Mixture Interpretation **Discussion**

John M. Butler, Ph.D.
National Institute of Standards and Technology

CE User's Group Meeting (Ammendale, MD)
April 10, 2008

Planned Promega 2008 Meeting Troubleshooting Workshop

- Title: **"Principles of Interpretation and Troubleshooting of Forensic DNA Typing Systems"**
- Instructors: **John Butler (NIST) and Bruce McCord (FIU)**
- Date: **October 16, 2008** with Promega Int. Symp. Human ID

The workshop will consist of three parts:
(1) a through examination of theoretical issues with capillary electrophoresis PCR amplification of short tandem repeat markers
(2) a discussion of how to properly set instrument parameters to interpret data (including mixtures), and
(3) **a review of specific problems seen by labs** submitting problematic data and commentary on possible troubleshooting solutions.

Seeking input of problems observed with CE systems

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…

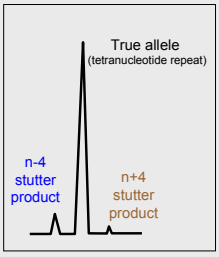
- **Type of sample (biological material if ID'd)**
- **Type of substrate**
- **Quantity amp'd**
- **Minimum # of contributors (1, 2, 3, 4, or >4)**
- **Predominant type (major profile) determined?**
- **Stats reported**
- **Comments**

We would love to have your lab mixture numbers…

Email information to Ann.Gross@state.mn.us

N+4 Stutter Evaluation Summaries

- **Mass State Police DNA Lab**
- **Trying to collect data from as many laboratories as possible** to characterize N + 4 stutter percentages in various platforms.
- Please email information to **rebecca.post@pol.state.ma.us**



N-4 Stutter % of	main allele		N+4		N+4 Stutter % of
	allele	rfu	'allele'	rfu	
6.43%	19	4664	20	57	1.22%

http://www.cstl.nist.gov/biotech/strbase/validation/N+4_stutter_spreadsheet.xls

Topics for Discussion

- SWGDAM Mixture Interpretation Committee progress
- Different statistical approaches: CPE or LR
- ISFG Mixture Interpretation Recommendations
  - UK response
  - German categories for mixtures
- Validation as it relates to mixture interpretation
  - Stochastic threshold vs analytical threshold
- Low-level DNA and mixtures
- Important elements of interpretation guidelines

SWGDAM Mixture Interpretation Subcommittee

- **John Butler** (NIST) - chair
- **Gary Sims** (CA DOJ) - co-chair
- Mike Adamowicz (CT)
- Jack Ballantyne (UCF/NCFS)
- George Carmody (Carleton U)
- Cecelia Crouse (PBSO)
- Allison Eastman (NYSP)
- Roger Frappier (CFS-Toronto)
- Ann Gross (MN BCA)
- Phil Kinsey (MT)
- Jeff Modler (RCMP)
- Gary Shutler (WSP)

Everyone not at every meeting…

Have met 3 times:
Jan 2007
July 2007
Jan 2008

Through the Jan 2008 meeting we have also had to deal with Y-STR issues – which has limited our focus on mixtures

Additional Participants (Jan 2008)
Bruce Heidebrecht (MD)
Steve Lambert (SC)

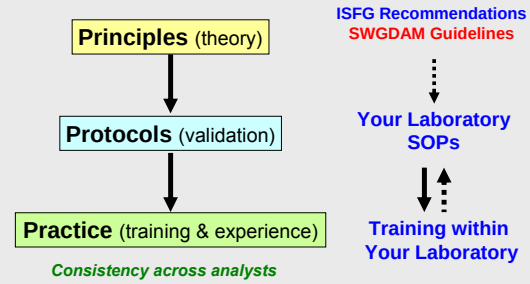
Started in January 2007

Progress and Plans for Mixture Committee

- **Guidelines in process** of being discussed and written
- Collecting data on number and type of mixture cases observed in various labs
- Plan to create a training workbook with worked examples
- Considering flow charts to aid mixture interpretation
- Have discussed responses to ISFG Recommendations

I invite your input as to what should be included in the guidelines…

Elements of DNA Mixture Interpretation



We discussed and would advocate periodic training to aid accuracy and efficiency within your laboratory.

