DNA Mixture Interpretation:

Principles and Practice in Component Deconvolution and Statistical Analysis

Background and Introductory Information

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

John M. Butler
Ann Marie Gross
Gary G. Shutler

Purpose for Teaching Workshop

We hope that participants:

- Gain a better understanding of the current approaches being used throughout the community for mixture interpretation
- See worked examples of mixture component deconvolution and statistical analysis
- Come away with ideas to improve your laboratory's interpretation guidelines and training regarding mixtures in forensic casework

Workshop Presenters

Ann Marie Gross
MN BCA

John M. Butler
NIST

George Carmody
Carleton University/
Statistical Consultant

Gary Shutler
Wash State Police
Crime Lab

Angie Dolph
Marshall University
(NIST Summer Intern)

Joanne B. Sgueglia
Mass State Police
Crime Lab

Tim Kalafut
US Army
Crime Lab

Morning Agenda - **Theory**

Background and Introductory Information
8:30 a.m. – 9:00 a.m. – John Butler

Survey Results on Numbers and Types of Casework Mixtures
9:00 a.m. – 9:15 a.m. – Ann Gross

Principles in Mixture Interpretation
9:15 a.m. – 10:15 a.m. – John Butler

***10:15 a.m. – 10:30 a.m.* BREAK**

Strategies for Mixture Deconvolution with Worked Examples
10:30 a.m. – 11:30 a.m. – John Butler

Different Approaches to Statistical Analysis of Mixtures
11:30 a.m. – 12:00 p.m. – George Carmody

***12:00 p.m. – 1:15 p.m.* LUNCH**

Afternoon Agenda – **Practical Application**

Real Case Example – Importance of Properly Stating Your Conclusions
1:15 p.m. – 1:30 p.m. – Gary Shutler

Variability between Labs in Approaches & Mixture Interlaboratory Studies
1:30 p.m. – 2:15 p.m. – John Butler

Validation Studies and Preparing Mixture Interpretation Guidelines
2:15 p.m. – 2:45 p.m. – Joanne Sgueglia

***2:45 p.m. – 3:00 p.m.* BREAK**

Testing of Mixture Software Programs
3:00 p.m. – 3:15 p.m. – Angela Dolph

DNA_DataAnalysis Software Demonstration
3:15 p.m. – 4:00 p.m. – Tim Kalafut

Training Your Staff to Consistently Interpret Mixtures
4:00 p.m. – 4:45 p.m. – Panel Discussion with Ann Gross, Gary Shutler, Joanne Sgueglia

***4:45 p.m. – 5:00 p.m.* – Questions and Answers as needed**

Why this Workshop? Why Now?

- NIST has conducted a series of four different **mixture interpretation interlaboratory studies** between 1997-2005 http://www.cstl.nist.gov/biotech/strbase/interlab.htm

Will be discussed this afternoon

- SWGDAM Mixture Interpretation Committee formed in January 2007 – *we want your input as guidelines and training materials are developed*
- Feedback from SAFS and NEAFS mixture workshops conducted in Fall 2007

Recent Mixture Workshops

Conducted by John Butler

Helpful feedback obtained from workshop participants

Southern Association of Forensic Scientists (SAFS)

September 11, 2007 (Atlanta, GA)

- **Mixture Interpretation (theory)**
- Along with Software discussion (Rhonda Roby) and demonstration (Tom Overson/Tim Kalafut)
- **33 attendees from 13 different labs**

Northeastern Association of Forensic Scientists (NEAFS)

November 2-3, 2007 (Bolton Landing, NY)

- **The Cutting Edge of DNA Testing: Mixture Interpretation, miniSTRs, and Low Level DNA**
- **42 attendees from 13 different labs**

NEAFS Workshop materials (70 pages) available on STRBase:
http://www.cstl.nist.gov/biotech/strbase/pub_pres/NEAFS2007_CuttingEdgeDNA.pdf

Overview of Planned Workshop Flow

Principles (theory)

↓

Protocols (validation)

↓

Practice (training & experience)

At the end of the day, you have to draw a conclusion with your data…

John Butler and George Carmody to cover theory

Joanne Sgueglia to review protocol development based on validation

Panel Discussion

Gary Shutler to discuss case conclusions

Mixture Basics

From J.M. Butler (2005) *Forensic DNA Typing, 2nd Edition*, p. 154

- Mixtures arise when two or more individuals contribute to the sample being tested.
- Mixtures can be challenging to detect and interpret without extensive experience and careful training.

