quality Assurance Standards (2009) cover the topic:

- FBI QAS Standard 9.6.1:
  - The laboratory **shall verify** that all control results meet the laboratory's interpretation guidelines for all reported results.
- SWGDAM Interpretation Guidelines 1.3.1:
  - The laboratory **must establish** criteria for evaluation of the following controls, including but not limited to: reagent blank and positive and negative amplification controls.

Gill et al. (2006) and SWGDAM (2010)

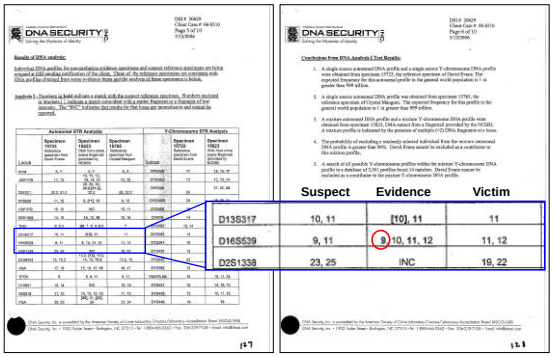
- Establish Stochastic Thresholds for use in interpreting data.
- What's the big deal about thresholds?

Don't Call Peaks Below Your Validated Threshold!



Data from Brad Bannon (Duke lacrosse player defense attorney)

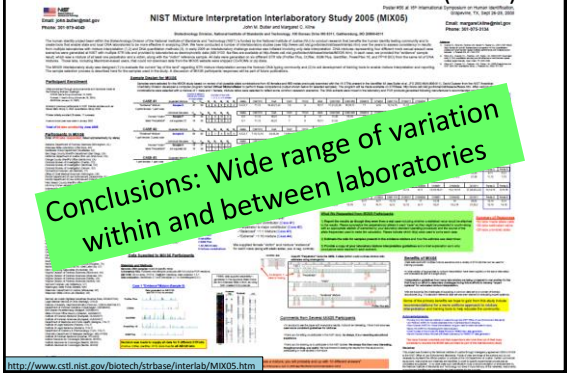
May 12, 2006: DNA Security Report



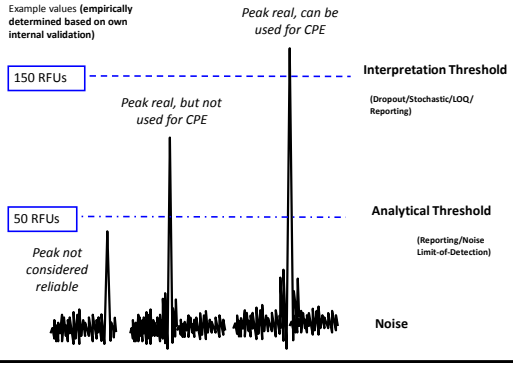
	Suspect	Evidence	Victim
D13S317	10, 11	[10], 11	11
D16S539	9, 11	9, 10, 11, 12	11, 12
D2S1338	23, 25	INC	19, 22

Data from Brad Bannon (Duke lacrosse player defense attorney)

MIX05 Poster Presentation at ISHI (2005)



Different Thresholds



"On the Threshold of a Dilemma"

- Gill and Buckleton (2010)
- Although most labs use thresholds of some description, this philosophy has always been problematic because there is an inherent illogicality which we call the falling off the cliff effect.

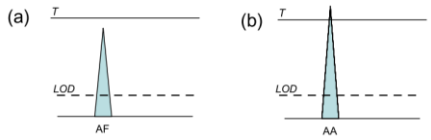
JOURNAL OF FORENSIC SCIENCES

J Forensic Sci, January 2010, Vol. 55, No. 1
doi: 10.1111/j.1556-4029.2009.01257.x
Available online at: interscience.wiley.com

Commentary on: Budowle B, Onorato AJ, Callaghan TF, Della Manna A, Gross AM, Guerrieri RA, Luttman JC, McClure DL. Mixture interpretation: defining the relevant features for guidelines for the assessment of mixed DNA profiles in forensic casework. J Forensic Sci 2009;54(4):810–21.