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)

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

ISFG Executive Committee

President
Niels Morling
(Copenhagen, Denmark)

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Peter Schneider
(Köln, Germany)

Working Party Representative
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Wolfgang Mayr
(Vienna, Austria)

Angel Carracedo
FSI Genetics **Editor-in-Chief**
(former ISFG President, VP)
(Santiago de Compostela, Spain)

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

My perspective…

Hierarchy of Rules for Forensic DNA Labs

	United States	Europe
Audits	FBI (DAB) Quality Assurance Standards	ENFSI Policies
	NDIS Procedures	ISFG Recommendations (DNA Commission)
Validation	SWGDAM Guidelines	National Recommendations
	Laboratory Protocols (SOPs)	Laboratory Protocols (SOPs)
Training & Experience	Individual Analyst Practice	Individual Analyst Practice
	Each Case Report	Each Case Report

Hopefully each conforms to the levels above it…

UK Response to ISFG Mixture Recommendations

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


Available online at www.sciencedirect.com
ScienceDirect
Letter to the Editor
National recommendations of the Technical UK DNA working group on mixture interpretation for the NDNAD and for court going purposes

Using the published UK response as a model, let us review the nine ISFG Recommendations on mixture interpretation…

From Report to the **Virginia Scientific Advisory Committee** by the DNA Subcommittee – Addendum January 8, 2008

(authored by Dr. Norah Rudin and Dr. Artie Eisenberg)

- "Among the many reasons that Forensic DNA analysis has become the gold standard for forensic science is the relatively discrete nature of the data. For strong, single source samples, a profile can readily be determined, and is subject to little or no analyst judgment. **However, ambiguity may arise when interpreting more complex samples, such as those containing multiple contributors, of poor quality (e.g. degraded or inhibited DNA), of low quantity (e.g. contact samples), or various combinations of these challenging situations**…"

http://www.dfs.virginia.gov/about/minutes/saCommittee/20080108.pdf

From Report to the **Virginia Scientific Advisory Committee** by the DNA Subcommittee – Addendum January 8, 2008

(authored by Dr. Norah Rudin and Dr. Artie Eisenberg)

- "…**These kinds of samples are encountered with increasing frequency, as the sensitivity of the technology has increased, and as law enforcement has become more sophisticated about the kinds of samples they submit for analysis**. Difficult samples are also frequently encountered when reanalyzing historical cases, in which samples were not collected and preserved using the precautions necessary for DNA analysis…"

"Cold cases" or Innocence Project samples…

http://www.dfs.virginia.gov/about/minutes/saCommittee/20080108.pdf

From Report to the **Virginia Scientific Advisory Committee** by the DNA Subcommittee – Addendum January 8, 2008

(authored by Dr. Norah Rudin and Dr. Artie Eisenberg)

- "It is for these types of challenging samples, where the evidence profile may not exactly "match" a reference profile, that confirmation bias becomes a concern. **The interpretation of an evidentiary DNA profile should not be influenced by information about a subject's DNA profile.** Each item of evidence must be interpreted independently of other items of evidence or reference samples. Yet forensic analysts are commonly aware of submitted reference profiles when interpreting DNA test results, creating the opportunity for confirmatory bias, despite the best intentions of the analyst…"

http://www.dfs.virginia.gov/about/minutes/saCommittee/20080108.pdf

DNA Mixture Interpretation:
Principles and Practice in Component Deconvolution and Statistical Analysis

Principles in Mixture Interpretation

Handouts available on STRBase at
http://www.cstl.nist.gov/biotech/strbase/training/AAFS2008_MixtureWorkshop.htm

AAFS 2008 Workshop #16
Washington, DC
February 19, 2008

John M. Butler

john.butler@nist.gov

Two Parts to Mixture Interpretation

- Determination of alleles present in the evidence and **deconvolution of mixture components** where possible
  - Many times through comparison to victim and suspect profiles
- **Providing some kind of statistical answer** regarding the weight of the evidence
  - There are multiple approaches and philosophies

Software tools can help with one or both of these…

Steps in the Interpretation of Mixtures
(Clayton *et al.* 1998)

Step #1 Identify the Presence of a Mixture
↓
Step #2 Designate Allele Peaks
↓
Step #3 Identify the Number of Potential Contributors
↓
Step #4 Estimate the Relative Ratio of the Individuals Contributing to the Mixture
↓
Step #5 Consider All Possible Genotype Combinations
↓
Step #6 Compare Reference Samples


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]

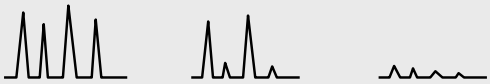
Clayton *et al.* (1998) *Forensic Sci. Int.* 91:55-70

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**

Type of mixture and interpretation

- **Type A:** Mixed profile without stochastic effects, a biostatistical analysis has to be performed
- **Type B:** Profile of a major contributor can be unambiguously described and interpreted as a profile from an unmixed stain
- **Type C:** due to the complexity of the mixture, the occurrence of stochastic effects such as allele and locus drop-outs have to be expected:
  - a clear decision to include or exclude a suspect may be difficult to reach, thus a biostatistical interpretation is not appropriate.