Even more challenging with poor quality data when degraded DNA is present…

- Differential extraction can help distinguish male and female components of many sexual assault mixtures.

Y-chromosome markers can help here in some cases…

More on Mixtures...

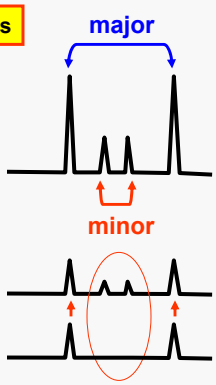
Most mixtures encountered in casework are 2-component mixtures arising from a combination of victim and perpetrator DNA profiles

Ann Gross will discuss some recent collected casework summaries

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**

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

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

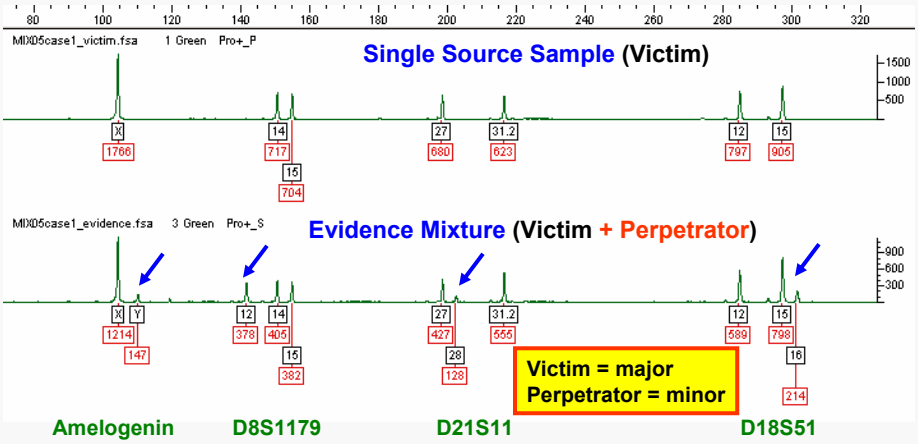


Some mixture interpretation strategies involve using victim (or other reference) alleles to help isolate obligate alleles coming from the unknown portion of the mixture

http://www.cstl.nist.gov/biotech/strbase/interlab/MIX05.htm

MIX05 Case #1; Profiler Plus green loci

Example Mixture Data (MIX05 Study-Profiler Plus)



Obligate Alleles (not present in the victim reference)

Amelogenin	D8S1179	D21S11	D18S51
Y	12	28	16

True "Perpetrator" Profile

Amelogenin	D8S1179	D21S11	D18S51
X,Y	12,12	28,31.2	15,16

UPDATED SLIDE

Sources of DNA Mixtures

- **Two (or more) individuals** contribute to the biological evidence examined in a forensic case (e.g., sexual assault with victim and perpetrator or victim, consensual sexual partner, and perp)

  Victim Reference and Spouse or Boyfriend Reference

- **Contamination** of a single source sample from
  - evidence collection staff
  - laboratory staff handling the sample
  - Low-level DNA in reagents or PCR tubes or pipet tips

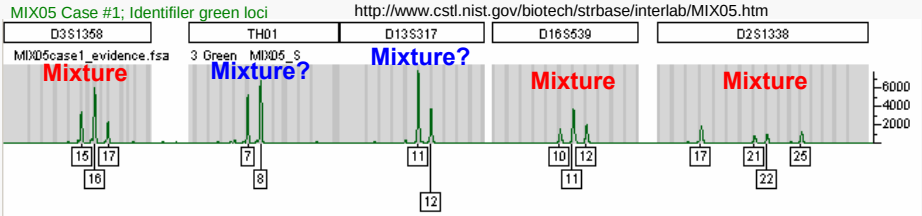
  Examine Staff Profiles (Elimination Database), etc.