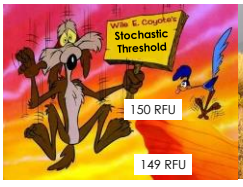
"Falling off the Cliff Effect"

- If T = an arbitrary level (e.g., 150 rfu), an allele of 149 rfu is subject to a different set of guidelines compared with one that is 150 rfu even though they differ by just 1 rfu (Fig. 1).



Gill and Buckleton *JFS* 55: 265-268 (2010)

Falling off the Cliff vs. Gradual Decline



Gill and Buckleton *JFS*
55: 265-268 (2010)

- "The purpose of the ISFG DNA commission document was to provide a way forward to demonstrate the use of ***probabilistic models to circumvent the requirement for a threshold*** and to safeguard the legitimate interests of defendants."

2010 – 2012 Mixture Workshops with Cotton, Word, Grgicak, and Butler

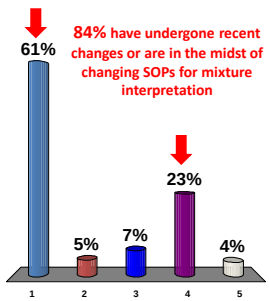
Used in ISHI 2011 workshop and FL, TX, MI, and AZ regional workshops

- **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

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)

Data from 150 responses
ISHI Mixture Workshop (Oct 2011)

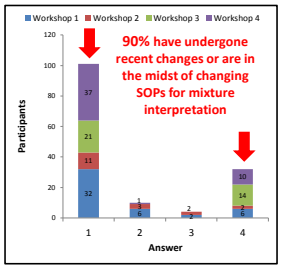


84% have undergone recent changes or are in the midst of changing SOPs for mixture interpretation

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)

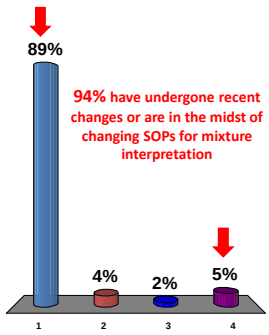


From ISHI 2011 poster "Impact of the SWGDAM Mixture Interpretation Guidelines: Successes, Issues and Suggested Future Directions"

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=121 from 7 different labs
NYC Apr 2012



The Basics of Mixture Statistics

Statistical Approaches with Mixtures

See Ladd *et al.* (2001) *Croat Med J.* 42:244-246

"Exclusionary" Approach	"Inferred Genotype" Approach
Random Man Not Excluded (RMNE)	Random Match Probability [modified] (mRMP)
Combined Prob. of Inclusion (CPI)	Likelihood Ratio (LR)
Combined Prob. of Exclusion (CPE)	
"Allele-centric"	"Genotype-centric"

Exclusionary Approach

Statistical Methods in Medical Research 1993; **2**: 241–262

Forensic inference from genetic markers

B Devlin Department of Epidemiology and Public Health, Yale University School of Medicine

Section 5.1 Exclusion probability

- Discussion about exclusion probabilities in Paternity cases.

Two types:

(1) Conditional Exclusion Probability - excluding a random man as a possible father, given the mother-child genotypes for a particular case.

(2) Average Exclusion Probability – excluding a random man as a possible father, given a randomly chosen mother-child pair.

Statistical Methods in Medical Research 1993; **2**: 241–262

Forensic inference from genetic markers

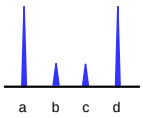
B Devlin Department of Epidemiology and Public Health, Yale University School of Medicine

Section 5.1 Exclusion probability

"The interpretation of conditional exclusion probability is obvious, which accounts for its value in the legal arena. Unlike [LR], however, it is not fully efficient."