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

Biostatistical approaches

- Calculation of the **probability of exclusion** for a randomly selected stain donor* [P(E)]
  (*RMNE - "random man not excluded")
- Calculation of the **likelihood ratio** [LR] based on defined hypotheses for the origin of the mixed stain

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

Which approach should be used?

- If the basis for clearly defined and mutually exclusive hypotheses is given, i.e.:
  - the number of contributors to the stain can be determined,
  - unambiguous DNA profiles across all loci are observed (type A mixtures, or type B, if the person considered as "unknown" contributor is part of the minor component of the mixture),

  then the calculation of a likelihood ratio is appropriate.

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

Which approach should be used?

- If major/minor contributors cannot be identified based on unambiguous DNA profiles, or if the the number of contributors cannot be determined, then the calculation of the probability of exclusion is appropriate.
- The calculation of P(E) is always possible for type A and type B mixtures.

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

Not acceptable …

- … is the inclusion of a genotype frequency of a non-excluded suspect into the report, if the given mixed stain does not allow a meaningful biostatistical interpretation.
  - this would lead to the wrongful impression that this genotype frequency has any evidentiary value regarding the role of the suspect as a contributor to the mixed stain in question.

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

Conclusions

- The likelihood ratio has a significant weight of evidence, as it relates directly to the role of the suspect in the context of the origin of the stain.
- The exclusion probability makes a general statement without relevance to the role of the suspect.
- However, this does not imply that P(E) is always more "conservative" in the sense that the weight of evidence is not as strong compared to the LR.

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

GEDNAP 32

Mixture interpretation exercise:

- 3 person mixture without major contributor
- Person A from group of reference samples was not excluded
- Allele frequencies for eight German database systems provided for exercise
- German-speaking GEDNAP participants invited to participate based on published recommendations

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

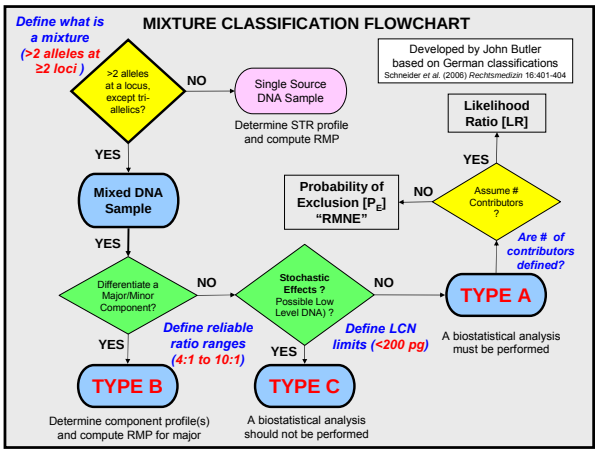
GEDNAP 32

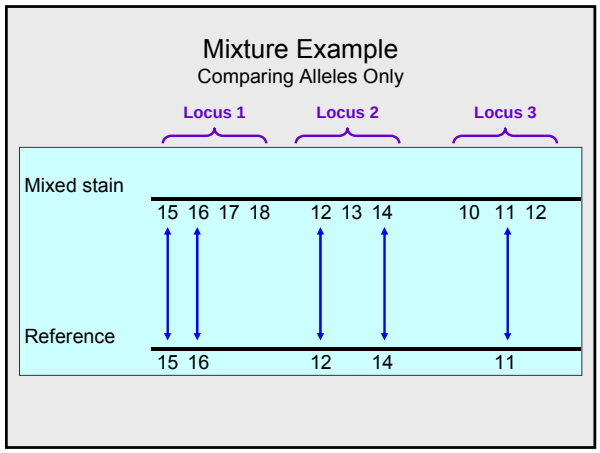
Results:

