NIST Human Identity Team Projects

John M. Butler
National Institute of Standards and Technology
Human Identity Project Team
"Leading the Way in Forensic DNA…"

Presentation to USACIL
November 14, 2006
Forest Park, GA

NIST and NIJ Disclaimer

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

Points of view are mine and do not necessarily represent the official position or policies of the US Department of Justice or the National Institute of Standards and Technology.

Certain 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 National Institute of Standards and Technology nor does it imply that any of the materials, instruments or equipment identified are necessarily the best available for the purpose.

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

Presentation Outline

- Team Members and Projects
- Training Workshops Conducted
- Technology Efforts
  - miniSTRs
  - Y-STRs
  - mtDNA
  - DNA Quantitation (qPCR)
  - STR Allele Sequencing
  - SNPs
  - Expert systems
  - Validation
  - Software
- Mixture Interpretation Interlab Study - MIX05

NIST History and Mission

- National Institute of Standards and Technology (NIST) was created in 1901 as the National Bureau of Standards (NBS). The name was changed to NIST in 1988.
- NIST is **part of the U.S. Department of Commerce** with a mission to develop and promote measurement, standards, and technology to enhance productivity, facilitate trade, and improve the quality of life.
- NIST supplies over 1,300 Standard Reference Materials (SRMs) for industry, academia, and government **use in calibration of measurements**.
- **NIST defines time for the U.S.**

$532 for 3 jars


DNA typing standard

NIST Gaithersburg Campus


Administration (Building 101)

Located in Gaithersburg, Maryland, on approximately 234 hectares (578 acres) just off Interstate 270 about **25 miles northwest of Washington, D.C.**

http://www.nist.gov

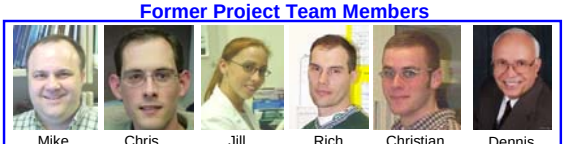
~2,500 staff


Advanced Chemical Sciences Laboratory (Building 227)

NIST Human Identity Project Team


John Butler | **Margaret Kline** | **Jan Redman** | **Pete Vallone** | **Becky Hill** | **Amy Decker** | **Dave Duewer**

Former Project Team Members


Mike Coble – **AFDIL**
Chris DeAngelis – **Medical School**
Jill Appleby – **NC SBI**
Rich Schoske – **Air Force**
Christian Ruitberg – **Pharma**
Dennis Reeder – **Retired/ABI**

- **27 publications** (published or submitted) since Jan 2006
- **45 presentations** and **10 workshops** to the community since Jan 2006
- **Training workshops**: **AAFS, MAAFS, MAFS, NEAFS, OCME, MASP, NYSP, MN BCA (slides available on STRBase)**
- **PDI Workshops: Validation, mtDNA, qPCR**

All NIST publications and presentations available on STRBase: http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

Training Workshops Conducted

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

John Butler (and Bruce McCord, Robyn Ragsdale, Pete Vallone, or Mike Coble)

Sept 29-30, 2004

Advanced Topics in STR DNA Analysis

February 20, 2006

University at Albany – Northeast Regional Forensics Institute

June 13-14, 2005

April 27-28, 2006

May 3, 2006

Applied Biosystems HID University

May 10, 2006

PRESIDENT'S DNA INITIATIVE

August 24-26, 2005

March 13-15, 2006

July 26-27, 2006

October 11, 2006

April 4, 2006

Chiapas

August 17, 2006

November 1, 2006

FBI LABORATORY

May 19, 2005

June 8, 2005

June 6, 2006

BCA

August 7, 2006

AAFS Workshop #6 (Feb 2006, Seattle)

Advanced Topics in STR DNA Analysis

Instructors: John Butler and Bruce McCord

For DNA analysts using the ABI 310 or ABI 3100 who would like to better understand the underlying issues and science involved with STR DNA typing

- **STR Biology, Markers, and Methods**
- **Capillary Electrophoresis Instrumentation: Theory and Application**
- **Validation Aspects to Consider in Bringing a New STR Kit "On-line"**
- **CE Troubleshooting**
- **STR Mixture Interpretation**
- **DNA Quantitation with Real-Time qPCR**
- **Low-copy Number Issues**
- **Y-STRs and mtDNA**

Available at http://www.cstl.nist.gov/biotech/strbase/training.htm

Training Materials Available on STRBase

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

John Butler Pete Vallone

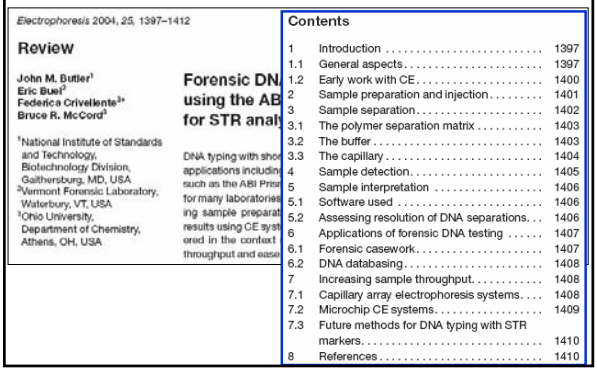
Training Materials/Review Articles

- Workshops on **STRs and CE** (ABI 310/3100) and Other Issues
  - John Butler with Bruce McCord, FIU
  - AAFS, NYSP, MASP, NEAFS, MAAFS, NYC OCME, MN BCA, Mexico
- PDI/NFSTC Workshops
  - **Validation** (John Butler with Robyn Ragsdale, FDLE) – Aug 2005
  - **mtDNA** (Mike Coble with Suni Edson, AFDIL) – Mar 2006
  - **qPCR** (Pete Vallone with Cristian Orrego, CA DOJ) – July 2006
- PowerPoint slides from *Forensic DNA Typing*, 2nd Edition
  - >150 slides available now (~1,000 planned) for download
  - **http://www.cstl.nist.gov/biotech/strbase/FDT2e.htm**
- Review articles
  - ABI 310 and 3100 chemistry – *Electrophoresis* 2004, 25, 1397-1412
  - Core STR loci – *J. Forensic Sci.* 2006, 51, 253-265

http://www.cstl.nist.gov/biotech/strbase/training.htm
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

Review Article on STRs and CE

pdf available from http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm



Electrophoresis 2004, 25, 1397–1412

Review

John M. Butler[1]
Eric Buel[2]
Federica Crivellente[3]*
Bruce R. McCord[3]

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[2]Vermont Forensic Laboratory, Waterbury, VT, USA
[3]Ohio University, Department of Chemistry, Athens, OH, USA

Forensic DN
using the AB
for STR anal

DNA typing with sho
applications including
such as the ABI Prism
for many laboratories
ing sample preparat
results using CE syst
ered in the context
throughput and ease

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Analytical Chemistry Application Review

June 15, 2005 issue of *Analytical Chemistry*

Forensic Science

T. A. Brettell*

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J. M. Butler

National Institute of Standards and Technology, Gaithersburg, Maryland 20899-8311

R. Saferstein

Box 1334, Mount Laurel, New Jersey 08054

Review Contents
- Forensic DNA Analysis
  - Collection, Characterization, Preservation, Extraction, and Quantitation of Biological Material
  - Short Tandem Repeats
  - Single-Nucleotide Polymorphisms
  - Y-STR Typing, Gender Identification, and X-Chromosome Analysis
  - Mitochondrial DNA Typing
  - Nonhuman DNA Typing Systems and Microbial Forensics
  - DNA Databases
  - Interpretation and Statistical Weight of DNA Typing Results
  - General Reviews

250 articles referenced covering forensic DNA analysis during 2003-2004

Support to the Community

…Bringing traceability and technology to the scales of justice…

- Conduct interlaboratory studies
- Perform beta-testing of new human identity testing products
- Provide input to
  - Scientific Working Group on DNA Analysis Methods (**SWGDAM**)
  - Department of Defense Quality Assurance Oversight Committee for DNA Analysis
  - American Prosecutor's Research Institute (**APRI**) DNA Forensics Program "Course-in-a-Box" for training lawyers
  - WTC Kinship and Data Analysis Panel (**KADAP**)
  - 2005 Hurricane Victim DNA Identification Expert Group (**HVDIEG**)
  - NIJ Expert System Testbed (**NEST**) Project