Statistical Approaches with Mixtures

- **Random Man Not Excluded (CPE/CPI)** - The probability that a random person (unrelated individual) would be included/excluded as a contributor to the observed DNA mixture.

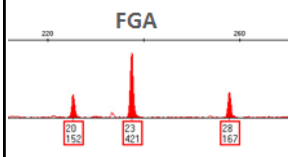


$$CPI = (f(a) + f(b) + f(c) + f(d))^2$$

$$CPI = PI_{M1} \times PI_{M2} \cdots$$

$$CPE = 1 - CPI$$

RMNE example with FGA

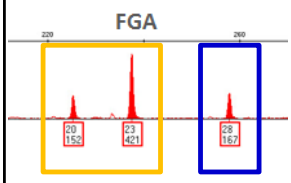


Possible Combinations

20, 28 and 23, 23
20, 23 and 23, 28

Assume ST = 150 RFU

RMNE example with FGA



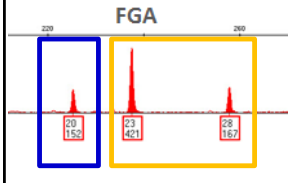
Possible Combinations

20, 28 and 23, 23
20, 23 and 23, 28

20, 23 and 28, 28

Assume ST = 150 RFU

RMNE example with FGA



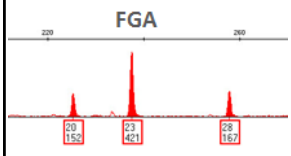
Possible Combinations

20, 28 and 23, 23
20, 23 and 23, 28

20, 23 and 28, 28
20, 20 and 23, 28

Assume ST = 150 RFU

RMNE example with FGA



Possible Combinations

20, 28 and 23, 23
20, 23 and 23, 28

20, 23 and 28, 28
20, 20 and 23, 28

$PI = (p + q + r)^2$
$PI = (f_{20} + f_{23} + f_{28})^2$
$PI = (0.145 + 0.158 + 0.013)^2$
$PI = (0.316)^2$
$PI = 0.099$
$PE = 1 - CPI = 0.901$

Assume ST = 150 RFU

"Advantages and Disadvantages" RMNE

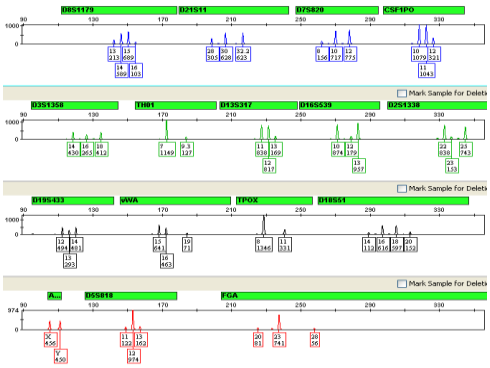
RMNE (CPE/CPI)

Advantages
- Does not require an assumption of the number of contributors to a mixture
- Easier to explain in court
- Deconvolution is not necessary

Disadvantages
- Weaker use of the available information (robs the evidence of its true probative power because this approach does not consider the suspect's genotype).
- Alleles below ST cannot be used for statistical purpose
- There is a potential to include a non-contributor

Summarized from John Buckleton, *Forensic DNA Evidence Interpretation*, p. 223
Buckleton and Curran (2008) *FSI-G* 343-348.