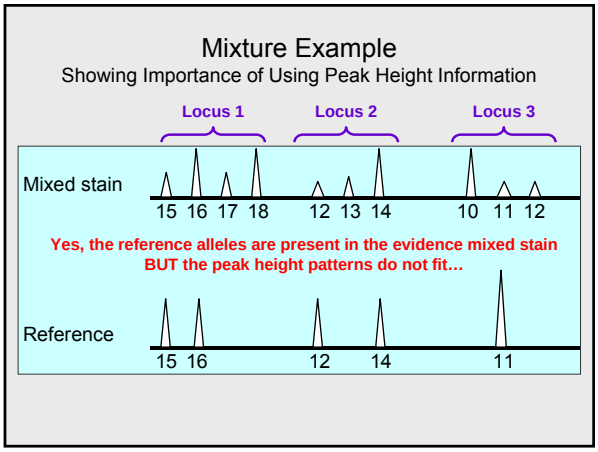
- 22 labs submitted results (from approx. 80 German-speaking GEDNAP participants)
- Calculations submitted were all correct and consistent:
  - 15x LR approach:
    - Person A + 2 unknown vs. 3 unknown contributors
  - 11x RMNE calculation
- Will be offered again next time

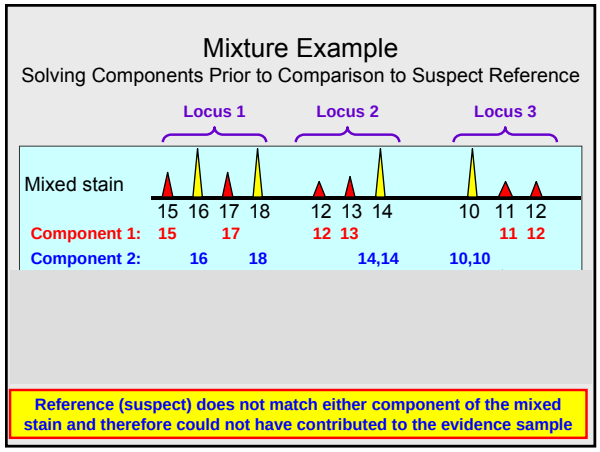
Training and Specific Guidelines/Classification Schemes yielded consistent results among laboratories

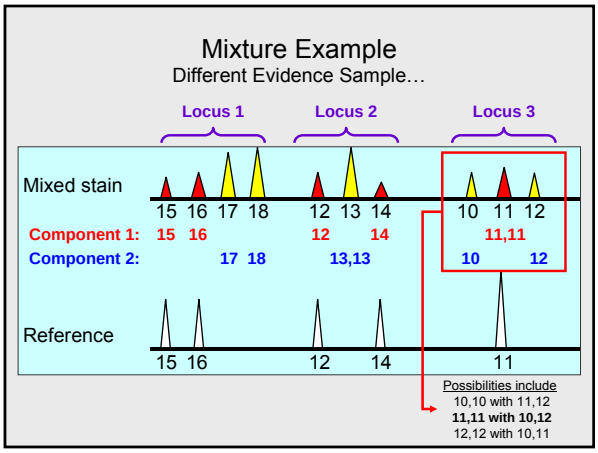
Slide from Peter Schneider (presented at EDNAP meeting in Krakow in April 2007)

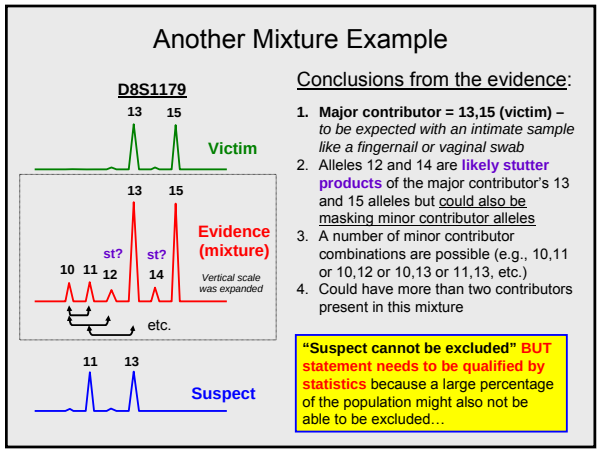

MIXTURE CLASSIFICATION FLOWCHART
Define what is a mixture (>2 alleles at ≥2 loci)
>2 alleles at a locus, except tri-allelics?
NO
Single Source DNA Sample
Determine STR profile and compute RMP
Developed by John Butler based on German classifications
Schneider et al. (2006) Rechtsmedizin 16:401-404
Likelihood Ratio [LR]
YES
Mixed DNA Sample
Probability of Exclusion [PE] "RMNE"
NO
Assume # Contributors ?
Are # of contributors defined?
YES
Differentiate a Major/Minor Component?
NO
Stochastic Effects ? Possible Low Level DNA) ?
NO
TYPE A
A biostatistical analysis must be performed
Define reliable ratio ranges (4:1 to 10:1)
YES
Define LCN limits (<200 pg)
YES
TYPE B
Determine component profile(s) and compute RMP for major
TYPE C
A biostatistical analysis should not be performed