National Institute of Justice

The Research, Development, and Evaluation Agency of the U.S. Department of Justice

Current Areas of NIST Effort with Forensic DNA

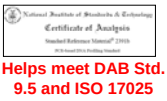
- **Standards**
  - Standard Reference Materials
  - Standard Information Resources (STRBase website)
  - Interlaboratory Studies
- **Technology**
  - Research programs in SNPs, miniSTRs, Y-STRs, mtDNA, qPCR
  - Assay and software development, expert system review
- **Training Materials**
  - Review articles and workshops on STRs, CE, validation
  - PowerPoint and pdf files available for download

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

Standard Reference Materials

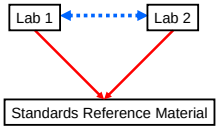
http://www.cstl.nist.gov/biotech/strbase/srm_tab.htm

Traceable standards to ensure accurate measurements in our nation's crime laboratories



Helps meet DAB Std. 9.5 and ISO 17025

SRM 2391b – CODIS STRs
SRM 2392-I – mtDNA
SRM 2395 – Y-STRs
SRM 2372 – DNA quantitation



Calibration with SRMs enables confidence in comparisons of results between laboratories

Information Resource

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

Includes information on:
- Core STR loci
- Validation
- STR reference list
- NIST publications
- miniSTRs
- Forensic SNPs
- Variant STR alleles
- Population data resources
- Addresses of scientists

Provides up-to-date information and has been used in court cases to support application of DNA technology

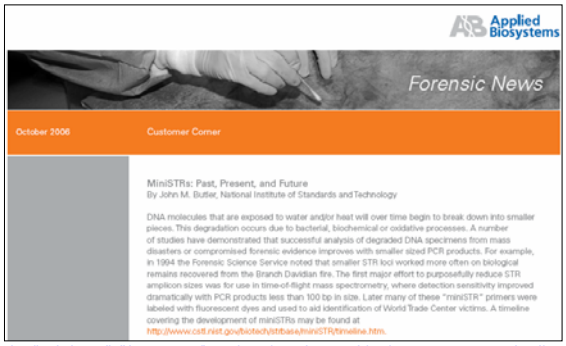
Recent STRBase Updates…

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

Technology: Research Programs

- miniSTRs
- Y-chromosome STRs
- mtDNA
- SNPs
- qPCR for DNA quantitation
- DNA stability studies
- Variant allele characterization and sequencing
- Software tools
- Expert System review
- Assay development with collaborators

miniSTR Overview Article



http://marketing.appliedbiosystems.com/images/enews/ForensicNews_Vol7/PDF/02A_CustomerCorner_Butler.pdf

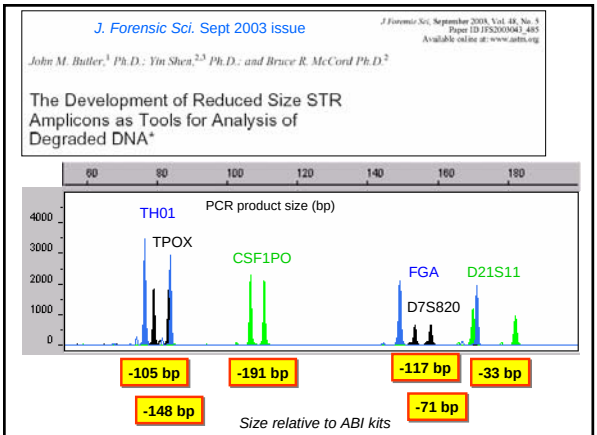
Timeline for miniSTRs

and Demonstrating the Value of Using Reduced Size Amplicons for Degraded DNA

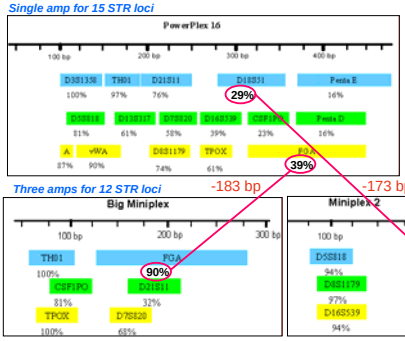
- 1994 – FSS finds that smaller STR loci work best with burned bone and tissue from Branch Davidian fire
- 1997 – New primers developed for time-of-flight mass spectrometry to make small STR amplicons
- 2001 – Work at NIST and OhioU with CODIS STRs; BodePlexes used in WTC investigation starting 2002
- 2004 – Work at NIST with **non-CODIS (NC) miniSTRs**
- 2006/07 – Applied Biosystems to release 9plex MiniFiler

http://www.cstl.nist.gov/biotech/strbase/miniSTR/timeline.htm

J. Forensic Sci. Sept 2003 issue

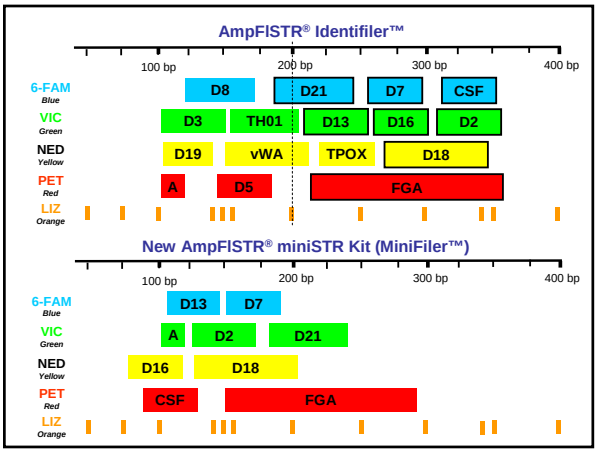


Comparison of PCR Amplification Success Rates with Commercial Kit vs. miniSTR Assays



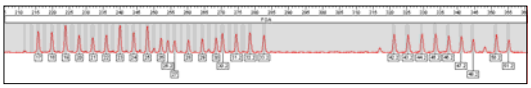
Study with 31 bones from the "Body Farm" (Knoxville, TN) and Franklin County Coroner's Office (OH)

Opel K. L.; Chung, D. T.; Drábek, J.; Tatarek, N. E.; Jantz, L. M.;. McCord, B.R. (2006) The Application of Miniplex Primer Sets in the Analysis of Degraded DNA from Human Skeletal Remains. *J. Forensic Sci.* 51(2): 351-356.

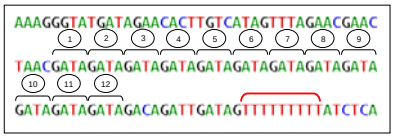


Why Go Beyond the CODIS Loci?

(1) Large Allele Ranges (e.g. FGA)



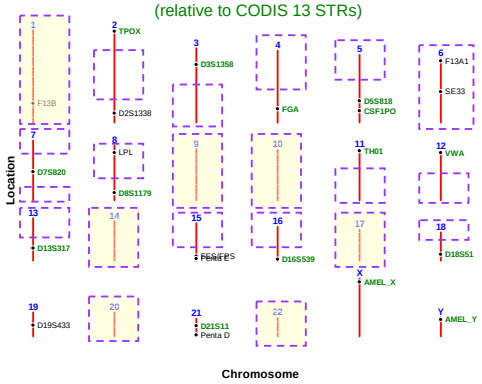
(2) "Unclean" Flanking Sequences (e.g. D7S820)



Butler, JM, Shen, Y., McCord, BR (2003) *JFS* 48(5): 1054-1064

Locations of Focus for New miniSTR Loci

(relative to CODIS 13 STRs)



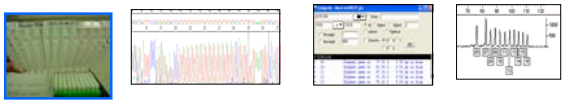
Characterization of New miniSTR Loci

"Computer Work"