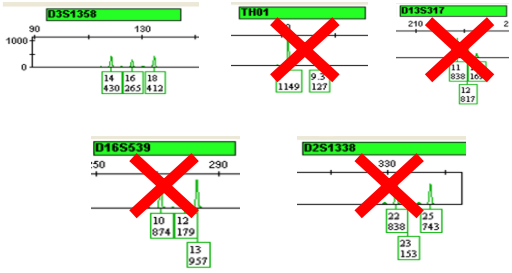
2-Person Mixture



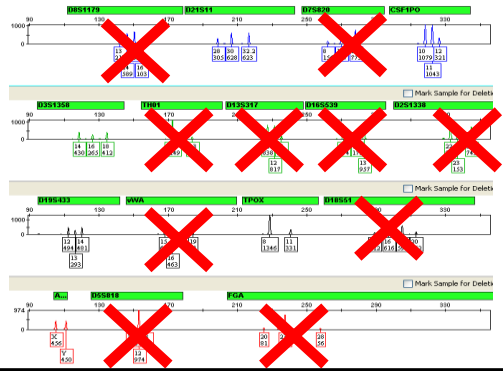
If CPI/CPE Stats are Used

Since exclusionary statistics cannot adjust for the possibility of dropout, and does not take the number of contributors into account, any loci with alleles below the stochastic threshold cannot be used in the CPI statistic.

If CPI/CPE Stats are Used (ST = 200 RFU)



If CPI/CPE Stats are Used



If CPI/CPE Stats are Used

Can use	Cannot use	
D21	D8	D2
CSF	D7	vWA
D3	TH01	D18
D19	D13	D5
TPOX	D16	FGA

Impact: discarding 2/3 of the data

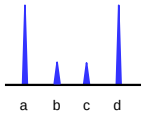
If CPI/CPE Stats are Used

- CPI statistics using FBI Caucasian Frequencies
- 1 in 71 Caucasians included
- 98.59% Caucasians excluded

modified Random Match Probability

Statistical Approaches with Mixtures

- **Random Match Probability (RMP)** – The major and minor components can be successfully separated into individual profiles. A random match probability is calculated on the evidence as if the component was from a single source sample.



$RMP_{minor} = 2pq$

$= 2 \times f(b) \times f(c)$

2013 JFS Article

JOURNAL OF FORENSIC SCIENCES

J Forensic Sci, March 2013, Vol. 58, No. 2
doi: 10.1111/1556-4029.12067
Available online at: onlinelibrary.wiley.com

TECHNICAL NOTE

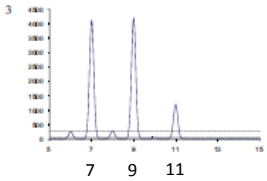
CRIMINALISTICS

Todd Bille,[1] M.Sc.; Jo-Anne Bright,[2] M.Sc.; and John Buckleton,[2] Ph.D.

Application of Random Match Probability Calculations to Mixed STR Profiles

When data is above ST

K = 7,9
S = 7,11
U = 7,11
9,11 or
11,11

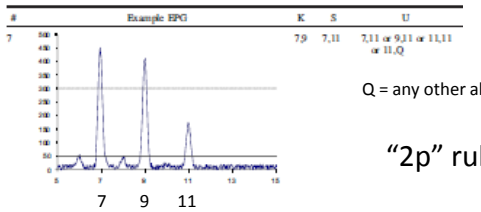


7 9 11

mRMP = $2f_7 f_{11} + 2f_9 f_{11} + (f_{11})^2$

CPI = $(f_7 + f_9 + f_{11})^2$

When data is below ST



7 9 11

Q = any other allele

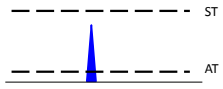
"2p" rule

CPI = n/a

mRMP = 2p

The "2p" Rule

- The "2p" rule can be used to statistically account for zygosity ambiguity – i.e. is this single peak below the stochastic threshold the result of a homozygous genotype or the result of a heterozygous genotype with allele drop-out of the sister allele?



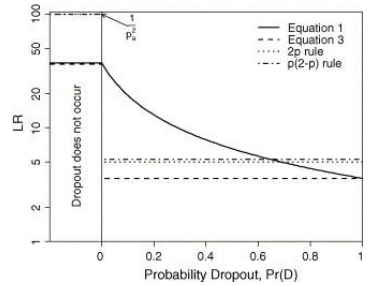
The "2p" Rule

- "This rule arose during the VNTR era. At that time many smaller alleles "ran off the end of the gel" and were not visualised."