Mixture Example
Comparing Alleles Only
Locus 1
Locus 2
Locus 3
Mixed stain
15 16 17 18 12 13 14 10 11 12
Reference
15 16 12 14 11


Mixture Example
Showing Importance of Using Peak Height Information
Locus 1
Locus 2
Locus 3
Mixed stain
15 16 17 18 12 13 14 10 11 12
Yes, the reference alleles are present in the evidence mixed stain
BUT the peak height patterns do not fit…
Reference
15 16 12 14 11

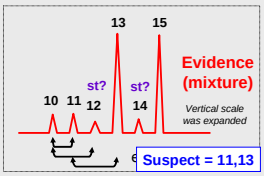

Mixture Example
Solving Components Prior to Comparison to Suspect Reference
Locus 1
Locus 2
Locus 3
Mixed stain
15 16 17 18 12 13 14 10 11 12
Component 1: 15 17 12 13 11 12
Component 2: 16 18 14,14 10,10
Reference (suspect) does not match either component of the mixed stain and therefore could not have contributed to the evidence sample


Mixture Example
Different Evidence Sample…
Locus 1
Locus 2
Locus 3
Mixed stain
15 16 17 18 12 13 14 10 11 12
Component 1: 15 16 12 14 11,11
Component 2: 17 18 13,13 10 12
Reference
15 16 12 14 11
Possibilities include
10,10 with 11,12
11,11 with 10,12
12,12 with 10,11


Another Mixture Example
D8S1179
13 15
Victim
13 15
st?
st?
10 11 12 14
Evidence (mixture)
Vertical scale was expanded
etc.
11 13
Suspect
Conclusions from the evidence:
1. Major contributor = 13,15 (victim) – to be expected with an intimate sample like a fingernail or vaginal swab
2. Alleles 12 and 14 are likely stutter products of the major contributor's 13 and 15 alleles but could also be masking minor contributor alleles
3. A number of minor contributor combinations are possible (e.g., 10,11 or 10,12 or 10,13 or 11,13, etc.)
4. Could have more than two contributors present in this mixture
"Suspect cannot be excluded" BUT statement needs to be qualified by statistics because a large percentage of the population might also not be able to be excluded…

Probability of Exclusion Calculation
for a Single STR Locus

The case may grow stronger against a suspect with information from additional STR loci…



From VA DFS STR Allele Frequencies
http://www.dfs.virginia.gov/manuals/manuals.cfm?id=5

D8S1179 alleles	AA (n=384)	C (n=346)	H (n=366)
10	0.0287	0.1069	0.0820
11	0.0495	0.0925	0.0465
12	0.1094	0.1416	0.1093
13	0.2422	0.3093	0.3224
14	0.2969	0.1965	0.2623
15	0.1849	0.0896	0.1202
SUM	0.9115	0.9364	0.9426
Sq SUM = PI	0.8308	0.8769	0.8886
PE = 1-PI	0.1692	0.1231	0.1114
PE (%)	**16.9%**	**12.3%**	**11.1%**
	African Am.	Caucasians	Hispanics

"Suspect cannot be excluded" BUT we would expect to see, for example, only 11.1% of Hispanics excluded (or 88.9% cannot be excluded) based on results at this one locus

The fact that in this case a suspect is included is not very informative because ~9 out of 10 people examined from any population could potentially be included in the evidence mixture…

The Statistic (Determining the Weight of the Evidence) Should Be **Calculated from the Evidence**

Evidence (partial profile):

	Type	Statistic
Locus 1	16,17	1 in 9
Locus 2	17,18	1 in 9
Locus 3	21,22	1 in 12
Locus 4	12,14	1 in 16
Locus 5	28,30	1 in 11

		Product = 1 in 171,000

Match Observed at All Loci that May Be Compared

Reference (full profile):