Reference elimination samples are useful in deciphering both situations due to possibility of intimate sample profile subtraction

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.



NEW SLIDE

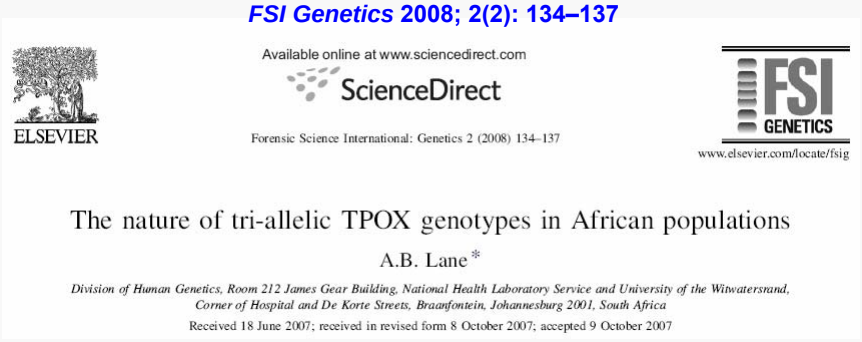
Detecting Mixtures

- Review and compile information from the entire profile – **don't just focus on a single locus!**
- **Tri-allelic patterns exist** in single source samples
  - **145 different tri-alleles recorded for the 13 core CODIS loci** on STRBase as of Jan 22, 2008
  - CSF1PO (5), FGA (22), TH01 (1), TPOX (15), VWA (18), D3S1358 (6), D5S818 (4), D7S820 (7), D8S1179 (11), D13S317 (8), D16S539 (8), D18S51 (21), D21S11 (19)
- A mixture often declared when **>2 peaks in ≥2 loci**

NEW SLIDE

TPOX Tri-Allelic Patterns

***FSI Genetics* 2008; 2(2): 134–137**



Available online at www.sciencedirect.com
ScienceDirect
Forensic Science International: Genetics 2 (2008) 134–137
www.elsevier.com/locate/fsig

The nature of tri-allelic TPOX genotypes in African populations

A.B. Lane *

Division of Human Genetics, Room 212 James Gear Building, National Health Laboratory Service and University of the Witwatersrand, Corner of Hospital and De Korte Streets, Braanfontein, Johannesburg 2001, South Africa

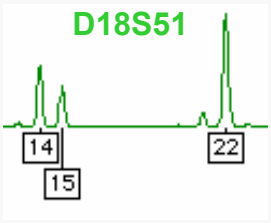
Received 18 June 2007; received in revised form 8 October 2007; accepted 9 October 2007

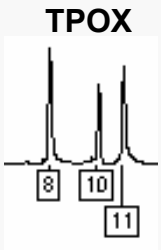
Approximately 2.4% of indigenous South Africans have three rather than two TPOX alleles. Data collected during routine paternity testing revealed that **the extra allele is almost always allele 10** and that it segregates independently of those at the main TPOX locus. Approximately twice as many females as males have tri-allelic genotypes which suggested that **the extra allele is on an X chromosome.**

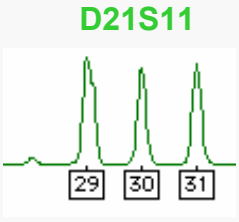
NEW SLIDE

Three-Peak Patterns

Clayton *et al.* (2004) A genetic basis for anomalous band patterns encountered during DNA STR profiling. *J Forensic Sci.* 49(6):1207-1214







"Type 1"

Sum of heights of two of the peaks is equal to the third

Most common in D18S51 and …..

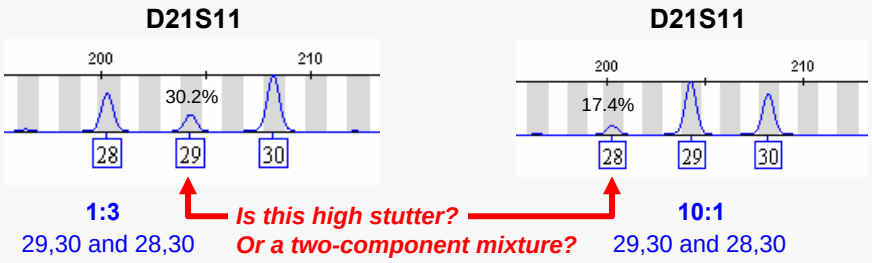
"Type 2"

Balanced peak heights

Most common in TPOX and D21S11

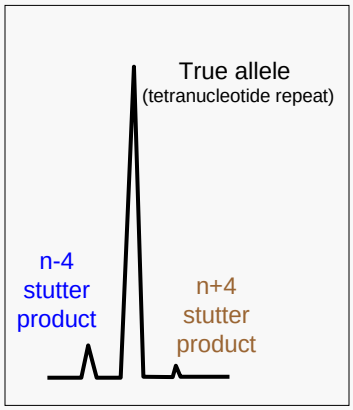
Mixtures: Issues and Challenges

- 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



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

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

Worked examples will be presented

- **Providing some kind of statistical answer** regarding the weight of the evidence
  - There are multiple approaches and philosophies

George Carmody will explain

Software tools can help with one or both of these…

Status of Software for Mixture Interpretation

- NIJ Expert System Testbed (**NEST**) Project
  - Evaluating software programs for DNA analysis of single-source (Phase I) and mixtures (Phase II)
  - **http://forensics.marshall.edu/NEST/NEST-Intro.html**

Angie Dolph will review efforts of NEST and NIST

- US Army Crime Laboratory (**USACIL**)
  - Commonly deal with complex sexual assaults
  - Developed software for aiding mixture interpretation and statistical analysis

Tim Kalafut to demonstrate their software

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Responses to Questions
from a Previous Mixture Workshop (Fall 2007)

What are the biggest obstacles you face in your lab in terms of mixture interpretation?

- Trying to be consistent in my interpretation and with coworkers
- Consistency between analysts
- No consistency – based on analysts discretion/experience; due to lack of consistent training
- Vague SOP leading to inconsistency between analysts due to differences in how "conservative" or not each analyst is
- There is a lot of "individual interpretation" in our lab
- Varying opinions between interpreting analysts due to lack of uniform guidelines
- Resistance to change from other analysts/supervisors
- Getting management to commit to guidelines that will be followed by everyone

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Responses to Questions
from a Previous Mixture Workshop (Fall 2007)

What are the biggest obstacles you face in your lab in terms of mixture interpretation?

- Where to draw the line without throwing away valuable data
- Partial minor contributors
- Stochastic effects in minor components
- STATS and presenting them in court so that the jury will understand them
- When to do stats and what stats to do in different cases
- Lack of concrete/uniform guidelines from statisticians

UPDATED SLIDE

Audience – Who Is Here Today?

- 199 registered (as of 2/4/08)
  - **Forensic DNA analysts and technical leaders** from **37 different states**, AFDIL, USACIL, and FBI
  - Individuals from 7 countries outside of U.S.
  - Private labs and consultants
  - Commercial suppliers: Applied Biosystems, Promega
  - College professors and students
  - Lawyers (prosecution and defense)
  - Defense experts
- Las Vegas, New York, Miami (West Palm Beach) – so all *CSI* sites are covered!

Dialogue between scientists and lawyers is essential and more education can only help…

Questions ???

- **Due to the volume of material we are trying to cover, we will not have time to stop and answer extensive questions during the presentations**
- Please write your questions down
- Feel free to email us with your questions
- We will try to allow a few minutes at the end of each presentation, and we will be happy to stay afterwards and answer questions

Other Resources

- Mixture literature listing <hyperlink>
- **http://www.cstl.nist.gov/biotech/strbase/mixture.htm**

NIST and NIJ Disclaimer

Funding: Interagency Agreement 2003-IJ-R-029 between the National Institute of Justice and NIST Office of Law Enforcement Standards

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