Candidate STR marker selection (e.g. Marshfield Clinic Center of Medical Genetics) → Pull down sequence data from the web (e.g. NCBI) → Identify Chromosome Location (e.g. Human BLAT Search) → Screen for PCR Primers (e.g. Primer3) → Test primers for Multiplex-ability (e.g. AutoDimer - NIST)

"Laboratory Work"

Test Markers on Population samples | Sequence homozygotes to determine allele sizes | Build Macros for Genotyping | Construct Allelic Ladders

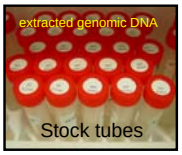


Standard U.S. Population Dataset

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

260 Caucasians, 260 African Americans, 140 Hispanics, 3 Asians = **663 males**

DNA extracted from whole blood (anonymous; self-identified ethnicities) received from Interstate Blood Bank (Memphis, TN) and Millennium Biotech Inc. (Ft. Lauderdale, FL)

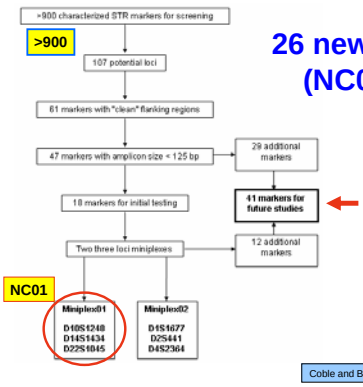


To date: (>100,000 allele calls)

Identifiler (15 autosomal markers + Amelogenin) (10,608)
Roche Linear Arrays (HV1/HV2 10 regions) (6,630)
Y STRs 22 loci—27 amplicons (17,388)
Y STRs 27 new loci (14,535)
Yfiler kit 17 loci (11,237)
Y SNPs 50 markers on sub-set of samples (11,498)
Orchid 70 autosomal SNPs on sub-set (13,230)
miniSTR testing-new loci and CODIS concordance (9,228)
New miniSTR loci – for 26 loci, 17,238 genotypes
mtDNA full control region sequences by AFDIL

Genotypes with various human identity testing markers

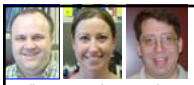
Initial Testing Results with Potential miniSTR Loci



26 new miniSTRs (NC01-NC09)

20 additional loci characterized across U.S. population groups

Coble and Butler (2005) *J. Forensic Sci.* 50(1): 43-53



Mike Coble | Becky Hill | John Butler

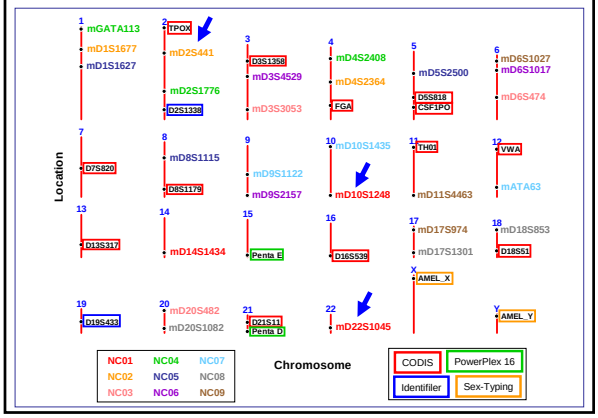
New miniSTR Non-CODIS (NC) Loci

No longer at NIST (AFDIL Research Section Chief since April 2006)

- 32 STR loci tested on NIST **665 U.S. population samples**
- **26 STR loci with allele sizes below 140 bp and good heterozygosities (above TPOX level)**
- All new STR loci are **physically unlinked** to the 13 CODIS core loci
- **Submitted articles** regarding primer sequences and locus characterization including population statistics
- **SRM 2391b components are being certified** through sequencing for D10S1248, D2S441, D22S1045; for reference purposes, genotypes for standard samples (9947A, 9948, 007, K562) will be made available on STRBase

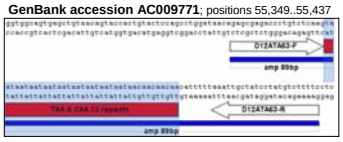
http://www.cstl.nist.gov/biotech/strbase/newSTRs.htm

Chromosomal Locations of New miniSTR Loci



Characterization of miniSTR **D12ATA63**

GenBank accession AC009771; positions 55,349..55,437



[FAM] – GAGCGAGACCCTGTCTCAAG
GAAAAGACATAGGATAGCAATTT

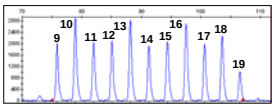
Chr 12 106.825 Mb (12q23.3)

Trinucleotide
[TAA][CAA] repeat
76 -106 bp
Alleles 9 -19

Allele	Caucasian (N = 260)	African Am (N = 259)	Hispanic (N = 140)
9	--	--	0.0036
10	0.0019	0.0154	0.0036
11	0.1385	0.1525	0.1500
12	0.2154	0.1004	0.1786
13	0.0173	0.1564	0.0286
14	0.1615	**0.3340**	0.2214
15	0.0577	0.0772	0.0714
16	**0.2981**	0.1004	**0.2643**
17	0.0981	0.0521	0.0679
18	0.0096	0.0058	0.0071
19	0.0019	0.0058	0.0036

Heterozygosity Values
U.S. Caucasian **0.842**
African American **0.788**
U.S. Hispanic **0.879**

D12ATA63 Allelic Ladder



Comparison of heterozygosity values on 26 non-CODIS loci across the U.S. samples examined in this study.

Locus	N	Heterozygosity (Overall)	Rank	African American	Caucasian	Hispanic
D9S2157	661	0.844	1	0.884	0.840	0.779
ATA63 (D12)	659	0.829	2	0.788	0.842	0.879
D10S1248 (NC01)	663	0.792	3	0.825	0.785	0.743
D22S1045 (NC01)	663	0.784	4	0.817	0.785	0.721
D2S441 (NC02)	660	0.774	5	0.798	0.780	0.721
D10S1435	663	0.766	6	0.798	0.770	0.700
D2S1776	654	0.763	7	0.740	0.801	0.734
D3S4529	660	0.761	8	0.752	0.723	0.829
D6S474	648	0.761	9	0.765	0.802	0.679
D5S2500	664	0.747	10	0.757	0.747	0.729
D1S1627	660	0.746	11	0.783	0.737	0.693
D1S1677 (NC02)	660	0.746	12	0.743	0.749	0.743
D6S1017	664	0.740	13	0.807	0.698	0.693
D3S3053	648	0.739	14	0.713	0.724	0.814
D9S1122	659	0.734	15	0.753	0.742	0.686
D17S974	664	0.732	16	0.757	0.702	0.743
D11S4463	664	0.730	17	0.780	0.676	0.743
D4S2408	654	0.722	18	0.752	0.709	0.691
D18S853	664	0.711	19	0.772	0.645	0.721
D20S1082	664	0.696	20	0.792	0.653	0.600
D14S1434 (NC01)	663	0.696	21	0.685	0.721	0.650
D20S482	648	0.691	22	0.673	0.689	0.729
GATA113 (D1)	654	0.668	23	0.673	0.632	0.727
D8S1115	664	0.663	24	0.629	0.660	0.729
D17S1301	664	0.649	25	0.626	0.717	0.564
D4S2364 (NC02)	660	0.511	26	0.385	0.551	0.664

European Labs Have Adopted the NIST-Developed NC miniSTRs

FSI (2006) **156(2):** 242-244

Short communication

The evolution of DNA databases—Recommendations for new European STR loci

Peter Gill [a,*], Lyn Fereday [b], Niels Morling [c], Peter M. Schneider [d]

[a] Forensic Science Service, Birmingham, UK
[b] Forensic Science Service, London, UK
[c] Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Denmark
[d] Institute of Legal Medicine, University of Cologne, Germany

Received 25 May 2005; accepted 26 May 2005

…recommended that existing multiplexes are re-engineered to enable small amplicon detection, and that three new mini-STR loci with alleles <130 bp (D10S1248, D14S1434 and D22S1045) are adopted as universal. This will increase the number of European standard Interpol loci from 7 to 10.