	Type	Statistic
Locus 1	16,17	1 in 9
Locus 2	17,18	1 in 9
Locus 3	21,22	1 in 12
Locus 4	12,14	1 in 16
Locus 5	28,30	1 in 11
Locus 6	14,16	1 in 26
Locus 7	12,13	1 in 9
Locus 8	11,14	1 in 31
Locus 9	9,9	1 in 32
Locus 10	9,11	1 in 14
Locus 11	6,6	1 in 19
Locus 12	8,8	1 in 3
Locus 13	10,10	1 in 21

		Product = 1 in 665 trillion

The reference sample is still a "match" – just not as much information is available from the evidence for comparison

Statistical Approaches with Mixtures

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

- **Inferring Genotypes of Contributors** - Separate major and minor components into individual profiles and compute the random match probability estimate as if a component was from a single source
- **Calculation of Exclusion Probabilities** - CPE/CPI (RMNE) – The probability that a random person (unrelated individual) would be excluded as a contributor to the observed DNA mixture
- **Calculation of Likelihood Ratio Estimates** – Comparing the probability of observing the mixture data under two (or more) alternative hypotheses; in its simplest form LR = 1/RMP

RMNE = Random Man Not Excluded (same as CPE)
CPE = Combined Probability of Exclusion (CPE = 1 – CPI)
CPI = Combined Probability of Inclusion (CPI = 1 – CPE)

Advantages and Disadvantages

RMNE (CPE/CPI)

- **Advantages**
  - Does not require an assumption of the number of contributors to a mixture
  - Easier to explain in court
- **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)
  - Likelihood ratio approaches are developed within a consistent logical framework

Likelihood Ratios (LR)

- **Advantages**
  - Enables full use of the data including different suspects
- **Disadvantages**
  - More difficult to calculate

John Buckleton, *Forensic DNA Evidence Interpretation*, p. 223

Assumptions for CPE/CPI Approach

- There is no allele dropout (i.e., all alleles are above stochastic threshold) – low-level mixtures can not reliably be treated with CPE
- All contributors are from the same racial group (i.e., you use the same allele frequencies for the calculations)
- All contributors are unrelated
- Peak height differences between various components are irrelevant (i.e., component deconvolution not needed) – this may not convey all information from the available sample data…

Likelihood Ratio (LR)

- LR is not a probability but a ratio of probabilities

DAB Recommendations on Statistics

February 23, 2000
Forensic Sci. Comm. 2(3); available on-line at
http://www.fbi.gov/hq/lab/fsc/backissu/july2000/dnastat.htm

"The DAB finds either one or both PE or LR calculations acceptable and strongly recommends that one or both calculations be carried out whenever feasible and a mixture is indicated"

- Probability of exclusion (PE)
  - Devlin, B. (1993) Forensic inference from genetic markers. *Statistical Methods in Medical Research*, 2, 241–262.
- Likelihood ratios (LR)
  - Evett, I. W. and Weir, B. S. (1998) *Interpreting DNA Evidence*. Sinauer, Sunderland, Massachusetts.

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

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


Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
Forensic Science International
DNA commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures

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

ISFG Recommendations on Mixture Interpretation

July 13, 2006 issue of *Forensic Science International*

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


ELSEVIER
DNA commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures
Abstract
The DNA commission of the International Society of Forensic Genetics (ISFG) was convened at the 21st congress of the International Society for Forensic Genetics held between 13 and 17 September in the Azores, Portugal. The purpose of the group was to agree on guidelines to encourage best practice that can be universally applied to assist with mixture interpretation. In addition the commission was tasked to provide guidance on low copy number (LCN) reporting. Our discussions have highlighted a significant need for continuing education and research into this area. We have attempted to present a consensus from experts but to be practical we do not claim to have conveyed a clear vision in every respect in this difficult subject. For this reason, we propose to allow a period of time for feedback and reflection by the scientific community. Then the DNA commission will meet again to consider further recommendations.
© 2006 Elsevier Ireland Ltd. All rights reserved.
Keywords: STR typing; Biostatistical analysis; Likelihood ratio; Probability of exclusion; Mixtures; ISFG DNA commission

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

Thoughts by Peter Gill on Recommendation #5

(ENFSI meeting, Krakow, Poland, April 19, 2007)