- Buckleton and Triggs (2006)

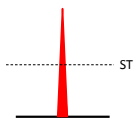
Is the 2*p* rule always conservative?"

The "2p" Rule



Stain = AA

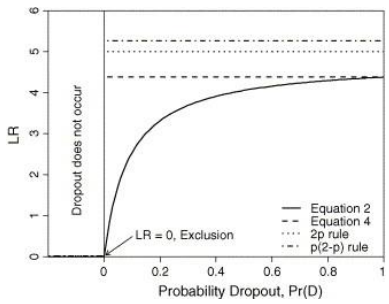
Suspect = AA



LR = 100

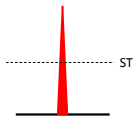
f(a) = 0.10 $1/p^2 = 100$ $1/2p = 5$

The "2p" Rule



Stain = AA

Suspect = AB



Exclusion

f(a) = 0.10 1/2p = 5

Likelihood Ratio

Statistical Approaches with Mixtures

- **Likelihood Ratio** - Comparing the probability of observing the mixture data under two (or more) alternative hypotheses

Likelihood Ratios in Forensic DNA Work

- We evaluate the evidence (*E*) relative to alternative pairs of hypotheses
- Usually these hypotheses are formulated as follows:
  - The probability of the evidence if the crime stain originated with the suspect or Pr(*E*|*S*)
  - The probability of the evidence if the crime stain originated from an unknown, unrelated individual or Pr(*E*|*U*)

$$LR = \frac{\Pr(E \mid S)}{\Pr(E \mid U)}$$

The numerator

The denominator

Slide information from Peter Gill

Likelihood Ratio (LR)

- Provides ability to express and evaluate both the prosecution hypothesis, H_p (the suspect is the perpetrator) and the defense hypothesis, H_d (an unknown individual with a matching profile is the perpetrator)

$$LR = \frac{H_p}{H_d}$$

- **The numerator, H_p, is usually 1** – since in theory the prosecution would only prosecute the suspect if they are 100% certain he/she is the perpetrator
- The denominator, **H_d,** is typically the profile frequency in a particular population (based on individual allele frequencies and assuming HWE) – i.e., **the random match probability**

Slide information from Peter Gill

FSI GENETICS

Forensic Science International: Genetics 2 (2008) 343–348

A discussion of the merits of random man not excluded and likelihood ratios

John Buckleton [a,*], James Curran [b]

[a] *ESR, PB 92021, Auckland, New Zealand*
[b] *Department of Statistics, University of Auckland, PB 92019, Auckland, New Zealand*

Received 15 January 2008; received in revised form 29 April 2008; accepted 1 May 2008

We conclude that the two matters that appear to have real force are:
(1) LRs are more difficult to present in court and
(2) the RMNE statistic wastes information that should be utilised.

To Summarize

- From 2000 – 2006, most DNA cases gave single source profiles and usually contained large quantities of DNA.
- The few mixtures encountered were two-person mixtures.
- Since 2006 – more and more cases are mixtures with low level DNA profiles.
- STR kits are more sensitive – we are doing cases today that we wouldn't touch 15 years ago.

To Summarize

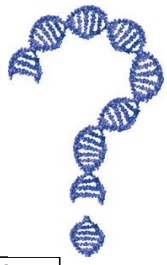
- In the U.S., most labs have adopted a CPI statistical approach. This approach suffers when alleles have dropped out of the evidence.
- Statistical approaches that consider GENOTYPES make better use of the data.

Acknowledgments

National Institute of Justice and NIST Law Enforcement Standards Office

Dr. Charlotte Word
Dr. Robin Cotton
Dr. John Butler

Dan Katz



Contact info:
mcoble@nist.gov
+1-301-975-4330