DNA Mixture Analysis:

Principles and Practice of Mixture Interpretation and Statistical Analysis Using the SWGDAM STR Interpretation Guidelines

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Background and Introductory Information

Michael D. Coble

John M. Butler

Todd W. Bille

AAFS 2011 Workshop #17

Chicago, IL

February 22, 2011

Purpose for Teaching Workshop

We hope that participants:

- Gain a better understanding of the basic principles and practice behind DNA mixture interpretation and statistical analysis utilizing the SWGDAM STR Interpretation Guidelines
- 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

Audience – Who Is Here Today?

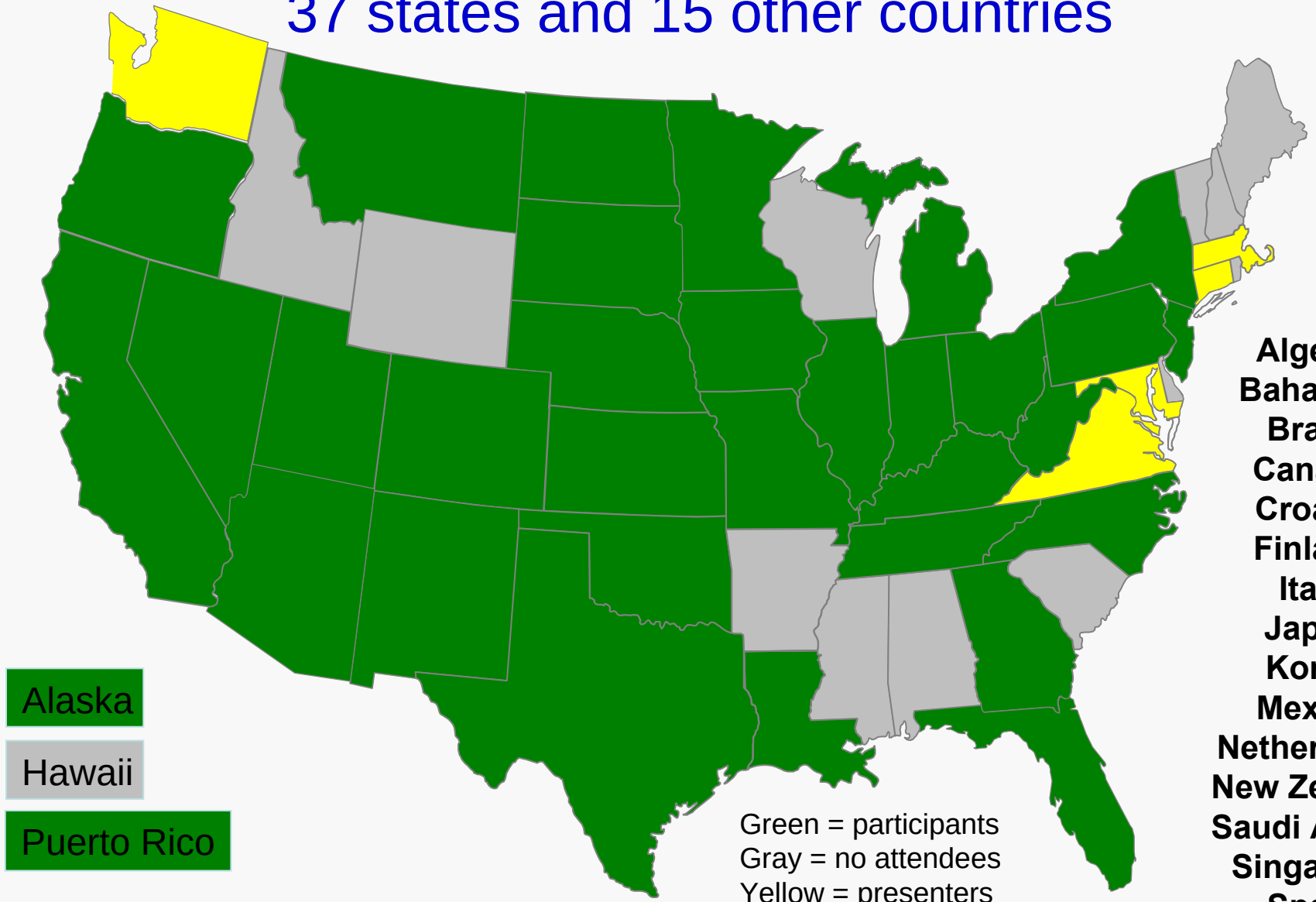
- **220 registered**
  - **Forensic DNA analysts and technical leaders** from **37 different states**, AFDIL, USACIL, ATF, and FBI
  - Individuals from 15 countries outside of U.S.
  - Private labs and consultants
  - Commercial suppliers: Applied Biosystems
  - College professors and students
  - Lawyers (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…

AAFS 2011 Mixture Workshop Attendees

37 states and 15 other countries



Algeria
Bahamas
Brazil
Canada
Croatia
Finland
Italy
Japan
Korea
Mexico
Netherlands
New Zealand
Saudi Arabia
Singapore
Spain

Workshop Presenters

Mike Coble
NIST

John M. Butler
NIST

Todd Bille
ATFE

Mike Adamowicz
University of
New Haven

Gary Shutler
Wash State Police
Crime Lab

Jennifer Gombos
Montgomery County
Crime Lab

Joanne B. Sgueglia
Mass State Police
Crime Lab

Ray Wickenheiser
Montgomery County
Crime Lab

Morning Agenda - **Principles**

Welcome and Introductory Information
8:30 a.m. – 8:40 a.m. – John Butler and Mike Coble

The SWGDAM STR Interpretational Guidelines and the Mixture Literature
8:40 a.m. – 9:30 a.m. – John Butler

Fundamentals of Interpreting STR Mixtures
9:30 a.m. – 10:30 a.m. – Mike Adamowicz

***10:30 a.m. – 10:45 a.m.* BREAK**

Developing Thresholds, Protocols and Validation Studies using the new SWGDAM Guidelines
10:45 a.m. – 11:15 a.m. – Joanne Sgueglia

Different Approaches to Statistical Analysis of Mixtures
11:15 a.m. – 12:00 p.m. – Todd Bille

***12:00 p.m. – 1:15 p.m.* LUNCH**

Afternoon Agenda – **Practical Applications**

Case Summary Analysis
1:15 p.m. – 1:30 p.m. – John Butler

Putting it all Together: A Case Example
1:30 p.m. – 2:00 p.m. – Mike Coble

Complex Mixtures – Strategies and Challenges
2:00 p.m. – 2:30 p.m. – Gary Shutler

A Survey of Mixture Interpretation Software
2:30 p.m. – 3:00 p.m. – Mike Coble

***3:00 p.m. – 3:15 p.m*. BREAK**

Updating Your Protocols – Lessons Learned
3:15 p.m. – 4:00 p.m. – Jennifer Gombos

Training Your Staff to Consistently Interpret Mixtures
4:00 p.m. – 4:45 p.m. – Ray Wickenheiser

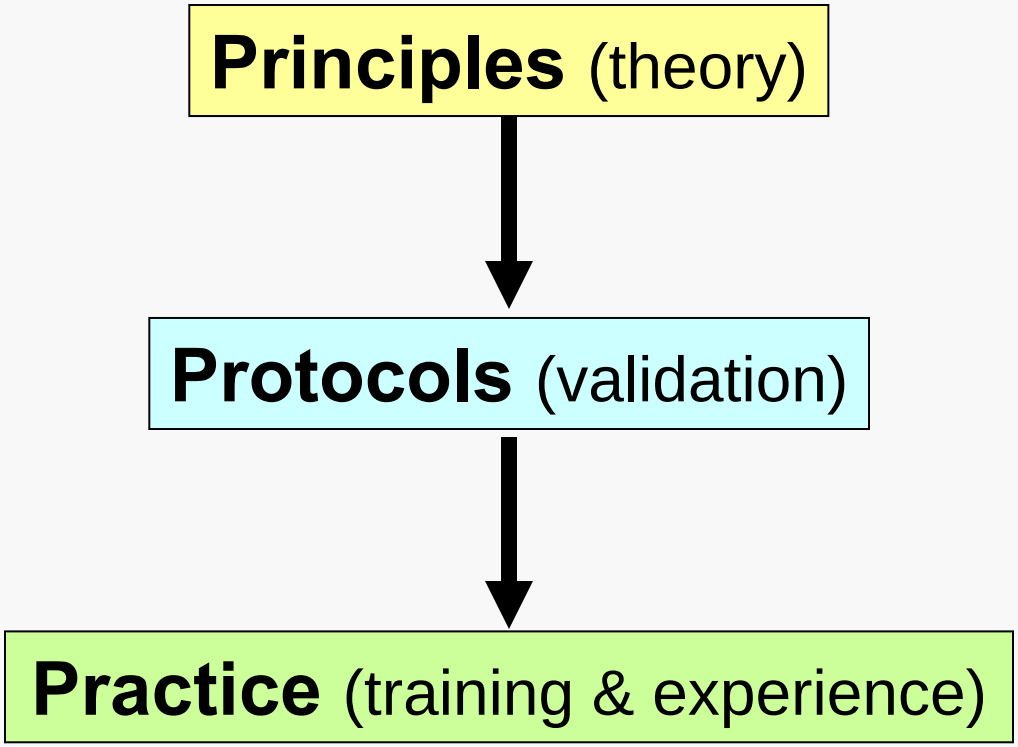
***4:45 p.m. – 5:00 p.m.* – Questions and Answers as needed**

Why this Workshop? Why Now?

- SWGDAM STR Interpretation Guidelines were approved in January 2010 and published in April 2010.

- The participants should gain a better understanding of applying the principles within the SWGDAM STR Interpretation Guidelines to validating mixture protocols, resolving DNA mixtures, developing strategies for statistical analysis, and reporting the results.

Overview of Planned Workshop Flow



John Butler, Mike Adamowicz and Todd Bille to cover theory

Joanne Sgueglia and Jennifer Gombos to review protocol development based on validation

Mike Coble, Gary Shutler and Ray Wickenheiser to discuss case conclusions

Mixture Basics

From J.M. Butler (2009) *Fundamentals of Forensic DNA Typing, 3nd Edition*, pp. 320-330

- 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...

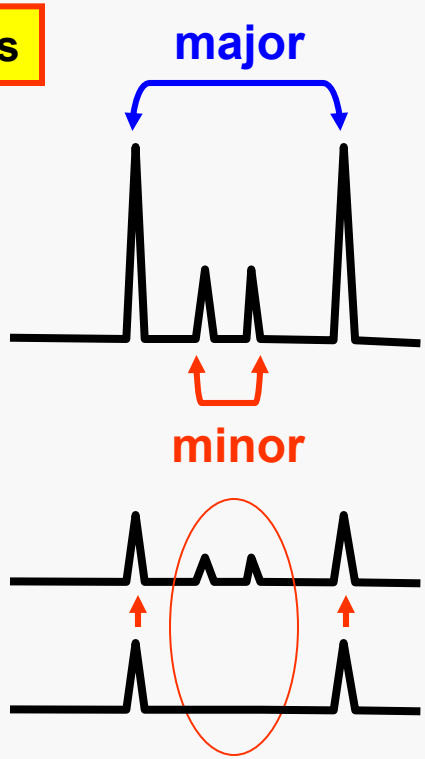
John Butler will discuss some recent collected casework summaries

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**

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

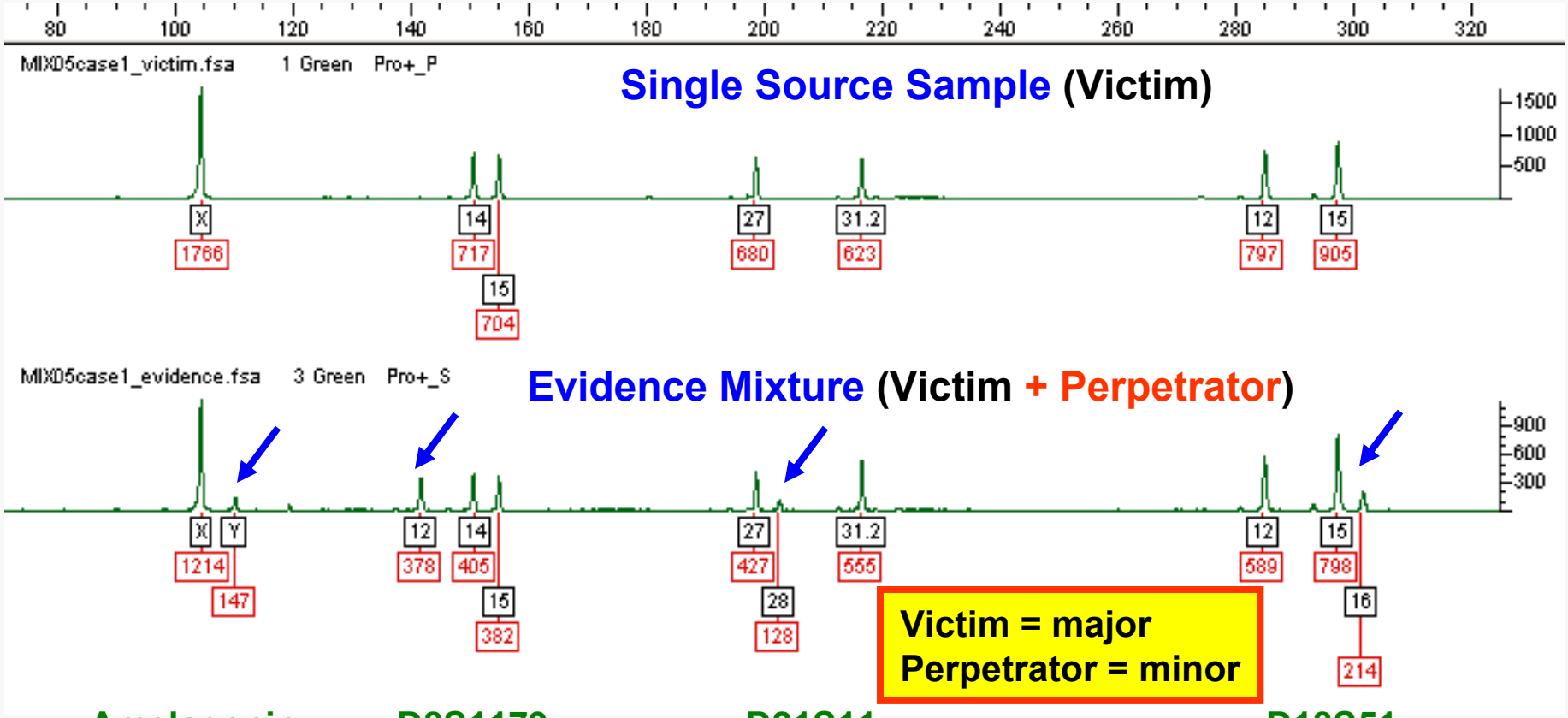


Some mixture interpretation strategies involve using victim (or other reference) alleles to help isolate obligate alleles coming from the unknown portion of the mixture

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

MIX05 Case #1; Profiler Plus green loci

Example Mixture Data (MIX05 Study-Profiler Plus)



Amelogenin **D8S1179** **D21S11** **D18S51**

Obligate Alleles (not present in the victim reference)

Y **12** **28** **16**

True "Perpetrator" Profile

X,Y **12,12** **28,31.2** **15,16**

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 (2009) *Fundamentals of Forensic DNA Typing, 3nd Edition*, pp. 320-330

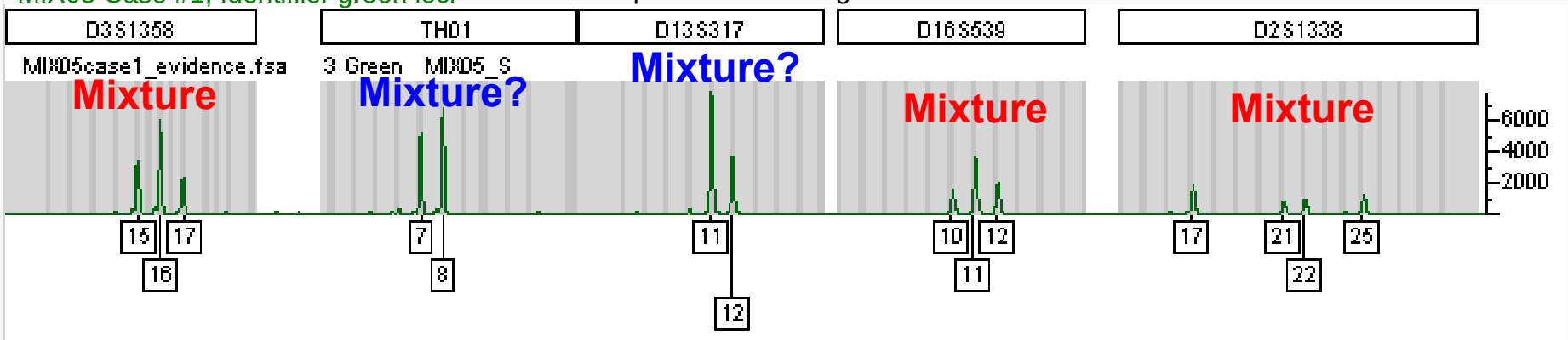
- The probability that a mixture will be detected improves with the use of more loci and genetic markers that have a high incidence of heterozygotes.

- 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.

MIX05 Case #1; Identifiler green loci

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



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
  - **173 different tri-alleles recorded for the 13 core CODIS loci** on STRBase as of Nov 11, 2010
  - CSF1PO (7), FGA (27), TH01 (3), TPOX (15), VWA (20), D3S1358 (9), D5S818 (7), D7S820 (10), D8S1179 (12), D13S317 (9), D16S539 (9), D18S51 (27), D21S11 (18)

- A mixture often declared when **>2 peaks in ≥2 loci**

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 *

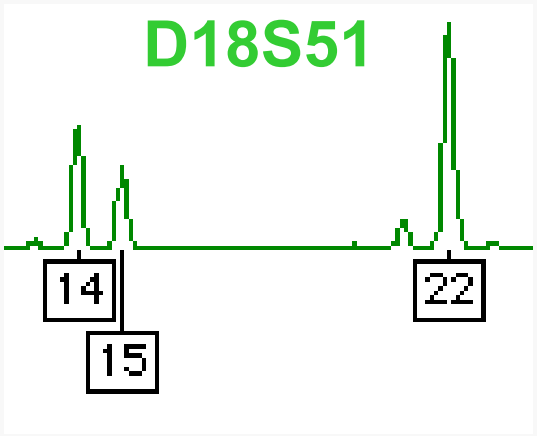
Division of Human Genetics, Room 212 James Gear Building, National Health Laboratory Service and University of the Witwatersrand, Corner of Hospital and De Korte Streets, Braanfontein, Johannesburg 2001, South Africa

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.**

Three-Peak Patterns

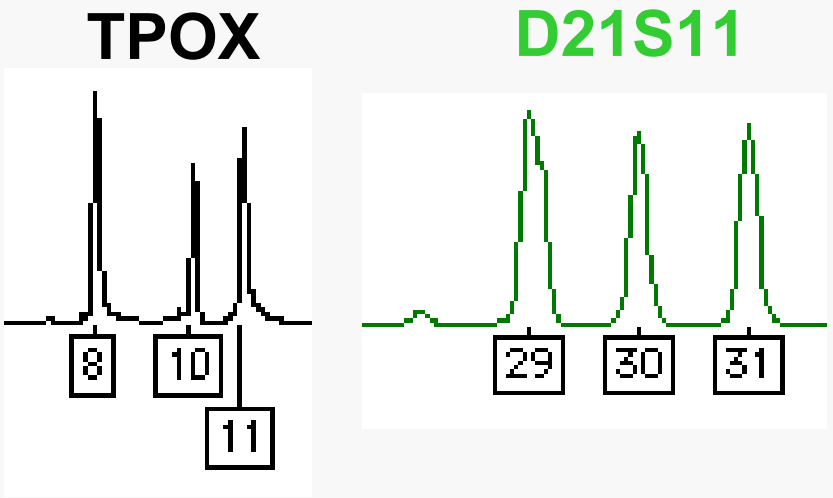
Clayton *et al.* (2004) A genetic basis for anomalous band patterns encountered during DNA STR profiling. *J Forensic Sci.* 49(6):1207-1214



"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

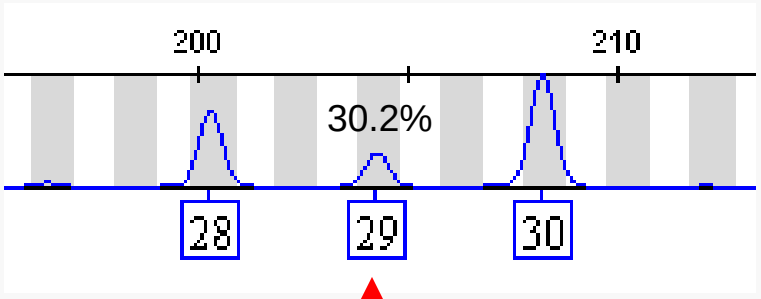
Most common in TPOX and D21S11

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

D21S11



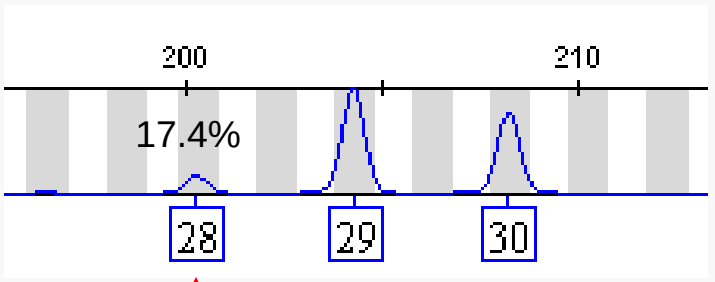
1:3

29,30 and 28,30

Is this high stutter?

Or a two-component mixture?

D21S11



10:1

29,30 and 28,30

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

Todd Bille will discuss

Software tools can help with one or both of these…

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 (in handout)
- SWGDAM STR Interpretation Guidelines (in handout)
- **http://www.cstl.nist.gov/biotech/strbase/mixture.htm**

NIST and NIJ Disclaimer

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