- Prosecution and defense each want to maximize their respective probabilities
- Recommendation 5 places ownership for each hypothesis.
- In order to perform the LR calculation(s), the forensic scientist decides on both the prosecution and defense hypotheses.
- Since the forensic scientists usually cannot discover the defense hypothesis before the trial (as they are typically working with the prosecution if the DNA matches…), assumptions must be clearly stated with the important caveat that you cannot perform calculations on the stand! (For example, you need three weeks warning to make and check calculations.)
- By anchoring the respective hypotheses to each side, the defense can change their hypothesis but the prosecution does not need to change theirs…
- It is worth noting that the likelihood ratio always goes up if the defense lowers their hypothesis (H_d gets lower with more possible combinations)

ISFG (2006) Recommendations

- Recommendation 6: If the crime profile is a major/minor mixture, **where minor alleles are the same size (height or area) as stutters of major alleles, then stutters and minor alleles are indistinguishable.** Under these circumstances alleles in stutter positions that do not support H_p should be included in the assessment.
- In general, stutter percentage is <15%

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

Consideration of Peak in Stutter Position

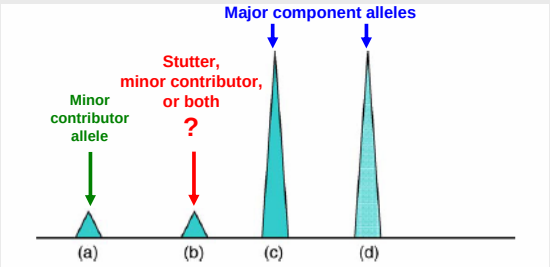


Fig. 4. *c* and *d* are unambiguous alleles, *b* is a minor allele in a stutter position and *a* is an unambiguous minor allele.

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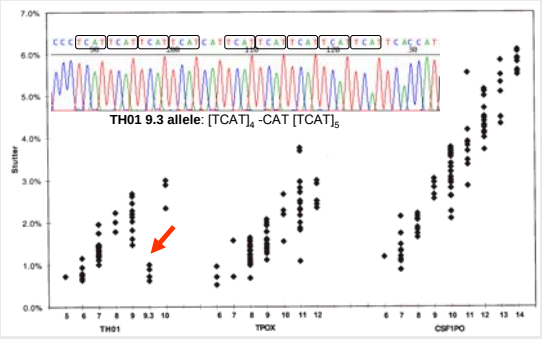
Recommendation 6:

- Stutters are locus-dependent…
- It is recommended that laboratories make their own maximum experimentally observed stutter sizes per locus determinations since the effects may be technique dependent.
- It is recommended that [maximum stutter percentages be] evaluated per locus.

Measured Stutter Percentages

Variable by Allele Length and Composition



Holt CL, Buoncristiani M, Wallin JM, Nguyen T, Lazaruk KD, Walsh PS. TWGDAM validation of AmpFISTR PCR amplification kits for forensic DNA casework. *J Forensic Sci* 2002; 47(1): 66-96.

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- ***Characterization of +4 base stutters***

We agreed to review +4 bp stutters, however, we note that their presence often relates to over-amplified samples. Preliminary experimental work suggests that they are low level and **generally less then 4% the size of the progenitor allele** (Rosalind Brown, personal communication). Note that 4 bp and +4 bp stutter cannot be distinguished from genetic somatic mutation without experimental work—furthermore, somatic mutations may give rise to peaks that are larger than those caused by stutter artifacts.

ISFG (2006) Recommendations

- Recommendation 7: If drop-out of an allele is required to explain the evidence under H_p: (S = ab; E = a), then the allele should be small enough (height/area) to justify this. Conversely, if a full crime stain profile is obtained where alleles are well above the background level, and the probability of drop-out approaches Pr(D) ≈ 0, then H_p is not supported.

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Recommendation 7:

- We recommend slight rewording…[with mention of companion allele]
- If a full crime-stain profile is obtained where alleles are well above the background level, and the probability of dropout Pr(D) approaches zero, then H_p is not supported (Figure 6).

Hypothetical Examples

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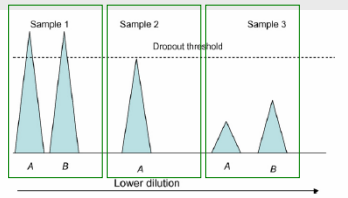

Fig. 4. Results from serial dilutions of the same sample genotype *AB*. The first result (sample 1) shows a locus where both alleles are represented in the profile. One or both of these alleles are above the dropout threshold and consequently are always present in the epg. The second result shows a result where dropout has occurred – the survivor allele is just below the dropout threshold hence this is a rare event, but not impossible. If *A* was just above the dropout threshold we would determine it to be a homozygote *AA* genotype. In the third sample, both alleles are well below the dropout threshold – it is an unambiguous, albeit unbalanced heterozygote. If only one allele was present, then we would have to consider the possibility of dropout of the partner. The same rationale can be applied to any analytical regime, e.g. 28 and 34 PCR cycles.