(D14 has been replaced with D2S441 from NC02)

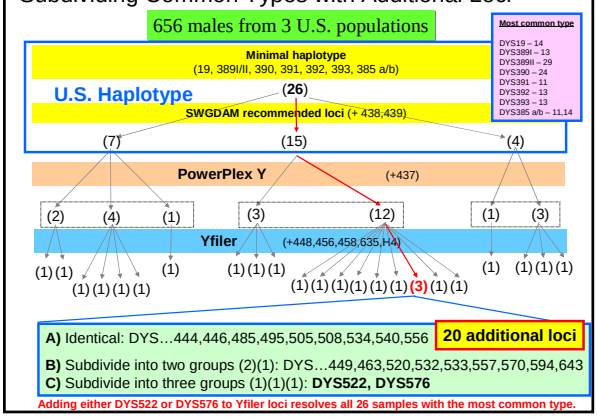
John Butler, Margaret Kline, Pete Vallone, Amy Decker

NIST Y-STR Goals

- Standardize information resources on Y-STRs and nomenclature for alleles
- Understand variation in U.S. populations so the best loci can be selected for commercial kits
- Construct multiplex assays to quickly evaluate loci
- Provide reference material for laboratory calibration (SRM 2395)

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

Subdividing Common Types with Additional Loci

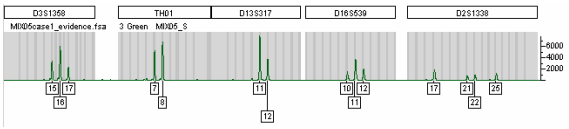


A) Identical: DYS…444,446,485,495,505,508,534,540,556 **20 additional loci**

B) Subdivide into two groups (2)(1): DYS…449,463,520,532,533,557,570,594,643

C) Subdivide into three groups (1)(1)(1): **DYS522, DYS576**

Adding either DYS522 or DYS576 to Yfiler loci resolves all 26 samples with the most common type.



Mixture Interpretation:
Lessons Learned from the MIX05 Interlaboratory Study

John M. Butler
National Institute of Standards and Technology

CODIS Conference – October 23, 2006
Arlington, VA

Presentation Outline

- Mixtures: issues and challenges
- MIX05 interlaboratory study (initiated at CODIS Conference Nov 15, 2004)
- Mixture interpretation variation – future role of expert systems
- Opportunities for community improvement and standardization regarding mixture interpretation

Other Session Speakers
Angelo DellaManna – case examples and CODIS search strategies with mixtures
Elizabeth Johnson – software demo of USACIL 2-component mixture ratio program

Mixtures: Issues and Challenges

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

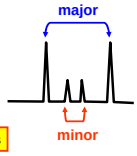
- Mixtures arise when two or more individuals contribute to the sample being tested.
- Mixtures can be challenging to detect and interpret without extensive experience and careful training. **Even more challenging with poor quality data when degraded DNA is present…**
- Differential extraction can help distinguish male and female components of many sexual assault mixtures. **Y-chromosome markers can help here in some cases…**

Principles of Mixture Interpretation

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

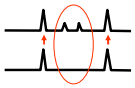
Torres *et al.* (2003) *Forensic Sci. Int.* 134:180-186 examined 1,547 cases from 1997-2000 containing **2,424 typed samples** of which **163 (6.7%) contained a mixed profile** with **only 8 (0.3%) coming from more than two contributors**

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



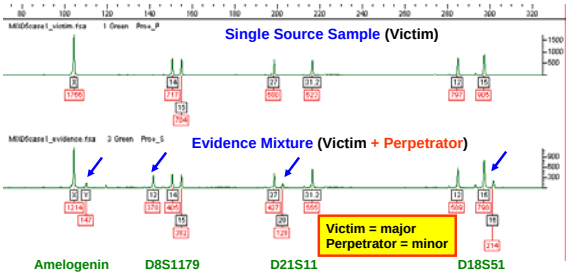
Ratios of the various mixture components stay fairly constant between multiple loci enabling deduction of the profiles for the major and minor components

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)



Obligate Alleles (not present in the victim reference)

Y	12	28	16

True "Perpetrator" Profile

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

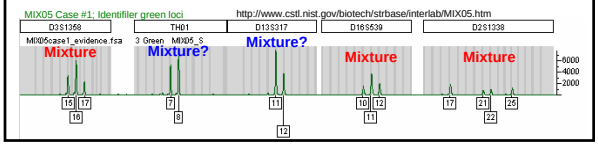
Mixtures: Issues and Challenges

- 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

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.



Two Parts to Mixture Interpretation

- **Deduction of alleles present in the evidence** (compared to victim and suspect profiles)
- **Providing some kind of statistical answer** regarding the weight of the evidence
  - An ISFG DNA Commission (Peter Gill, Bruce Weir, Charles Brenner, etc.) is evaluating the statistical approaches to mixture interpretation and has made recommendations

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

Forensic Science International 160 (2006) 90–101

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]

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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.

Keywords: STR typing; Biostatistical analysis; Likelihood ratio; Probability of exclusion; Mixtures; ISFG DNA commission

A High Degree of Variability Currently Exists with Mixture Interpretation

- **"If you show 10 colleagues a mixture, you will probably end up with 10 different answers"**
  - Peter Gill, Human Identification E-Symposium, April 14, 2005
- **Interlaboratory studies help to better understand why variability may exist between laboratories**
- Most analysts are only concerned about their own lab protocols and do not get an opportunity to see the big picture from the entire community that can be provided by a well-run interlaboratory study

NIST Initiated Interlaboratory Studies

Studies involving STRs	# Labs	Publications
Evaluation of CSF1PO, TPOX, and TH01	34	Kline MC, Duewer DL, Newall P, Redman JW, Reeder DJ, Richard M. (1997) Interlaboratory evaluation of STR triplex CTT. *J. Forensic Sci.* 42: 897-906
Mixed Stain Studies #1 and #2 (Apr–Nov 1997 and Jan–May 1999)	45	Duewer DL, Kline MC, Redman JW, Newall PJ, Reeder DJ. (2001) NIST Mixed Stain Studies #1 and #2: interlaboratory comparison of DNA quantification practice and short tandem repeat multiplex performance with multiple-source samples. *J. Forensic Sci.* 46: 1199-1210
MSS3 **Mixed Stain Study** #3 (Oct 2000-May 2001)	74	Kline, M.C., Duewer, D.L., Redman, J.W., Butler, J.M. (2003) NIST mixed stain study 3: DNA quantitation accuracy and its influence on short tandem repeat multiplex signal intensity. *Anal. Chem.* 75: 2463-2469. Duewer, D.L., Kline, M.C., Redman, J.W., Butler, J.M. (2004) NIST Mixed Stain Study #3: signal intensity balance in commercial short tandem repeat multiplexes, *Anal. Chem.* 76: 6928-6934.
DNA Quantitation Study (Jan-Mar 2004) QS04	80	Kline, M.C., Duewer, D.L., Redman, J.W., Butler, J.M. (2005) Results from the NIST 2004 DNA Quantitation Study, *J. Forensic Sci.* 50(3):571-578
Mixture Interpretation Study (Jan - Aug 2005) **MIX05**	**69**	***Data analysis currently on-going ...*** **Poster at 2005 Promega meeting (Sept 2005); available on STRBase**

Overall Lessons Learned from NIST MSS 1,2,&3

- Laboratories have instruments with different sensitivities
- **Different levels of experience and training plays a part in effective mixture interpretation**
- Amount of input DNA makes a difference in the ability to detect the minor component (labs that put in "too much" DNA actually detected minor components more frequently)

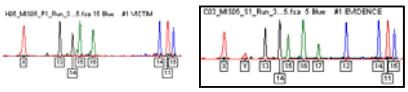
Purpose of MIX05 Study

- **Goal is to understand the "lay of the land" regarding mixture analysis across the DNA typing community**

- One of the primary benefits we hope to gain from this study is **recommendations for a more uniform approach to mixture interpretation** and training tools to help educate the community

Mixture Interpretation Interlab Study (MIX05)

- **Only involves interpretation of data – to remove instrument detection variability and quantitation accuracy issues**
- **94 labs enrolled** for participation
- **69 labs have returned results** (17 from outside U.S.)
- Four mock cases supplied with "victim" and "evidence" electropherograms (GeneScan .fsa files – that can be converted for Mac or GeneMapper; gel files made available to FMBIO labs)
- Data available with Profiler Plus, COfiler, SGM Plus, PowerPlex 16, Identifiler, PowerPlex 16 BIO (FMBIO) kits
- Summary of results will involve training materials to illustrate various approaches to solving mixtures



Perpetrator Profile(s) ??

Along with reasons for making calls and any stats that would be reported

MIX05 Study Design and Purpose

Interlab studies provide a "big picture" view of the community

- Permit a large number of forensic practioners to **evaluate the same mixture data**

- Provide multiple cases representing a range of mixture scenarios
- Generate data from multiple STR kits on the same mixture samples to compare performance for detecting minor components
- The primary variable should be the laboratory's interpretation guidelines rather than the DNA extraction, PCR amplification, and STR typing instrument sensitivity
- **Are there best practices in the field that can be advocated to others?**

Requests for Participants in MIX05

Mixtures representing four different case scenarios have been generated at NIST with multiple STR kits and provided to laboratories as electropherograms.

We would like to receive the following information:

1) **Report the results as though they were from a real case** including whether a statistical value would be attached to the results. *Please summarize the perpetrator(s) alleles in each "case" as they might be presented in court—along with an appropriate statistic (if warranted by your laboratory standard operating procedure) and the source of the allele frequencies used to make the calculation.* Please indicate which kit(s) were used to solve each case.

2) **Estimate the ratio for samples present in the evidence mixture** and how this estimate was determined.

3) **Provide a copy of your laboratory mixture interpretation guidelines** and a brief explanation as to why conclusions were reached in each scenario

A MIX05 Participant Noted…

"Things we do not do:

- **Calculate mixture ratios for casework**
  - **Calculation used for this study:** Find loci with 4 alleles (2 sets of sister alleles). Make sure sister alleles fall within 70%, then take the ratio of one allele from one sister set to one allele of the second sister set, figure ratios for all combinations and average. Use peak heights to calculate ratios.
- **Provide allele calls in reports**
- **Provide perpetrator(s) alleles or statistics in court without a reference sample to compare to the DNA profile obtained from the evidence. We will try to determine the perpetrator(s) profile for entry into CODIS."**

We recognize that some of the information requested in this interlab study may not be part of a lab's standard operating procedure

MIX05 Case Scenarios

Based on Identifiler 15 STR loci

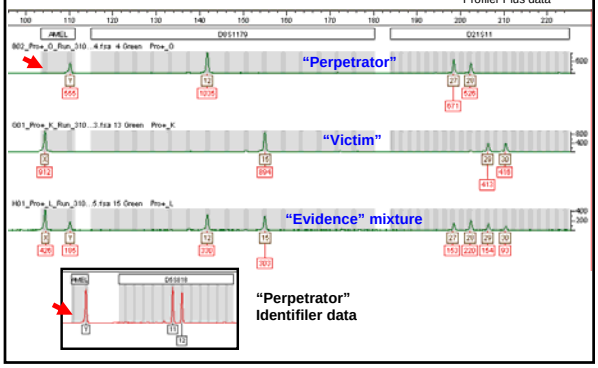
Genomic DNA samples with specific allele combinations ("evidence") were mixed in the following ratios:

	#alleles		#loci with #alleles				
	N all	N unq	N 1	N 2	N 3	N 4	N 5
Case #1 – victim is major contributor (3F:1M)	39	26	2	6	5	2	0
Case #2 – perpetrator is major contributor (1F:3M)	55	52	0	1	4	10	0
Case #3 – balanced mixture (1F:1M) • Male lacked amelogenin X	48	37	0	3	8	4	0
Case #4 – more extreme mixture (7F:1M) • Male contained tri-allelic pattern at TPOX	50	42	0	3	7	4	1

Female victim DNA profile was supplied for each case

Labs asked to deduce the perpetrator DNA profile – suspect(s) not provided

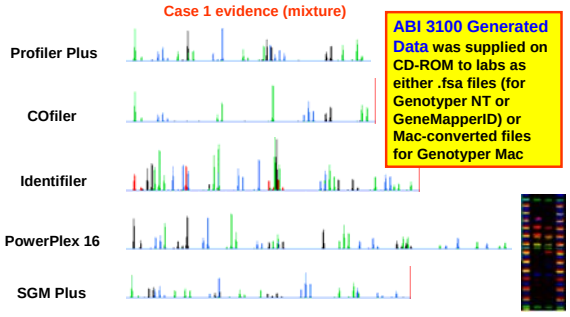
Amelogenin X allele is missing in male perpetrator DNA sample for MIX05 Case #3



"Perpetrator" Identifiler data

MIX05 Results on Multiple Kits

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



ABI 3100 Generated Data was supplied on CD-ROM to labs as either .fsa files (for Genotyper NT or GeneMapperID) or Mac-converted files for Genotyper Mac

FMBIO data was also made available upon request

Summary of MIX05 Responses

94 labs enrolled for participation

69 labs returned results (17 from outside U.S.)

50 labs made allele calls
39 labs estimated ratios
29 labs provided stats

All participants were supplied with all data and could choose what kits to examine based on their experience and lab protocols

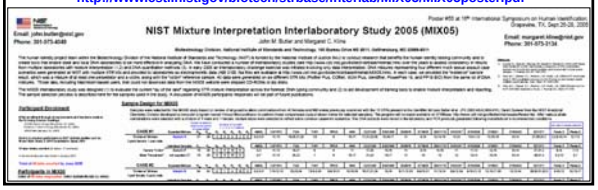
STR kit results used
- **34 ProfilerPlus/COfiler**
- **10 PowerPlex 16**
- **7 PP16 BIO**
- **5 Identifiler**
- **2 SGM Plus**
- **1 All ABI kit data**
- **9 Various combinations**

Generally Identifiler data was of poorer quality in the electropherograms we provided…which caused some labs to not return results (they indicated a desire for higher quality data through sample re-injection to reduce pull-up prior to data interpretation)

What MIX05 Participants Have Received Back from NIST…

- Certificate of participation in the interlab study
- Copy of the poster presented at the Promega Sept 2005 meeting displaying "correct" results for the perpetrator in each case scenario as well as an explanation of study design and preliminary results

http://www.cstl.nist.gov/biotech/strbase/interlab/MIX05/MIX05poster.pdf


NIST Mixture Interpretation Interlaboratory Study 2005 (MIX05)

When is a Sample a Potential Mixture?

According to several MIX05 participant interpretation guidelines

- Number of Observed Peaks
  - Greater than two peaks at a locus
  - More than two alleles are present at two or more loci, although three banded patterns can occur
  - Presence of 3 alleles at a single locus within a profile
  - 4 peaked patterns (if observed at any locus), 3 peaked patterns (if observed at two or more loci), significant imbalances (peak height ratios <60%) of alleles for a heterozygous genotype at two ore more loci with the exception of low template amplifications, which should be interpreted with caution
- Imbalance of heterozygote alleles
  - thresholds range from 50-70%
- Stutter above expected levels
  - generally 15-20%

These protocol differences can lead to variation in reported alleles and therefore the deduced profile and resulting statistics

Summary of Some MIX05 Reported Results

CASE #2		D3S1358	VWA	FGA	AMEL	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820	D16S539	THO1	TPOX	CSF1PO
True Perp	ZT79619	15,15	15,15	20,24	X,Y	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
LabID	Kit Used														
16	ProPlus/Cofiler	--	--	--	--	--	--	--	--	--	--	--	--	--	--
6	ProPlus/Cofiler	15	15	20,24	X,Y	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
91	SGM Plus	15	15	20,24	X,Y	11,13	28,32.2	17,18				10,11	7,9.3		
46	PP16	--	--	--	--	--	--	--	--	--	--	--	--	--	--
37	ProPlus/Cofiler	--	15	20	X,Y	13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
2	PP16	15	15,15	20,24	X,Y	11,13	28,32.2	17,18	8,13	INC	8,10	10,11	7,9.3	9,10	7,10
13	PP16 & Identifiler	15	15	20,24	--	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
34	ProPlus/Cofiler	15	15	20,24	--	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
70	Identifiler	15	15	20,24	X,Y	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
55	ProPlus/Cofiler	15	15	20,24	--	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
21	ProPlus/Cofiler	15,15	15,15	20,24	X,Y	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
73	ProPlus/Cofiler	15,15	15,15	20,24	X,Y	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
29	Identifiler	15	15	20,24	X,Y	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
54	All Kits	15,15	15,15	20,24	X,Y	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
90	ProPlus/Cofiler	15	15	20,24	X,Y	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
9	ProPlus/Cofiler	15	15	20,24	X,Y	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
4	ProPlus/Cofiler	15	15	20,24	X,Y	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
33	ProPlus/Cofiler	--	--	--	--	--	--	--	--	--	--	--	--	--	--
12	ProPlus/Cofiler	15	15	20,24	X,Y	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
67	PP16	15	15,16	20,24	X,Y	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
86	ProPlus/Cofiler	15,15	15,15	20,24	--	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
79	ProPlus/Cofiler	15,15	15,15	20,24	--	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
77	Identifiler	--	--	--	--	--	--	--	--	--	--	--	--	--	--
60	PP16	15	15	20,24	X,Y	11,13	28,32.2	17,18	8,13	12,14	8,10	10,11	7,9.3	9,10	7,10
61	Identifiler	--	--	--	--	--	--	--	--	--	--	--	--	--	--

Most calls were correct (when they were made)

Some Mixture Ratios Reported in MIX05

Many labs do not routinely report the estimated ratio of mixture components

LabID	Case1 (F:M)	Case2 (M:F)	Case3 (M:F)	Case4 (F:M)
13	2	5	<2	10
34	1.8--3.6	3.9--6.7	1.6--1.8	6.2--7.6
70				
55	68%:32%	85%:15%	64%:36%	
21				
73	2:1	6:1	2:1	not determined
29				
54	2:1	6:1	2:1	6:1
90	male23-39%	not determined	male64-71%	
9	3 or 4:1	4 or 5:1	1.4:1	~10:1
4	10:1	6:1	1:1	not determined
33	male60-78%	male80-90%	male58-71%	victim86%
12	male25%	male85%	male40-45%	unknown10%
67	1:2.3	6.4:1	2:1	1:6.8
86	2:1	6-6.5:1	1.6-2:1	4-4.5:1
79	~3:1 to ~2:1	~6:1 to ~4:1	~2:1*	a lot of victim
77				
60	2:1	5:1	2:1	10:1
61				

Some Reported Stats for MIX05 Case #1

Many of the 29 labs providing statistics used PopStats 5.7

		Case1		
LabID	Kits Used	Caucasians	African Americans	Hispanics
77	Identifiler	PE calculated	PE calculated	PE calculated
73	ProPlus/Cofiler	none provided	none provided	none provided
4	ProPlus/Cofiler	none provided	none provided	none provided
12	ProPlus/Cofiler	none provided	none provided	none provided
29	Identifiler	none provided	none provided	none provided
90	ProPlus/Cofiler	1.18E+15	2.13E+14	3.09E+15
34	ProPlus/Cofiler	2.40E+11	7.00E+09	9.80E+10
46	PP16	5.60E+09	3.80E+11	none provided
33	ProPlus/Cofiler	2.94E+08	1.12E+08	1.74E+09
6	ProPlus/Cofiler	40,000,000	3,500,000	280,000,000
9	ProPlus/Cofiler	1.14E+07	1.97E+07	1.54E+08
61	Identifiler	1.50E+06	260,000	2.40E+07
79	ProPlus/Cofiler	930,000	47,900	1,350,000
16	ProPlus/Cofiler	434,600	31,710	399,100

Some Differences in Reporting Statistics

		Case1		
LabID	Kits Used	Caucasians	African Americans	Hispanics
90	ProPlus/Cofiler	1.18E+15	2.13E+14	3.09E+15
34	ProPlus/Cofiler	2.40E+11	7.00E+09	9.80E+10
33	ProPlus/Cofiler	2.94E+08	1.12E+08	1.74E+09
6	ProPlus/Cofiler	40,000,000	3,500,000	280,000,000
9	ProPlus/Cofiler	1.14E+07	1.97E+07	1.54E+08
79	ProPlus/Cofiler	930,000	47,900	1,350,000
16	ProPlus/Cofiler	434,600	31,710	399,100

~10 orders of magnitude difference (10^5 to 10^{15}) based on which alleles were deduced and reported

Remember that these labs are interpreting the same MIX05 electropherograms

Questions for Consideration

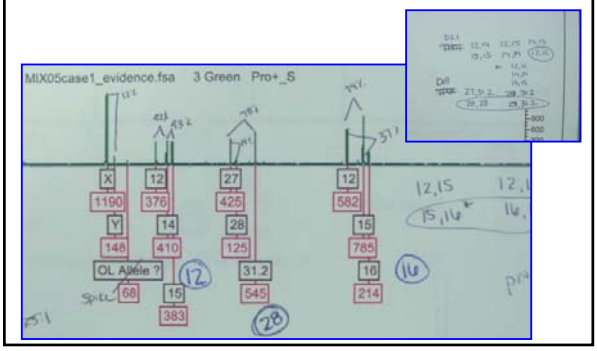
- Do you look at the evidence data first without considering the suspect's profile?
- Without a suspect, does your lab proceed with mixture interpretation?
- Do you have a decision point whereby you consider a mixture too complicated and do not try to solve it? If so, is the case declared inconclusive?
- What kind of training materials would benefit your lab in improving consistency in mixture interpretation?

Examples of MIX05 Report Formats

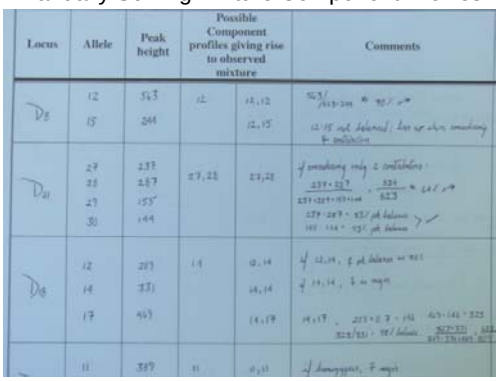
All examples with Case #1

(~3:1 mixture with female victim as the major component – and victim profile is provided)

Manual Solving of MIX05 Peak Ratios and Possible Mixture Combinations

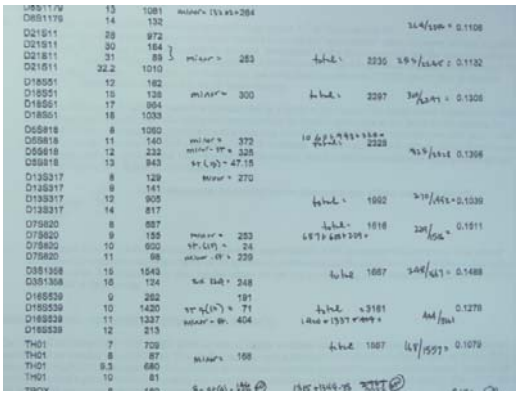


Manually Solving Mixture Component Profiles



Locus	Allele	Peak height	Possible Component profiles giving rise to observed mixture		Comments
D8	12	563	12	12,12	
	15	244		12,15	
D21	27	237	27,28	28,28	
	28	287			
	29	155			
	30	144			
D18	12	283	14	12,14	
	14	331		14,14	
	17	463		14,17	
	11	387	11	11,11	

Another MIX05 Participant Manually Solving a Mixture



Semi-Automated Locus-by-Locus Interpretation Performed by One MIX05 Participant

D21S11

Peak	A	B	C	D	Known type:	K1	K2
Allele	28	30	31	32.2		30	31
RFU	988	167	92	1025			

Possible combinations					Thresholds 70%	Thresholds 60%	Htrzyg. Pair 1	Htrzyg. Pair 2	Mixture ratio(1/2)
28	30	AND	31	32.2	N	N	16.90%	8.98%	
28	31	AND	30	32.2	N	N	9.31%	16.29%	
28	32.2	AND	30	31	N	N	96.39%	55.09%	Best fit

D18S51

Peak	A	B	C	D	Known type:	K1	K2
Allele	12	15	17	18		12	15
RFU	163	139	972	1047			

Possible combinations					Thresholds 70%	Thresholds 60%	Htrzyg. Pair 1	Htrzyg. Pair 2	Mixture ratio(1/2)	
12	15	AND	17	18	Y	Y	85.28%	92.84%	0.15	Known present
12	17	AND	15	18	N	N	16.77%	13.28%		
12	18	AND	15	17	N	N	15.57%	14.30%		

Excel spreadsheet used to examine possible component combinations

Different Reporting Formats for MIX05 Data

Different Reporting Formats for MIX05 Data

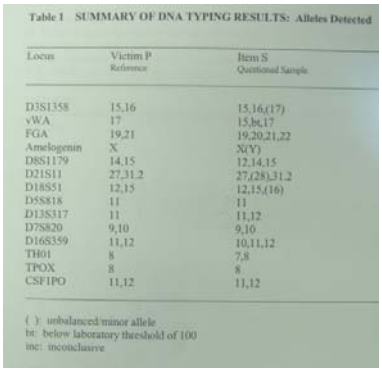


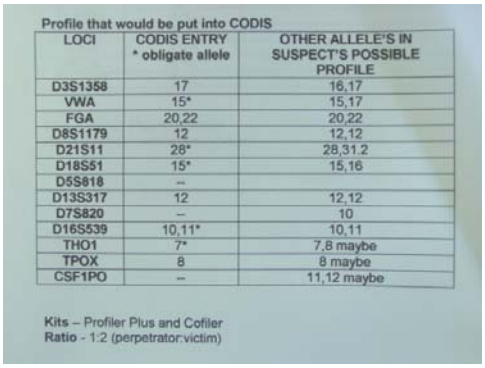
Table 1 SUMMARY OF DNA TYPING RESULTS: Alleles Detected

Locus	Victim P Reference	Item S Questioned Sample
D3S1358	15,16	15,16,(17)
vWA	17	15,bt,17
FGA	19,21	19,20,21,22
Amelogenin	X	X(Y)
D8S1179	14,15	12,14,15
D21S11	27,31.2	27,(28),31.2
D18S51	12,15	12,15,(16)
D5S818	11	11
D13S317	11	11,12
D7S820	9,10	9,10
D16S359	11,12	10,11,12
TH01	8	7,8
TPOX	8	8
CSF1PO	11,12	11,12

( ) unbalanced/minor allele
bt: below laboratory threshold of 100
inc: inconclusive

No attempt to deduce perpetrator alleles (foreign profile)

Different Reporting Formats for MIX05 Data



Profile that would be put into CODIS

LOCI	CODIS ENTRY * obligate allele	OTHER ALLELE'S IN SUSPECT'S POSSIBLE PROFILE
D3S1358	17	16,17
VWA	15*	15,17
FGA	20,22	20,22
D8S1179	12	12,12
D21S11	28*	28,31.2
D18S51	15*	15,16
D5S818	--	
D13S317	12	12,12
D7S820	--	10
D16S539	10,11*	10,11
THO1	7*	7,8 maybe
TPOX	8	8 maybe
CSF1PO	--	11,12 maybe

Kits – Profiler Plus and Cofiler
Ratio - 1:2 (perpetrator:victim)

Different Reporting Formats for MIX05 Data

	Items	
Locus	"S" Case 1 Evid.	"P" Case 1 Victim
D3S1358	15, 16, *	15, 16
D16S539	(10), 11, (12)	11, 12
AMEL	X, *	X
THO1	(7), 8	8
TPOX	8	8
CSF1PO	11, 12	11, 12
D7S820	9, 10	9, 10
vWA	(15), 17	17
FGA	19, 20, 21, 22	19, 21
D8S1179	12, 14, 15	14, 15
D21S11	27, 31.2, *	27, 31.2
D18S51	12, 15, (16)	12, 15,
D5S818	11	11
D13S317	11, 12	11

() indicates apparent minor peaks in a mixture.
* indicates peaks below the VFL threshold (150 rfus) for reporting.

Different Reporting Formats for MIX05 Data

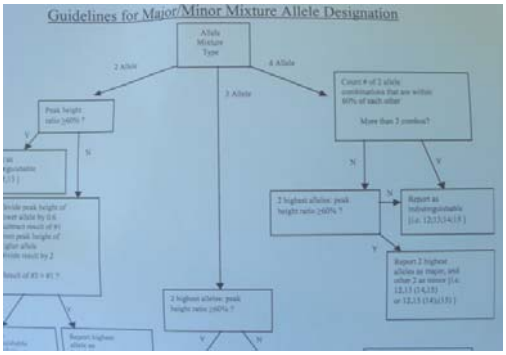
Case 1:

Item: description	D3S1358	VWA	FGA	AMEL	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820	D16S539	TH01	TPOX	CSF1PO
Pro+/CO_S: evid 1	15,16 (17)	15|17	19|20 21|22	X,X (Y)	12|14|15	27,31.2 (28)	12,15 (16)	11,11	11|12	9|10	10|11 |12	7|8	8,8	11,12
Pro+/CO_P: victim 1 reference	15,16	17,17	19,21	X,X	14,15	27,31.2	12,15	11,11	11,11	9,10	11,12	8,8	8,8	11,12
Male interpreted from evidence 1	17	15,15 15,17	20,22	X,Y	12,12	28	16	11,11	12,12	Nd	10,11	7,7 7,8	Nd	Nd

Two allele values separated by a comma represent a genotype. Genotype calls assume biallelic donors with no null alleles.
() Indicates minor allele detected.
Single numbers and numbers separated by "|" represent an allele only designation rather than a genotype.
Interpreted profile assumes that the victim is present in the evidence mixture of two people. More than one genotype may be listed where a single genotype could not be conclusively determined. Nd=not determined due to level of results.

The community would benefit from more uniform reporting formats and mixture solving strategies…

Some Protocols Have Flow Charts to Help Make Decisions in Mixture Resolution



Some Labs Do Not Attempt Mixture Interpretation

- **A number of laboratories chose not to report anything in the MIX05 study citing that without a suspect, mixtures are not examined.**
- Why does a National DNA Database such as CODIS exist and how can it be helpful and reach its full potential if casework mixtures are not examined and perpetrator alleles deduced (where possible)?

Value of the MIX05 Study

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

- Data sets exist with multiple mixture scenarios and a variety of STR kits that **can be used for training purposes**
- A wide variety of approaches to mixture interpretation have been applied on the **same data sets evaluated as part of a single study**
- **Interpretation guidelines from many laboratories are being compared to one another for the first time in an effort to determine challenges facing future efforts to develop "expert systems" for automated mixture interpretation**
- **We are exploring the challenges of supplying a common data set to a number of forensic laboratories** (e.g., if a standard reference data set was ever desired for evaluating expert systems)

Conclusions

(Opportunities for Improvement)

- It is worth taking a closer look at protocol differences between labs to see the impact on recovering information from mixture data
- Expert systems (when they become available and are used) should help aid consistency in evaluating mixtures and help produce more uniform reporting formats

Software Programs (Expert Systems) for Mixture Deconvolution

These programs do not supply stats (only attempt to deduce mixture components)

- Linear Mixture Analysis (LMA)
  - **Part of TrueAllele system** developed by Mark Perlin (Cybergenetics)
  - Perlin, M. W. and Szabady, B. (2001) Linear mixture analysis: a mathematical approach to resolving mixed DNA samples. *J.Forensic Sci.* 46(6): 1372-1378
- Least Squares Deconvolution (LSD)
  - Described by T. Wang (University of Tennessee) at Oct 2002 Promega meeting
  - Available for use at **https://lsd.lit.net/**
- PENDULUM
  - **Part of FSS i-3 software suite (i-STReam)**
  - Bill, M., Gill, P., Curran, J., Clayton, T., Pinchin, R., Healy, M., and Buckleton, J. (2005) PENDULUM-a guideline-based approach to the interpretation of STR mixtures. *Forensic Sci.Int.* 148(2-3): 181-189

USACIL program developed by Tom Overson

Future Plans

- Develop training information based on lessons learned from the MIX05 study
- Create other useful software tools like ***mixSTR*** and ***Virtual MixtureMaker*** to increase mixture interpretation capabilities of the forensic DNA typing community
- Conduct another interlab study in 2007 (MIX07)?
  - To try and capture improved knowledge regarding mixture interpretation and capabilities of expert systems

Some Final Thoughts…

- It is of the highest importance in the art of detection to be able to recognize out of a number of facts, which are incidental and which vital. Otherwise your energy and attention must be dissipated instead of being concentrated (Sherlock Holmes, *The Reigate Puzzle*).
- "**Don't do mixture interpretation unless you have to**" (Peter Gill, Forensic Science Service, 1998).
- Mixture interpretation consumes a large part of DNA analysts' time – software tools that improve consistency in analysis will speed casework reporting and hopefully cases solved


ELSEVIER

Forensic Science International

DNA mixtures in forensic casework: a 4-year retrospective study

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Received 27 March 2003; accepted 1 April 2003

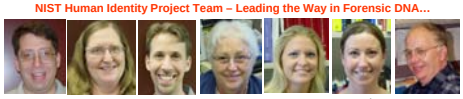
Conclusion

"Mixture interpretation theory is well established and used in forensic laboratories. Most mixtures detected in casework are satisfactorily solved. But from this revision we can conclude that the behaviour of each mixed sample can be different and multifactorial and occasionally its interpretation turns out to be complicated—sometimes paralleling the importance of the evidence in the resolution of the case. In some casework mixtures our experience has proved that theoretical assumptions from studies with laboratory samples, albeit very useful, can turn out to be impracticable. **We consider that more sharing of day to day forensic laboratory problems is needed to refine our technical procedures in the resolution of specially difficult evidence."**

Acknowledgments

Funding from interagency agreement 2003-IJ-R-029 between NIJ and the NIST Office of Law Enforcement Standards

NIST Human Identity Project Team – Leading the Way in Forensic DNA…


John Butler, Margaret Kline, Pete Vallone, Jan Redman, Amy Decker, Becky Hill, Dave Duewer

Role in MIX05

- Margaret Kline (running study, sample prep, data review)
- John Butler (study design and data review)
- Becky Hill (GeneMapper*ID* data review)
- Jan Redman (Access database entry, shipping)
- Dave Duewer (*Virtual MixtureMaker* to aid sample selection; **mixSTR program**)
- Chris Tomsey & Frank Krist (FMBIO Mac data)
- Kermit Channel & Mary Robnett (FMBIO NT data)

Mandy Sozer for early discussions on study design

The many forensic scientists and their supervisors who took time out of their busy schedules to examine the MIX05 data provided as part of this interlaboratory study

Validation Information

- President's DNA Initiative: **Validation Workshop (Aug 2005) with Robyn Ragsdale** – slides on STRBase; NFSTC working on DVD
- **ABI Roadshow/HID University: Validation Workshop (May 2006)** – slides available on STRBase
- We would love to have **more internal validation information for STRBase Validation Section** (e.g., Y-STRs)


Profiles in DNA (Promega Corporation), vol. 9(2), pp. 3-6

PROFILES IN DNA

VALIDATION

http://www.promega.com/profiles/902/ProfilesInDNA_902_03.pdf

Debunking Some Urban Legends Surrounding Validation Within the Forensic DNA Community

By John Butler
National Institute of Standards and Technology, Gaithersburg, Maryland, USA

Urban Legends of Validation…

Butler, J.M. (2006) *Profiles in DNA* vol. 9(2), pp. 3-6

#1: HUNDREDS OR THOUSANDS OF SAMPLES ARE REQUIRED TO FULLY VALIDATE AN INSTRUMENT OR METHOD

#2: VALIDATION IS UNIFORMLY PERFORMED THROUGHOUT THE COMMUNITY

#3: EACH COMPONENT OF A DNA TEST OR PROCESS MUST BE VALIDATED SEPARATELY

#4: VALIDATION SHOULD SEEK TO UNDERSTAND EVERYTHING THAT COULD POTENTIALLY GO WRONG WITH AN INSTRUMENT OR TECHNIQUE

#5: LEARNING THE TECHNIQUE AND TRAINING OTHER ANALYSTS ARE PART OF VALIDATION

#6: VALIDATION IS BORING AND SHOULD BE PERFORMED BY SUMMER INTERNS SINCE IT IS BENEATH THE DIGNITY OF A QUALIFIED ANALYST

#7: DOCUMENTING VALIDATION IS DIFFICULT AND SHOULD BE EXTENSIVE

#8: ONCE A VALIDATION STUDY IS COMPLETED YOU NEVER HAVE TO REVISIT IT

For example, RFU threshold values…

- Should thresholds be lowered below 150 RFU if instrument noise has been reduced in newer instruments?

Pete Vallone Dave Duewer

Software Tools from NIST

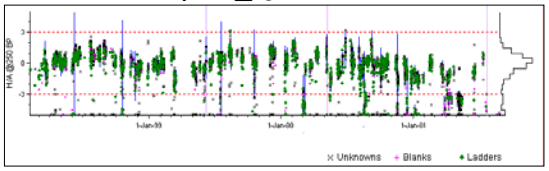
- AutoDimer – multiplex PCR primer screening tool

http://www.cstl.nist.gov/biotech/strbase/AutoDimerHomepage/AutoDimerProgramHomepage.htm

- mixSTR – mixture component resolution tool
- **Multiplex_QA** – quality assessment tool for monitoring instrument performance over time
- Tools to aid Expert System data review
  - DNA_FSSi3_Convert.xls (converts data format)
  - STR_MatchSamples.xls (compares samples)

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

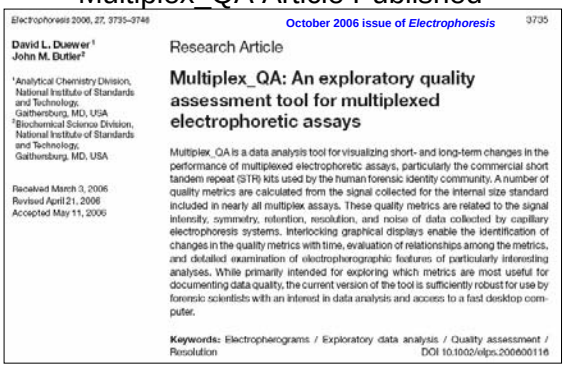
Multiplex_QA Overview



- **Research tool** that **provides quality metrics to review instrument performance over time** (e.g., examines resolution and sensitivity using internal size standard peaks)
- Runs with Microsoft Excel macros. Requires STR data to be converted with NCBI's BatchExtract program into numerical form.

Available for download from STRBase:
http://www.cstl.nist.gov/biotech/strbase/software.htm

Multiplex_QA Article Published



Electrophoresis 2006, 27, 3735–3746 — October 2006 issue of *Electrophoresis* — 3735

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Accepted May 11, 2006

Research Article

Multiplex_QA: An exploratory quality assessment tool for multiplexed electrophoretic assays

Multiplex_QA is a data analysis tool for visualizing short- and long-term changes in the performance of multiplexed electrophoretic assays, particularly the commercial short tandem repeat (STR) kits used by the human forensic identity community. A number of quality metrics are calculated from the signal collected for the internal size standard included in nearly all multiplex assays. These quality metrics are related to the signal intensity, symmetry, retention, resolution, and noise of data collected by capillary electrophoresis systems. Interlocking graphical displays enable the identification of changes in the quality metrics with time, evaluation of relationships among the metrics, and detailed examination of electropherographic features of particularly interesting analyses. While primarily intended for exploring which metrics are most useful for documenting data quality, the current version of the tool is sufficiently robust for use by forensic scientists with an interest in data analysis and access to a fast desktop computer.

Keywords: Electropherograms / Exploratory data analysis / Quality assessment / Resolution — DOI 10.1002/elps.200600116

User manual (127 pages) available for download from STRBase

Thank you for your attention…

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

Questions?

See also http://www.dna.gov/research/nist
http://www.cstl.nist.gov/biotech/strbase
john.butler@nist.gov