If Below Dropout Threshold…

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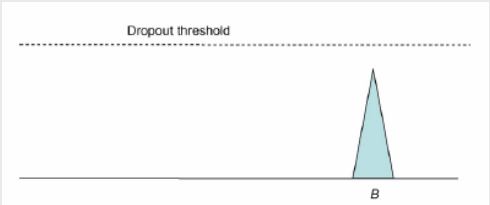

Fig. 5. In this example allele *B* is below the dropout threshold, hence we cannot be confident that it is from a homozygote *BB* individual. It could also be from an individual who is heterozygote, where the missing allele is any other allele. The probability *B*|unknown, H_d is 2Pr(*BF*), where the '*F*' designation is assigned a probability of 1 to take account of the possibility that any allele could have dropped out.

If Above Dropout Threshold…

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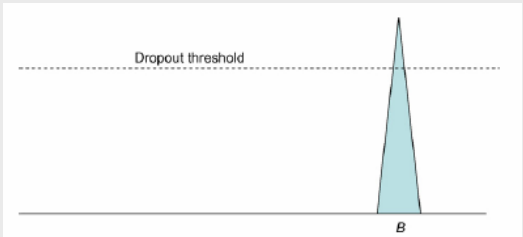

Fig. 6. In this example allele *B* is above the dropout threshold, hence we can be confident that it is from a homozygote *BB* individual. The probability of B|unknown, H_d is $Pr(B)^2$.

Setting Thresholds

- **Detection (analytical) threshold**
  - Dependent on instrument sensitivity
    ~50 RFU
  - Impacted by instrument baseline noise
- **Dropout (stochastic) threshold**
  - Dependent on biological sensitivity
    ~150-200 RFU
  - Impacted by assay and injection parameters

Determining the Dropout (Stochastic) Threshold

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- The dropout threshold can be determined experimentally for a given analytical technique from a series of pre-PCR dilutions of extracts of known genotype technique (it will probably vary between analytical methods). These samples can be used to determine the point where allelic dropout of a heterozygote is observed relative to the size of the survivor companion allele. The threshold is the maximum size of the companion allele observed. This is also the point where Pr(D) approaches zero (Fig. 4).

Dropout threshold will change depending on instrument and assay conditions (e.g., longer CE injection will raise dropout threshold)

ISFG (2006) Recommendations

- Recommendation 8: If the alleles of certain loci in the DNA profile are at a level that is dominated by background noise, then a biostatistical interpretation for these alleles should not be attempted.

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Recommendation 8:

- If there is a band below the experimental threshold where background noise might be prevalent, and it is distinct and clear from the background, then it should be recorded and available on the case file.

ISFG (2006) Recommendations

- Recommendation 9: In relation to low copy number, stochastic effects limit the usefulness of heterozygous balance and mixture proportion estimates. In addition, allelic drop-out and allelic drop-in (contamination) should be taken into consideration of any assessment.

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Recommendation 9:

- Case pre-assessment is necessary in order to determine the best scientific method to process a sample. To facilitate this, it is recommended that wherever possible, this should include quantification. Quantification is used to determine the optimum method to process—if low-level DNA, a sample would benefit from procedures to enhance sensitivity of detection. There may be reasons where quantification is not practicable, especially if low levels of DNA are expected, since the result itself may be compromised if a portion of the sample is sacrificed. At low DNA levels, the accuracy of the quantification test itself may be inefficient.

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Recommendation 9 (cont):

- It is possible that a given DNA profile may simultaneously comprise both 'conventional' and 'low-level' loci: for example, if degradation has occurred then low molecular weight loci may be above the dropout threshold, whereas high molecular weight loci may be below the dropout threshold.
- Similarly, if the sample is a mixture, then at a given locus there may be some alleles that are above the dropout threshold (from a major contributor) and others that are below the dropout threshold (from a minor contributor), i.e. different interpretation rationale may be simultaneously applied to different contributors within a locus.

Thank you for your attention…

Questions
or Comments?

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Our team publications and presentations are available at:
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm