18th Annual National CODIS Conference
(Norman, OK) – November 14, 2012

NIST Update

John M. Butler

NIST Applied Genetics Group
National Institute of Standards and Technology
Gaithersburg, Maryland

NIST Human Identity Project Teams
within the Applied Genetics Group

Forensic DNA Team

Funding from the **National Institute of Justice (NIJ)** through NIST Office of Law Enforcement Standards

John Butler

STRBase, Workshops & Textbooks

Mike Coble

Mixtures, mtDNA & Y

Becky Hill

Concordance & LT-DNA

Margaret Kline

SRM work, variant alleles & Cell Line ID

Office Manager
Patti Rohmiller

Guest Researcher

Manuel **Fonde**vila Alvarez

Data Analysis Support

Dave Duewer

DNA Biometrics Team

Funding from the **FBI S&T Branch** through NIST Information Access Division

Pete Vallone

Rapid PCR, Direct PCR & Biometrics

Erica Butts

ABI 3500 & DNA Extraction

Kevin Kiesler

PLEX-ID & NGS Exploration

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

NIST
Applied Genetics

APPLIED GENETICS Group

Major Programs Currently Underway

- **Forensic DNA**
  - STRBase website
  - New loci and assays (26plex)
  - **STR kit concordance**
  - Ancestry SNP assays
  - Low-template DNA studies
  - **Mixture interpretation research and training**
  - STR nomenclature
  - Variant allele cataloging and sequencing
  - ABI 3500 validation
  - Training workshops to forensic DNA laboratories
  - Validation experiments, information and software tools
  - **Textbooks – 3rd ed.** (3 volumes)
- **Clinical Genetics**
  - Huntington's Disease SRM
  - CMV SRM
  - Exploring future needs
- **DNA Biometrics**
  - **Rapid PCR methods**
  - Testing of rapid DNA systems
  - Plex-ID mtDNA base composition
- **Cell Line Authentication**
  - **ATCC documentary standard** (Margaret Kline & John Butler served on this international committee)

Aiding Cell Line Authentication

Katsnelson, A. (2010) *Nature News*, 465: 537 (3 June 2010)

Biologists tackle cells' identity crisis

DNA fingerprinting scheme aims to make sure researchers are working on the right cells.

Ever since biologists learned how to grow human cells in culture half a century ago, the cells have been plagued by a problem of identity: many commonly used cell lines are not actually what researchers think they are.

Cell-line misidentification has led to mistakes in the literature, misguided research based on those results and millions wasted in grant money. Last year, *Nature* described the situation as a scandal[1].

But a universal system for determining the identity of cell lines may now be in view. Next month, a working group led by the American Type Culture Collection (ATCC), a nonprofit biological repository based in Manassas, Virginia, that stores 3,600 cell lines from more than 150 species, plans to unveil standard-

ATCC
LEADING BIOLOGICAL STANDARDS

ATCC® Standards Development Organization

Designation: ASN-0002

Authentication of Human Cell Lines: Standardization of STR Profiling

The working group, composed of representatives from academia, government and industry,

a universally accepted approach will allow different facilities to compare their cell lines with each other, he adds.

Fingerprinting has its limits, cautions Michael Johnson, a cancer researcher at Georgetown University in Washington DC. "Just because a cell fingerprints out as the same [as another cell] doesn't mean they will behave the same," he says, noting that a cell's properties can also be affected by the way it has been grown, the number of times it has been cultured anew and small genetic changes that wouldn't show up in a fingerprint test. One classic example, he notes, is an immortalized breast cell line called MCF10A, which can form organized hollow structures similar to those found in mammary tissue; MCF10A cells currently distributed by

http://www.nature.com/news/2010/100602/pdf/465537a.pdf

Highlights Since Last CODIS Conference

- InDel work published
- PLEX-ID report available
- New DNA mixture training materials
- TrueAllele evaluation continues…
- New autosomal STR and Y-STR loci & kits
  - NIST U.S. population data set completed
- SRM 2372 recertification underway
- Rapid DNA efforts
- *Interpretation* book being written

Insertion/Deletion (InDel) Markers

Manuel **Fonde**vila Alvarez

Guest Researcher from Spain (Jan 2011 to July 2012)

Main Points:

- InDels (insertion-deletion) or DIPs (deletion-insertion polymorphisms) are short length polymorphisms, consisting of the presence or absence of a short (typically 1-50 bp) sequence
- Like SNPs, InDels have low mutation rate (value to kinship analysis), small amplicon target sizes (value with degraded DNA), and can be highly multiplexed
- Can be analyzed on CE instruments like STRs
- Studied **commercial 30plex** (Qiagen DIPlex) and a **home-brew 38plex** in **U.S. population samples**

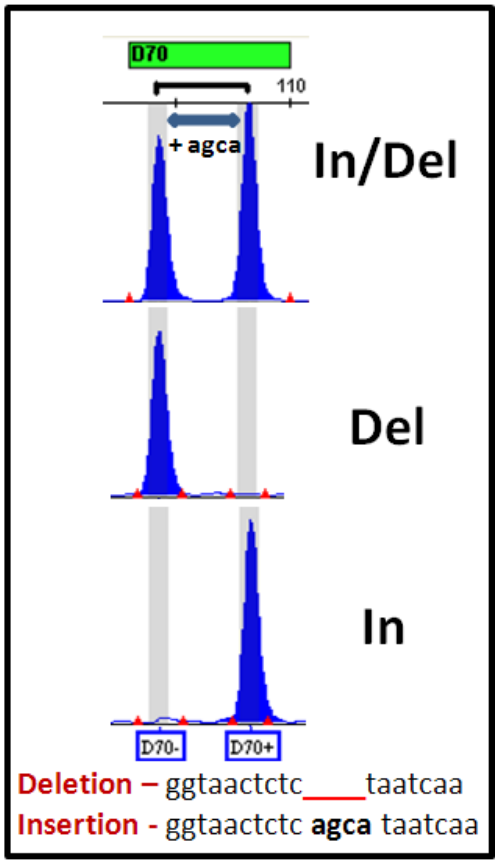
Int J Legal Med (2012) 126:725–737
DOI 10.1007/s00414-012-0721-7

Int. J. Legal Med. (2012) 126: 725-737

ORIGINAL ARTICLE

Forensic performance of two insertion–deletion marker assays

M. Fondevila · C. Phillips · C. Santos · R. Pereira · L. Gusmão · A. Carracedo · J. M. Butler · M. V. Lareu · P. M. Vallone



Performance Assessment of Plex-ID

Kevin Kiesler

Abbott Ibis Biosciences
Plex-ID System

NIST Report to the FBI:
Plex-ID Electrospray Time-of-Flight Mass Spectrometer for Mitochondrial DNA Base Composition Profiling

Experiments performed and report written by: Kevin Kiesler, M.S. (NIST)

Under the direction of: Dr. Peter Vallone (NIST)

- **In collaboration with FBI**
- **Evaluating ESI-TOF mass spectrometer for mtDNA**
- Base composition of the control region determined from 8 triplex PCRs
- Started running the Plex-ID platform mid-October 2011
- **136 page NIST report available on STRBase**

http://www.cstl.nist.gov/strbase/pub_pres/NIST-report-on-PlexID.pdf

Mixture Training Workshops


John Butler


Mike Coble

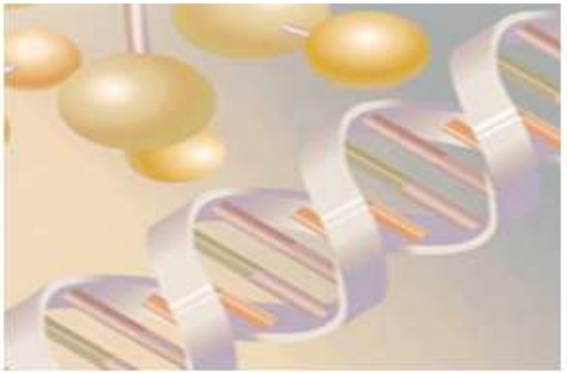
- Collaborators from Boston University (formerly Cellmark)
- ISHI 2012 workshop covered issues with thresholds, statistics, probabilistic genotyping, complex mixtures, court testimony, and assumptions made
  - Audience response systems (clickers) used to gather data from participants
- Slides are available on STRBase

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

SWGDAM Website and Resources Available

http://www.swgdam.org/resources.html

- Home
- ByLaws
- Members
- Committees
- Meetings
- Publications



Additional Resources

Beginning with the development or/and revision of its next draft guidance document(s), SWGDAM will make a "Draft for Comment" or other work product available for the purpose of receiving comments from the general public. This "Draft for Comment" solicitation will be open for a minimum of 60 days, usually through SWGDAM.org. SWGDAM will make all reasonable efforts to advise the forensic DNA community of the open comment period for a proposed guidance document or standard, guideline, best practice, study, or other recommendation and/or finding via as many avenues as possible to include posting notices through discipline-specific and related professional organizations. SWGDAM strongly encourages all interested parties to regularly monitor SWGDAM.org for the posting of such draft documents as well. All public comments received by SWGDAM will forwarded to the appropriate SWGDAM Committee for review and consideration as a part of its formal business practice for the development of the guidance documents or other work product.

The following information resources have been produced and reviewed by members of the ***Mixture Committee*** *of SWGDAM and are available at*
www.cstl.nist.gov/biotech/strbase/mixture/SWGDAM-mixture-info.htm

Link to http://www.cstl.nist.gov/biotech/strbase/mixture/SWGDAM-mixture-info.htm

Mixture Training Materials

Reviewed by SWGDAM Mixture Committee

SWGDAM Mixture Committee Resource Page

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*The following information resources have been produced and reviewed by members of the **Mixture Committee** of the Scientific Working Group on DNA Analysis Methods (SWGDAM) -- see* http://www.swgdam.org/resources.html *for additional information.*

Mixture Training Examples

- Download "Mixture 6" PowerPoint show (56 Mb)

  - with voice-over by Bruce Heidebrecht (Maryland State Police); may work best if file is first saved to your computer

- Download "Mixture IQAS2904" PowerPoint show (35 Mb)

  - with voice-over by Bruce Heidebrecht (Maryland State Police); may work best if file is first saved to your computer

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

December 2012 Issue of *FSI Genetics*

Contents lists available at SciVerse ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Editorial

Focus issue—Analysis and biostatistical interpretation of complex and low template DNA samples

Contents lists available at SciVerse ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

DNA commission of the International Society of Forensic Genetics: Recommendations on the evaluation of STR typing results that may include drop-out and/or drop-in using probabilistic methods

P. Gill[a,b,*], L. Gusmão[c], H. Haned[d], W.R. Mayr[e], N. Morling[f], W. Parson[g], L. Prieto[h], M. Prinz[i], H. Schneider[j], P.M. Schneider[k], B.S. Weir[l]

Some of the articles present in this issue…

Contents lists available at SciVerse ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Exploratory data analysis for the interpretation of low template DNA mixtures

H. Haned [a,*], K. Slooten [a,b], P. Gill [c,d]

[a] Netherlands Forensic Institute, Department of Human Biological traces, The Hague, The Netherlands
[b] VU University Amsterdam, Amsterdam, The Netherlands
[c] Norwegian institute of Public Health, Oslo, Norway
[d] University of Oslo, Norway

Contents lists available at SciVerse ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Validation of a DNA mixture statistics tool incorporating allelic drop-out and drop-in

Adele A. Mitchell *, Jeannie Tamariz, Kathleen O'Connell, Nubia Ducasse, Zoran Budimlija, Mechthild Prinz, Theresa Caragine

Department of Forensic Biology, Office of Chief Medical Examiner of The City of New York, 421 E 26th Street, New York, NY 10016, United States

TrueAllele Mixture Software Evaluation

Mike Coble

Main Points:

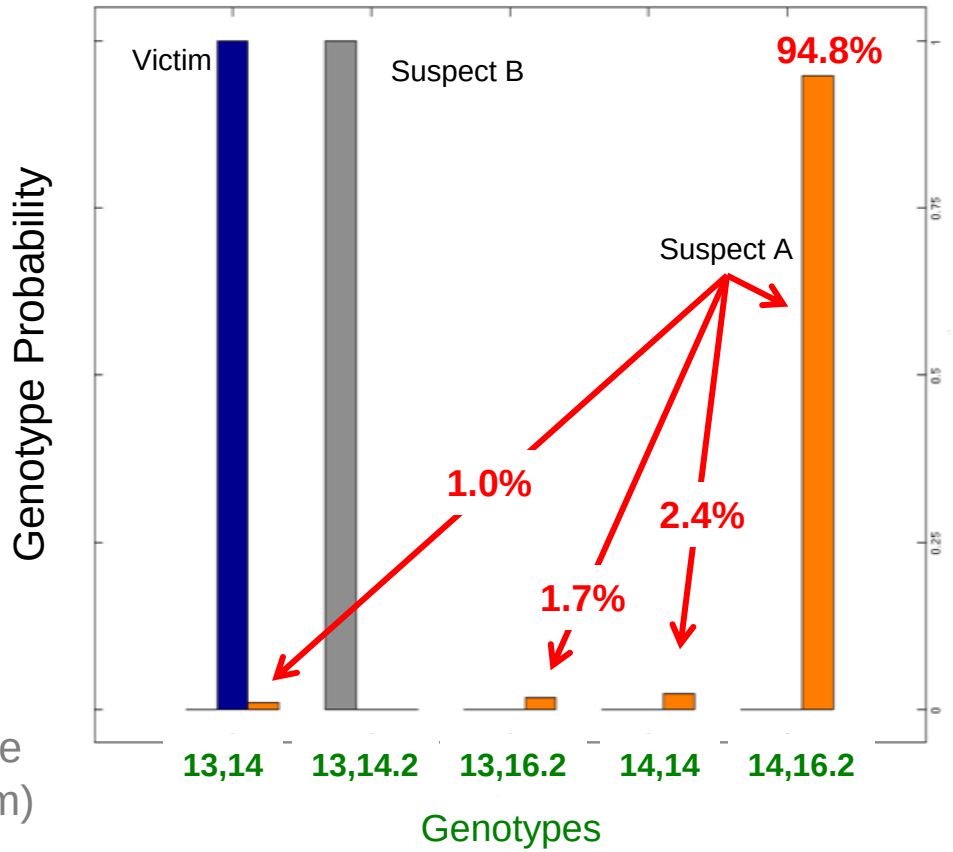
- Exploring the capabilities and limitations of a probabilistic genotyping approach
- Studying TrueAllele software with a number of different types of mixtures (including low-level and 3-4 person mixtures)
- Work being performed at NIST independently of Cybergenetics

Presentations/Publications:

- ISFG 2011 presentation
- Numerous mixture workshop talks (see http://www.cstl.nist.gov/strbase/mixture.htm)

D19S433 result from one replicate of 50,000 simulations

3 person mixture conditioning on the victim

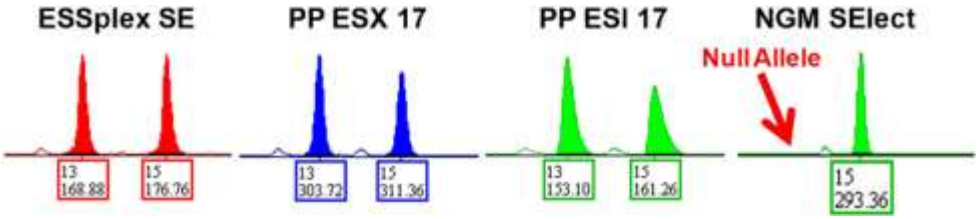


See also Perlin et al. (2011) Validating TrueAllele DNA mixture interpretation. *J. Forensic Sci.* 56(6):1430-1447

STR Kit Concordance Studies

Becky Hill

D18S51 Comparisons



D18S51 null allele with the NGM SElect kit as compared to the ESSplex SE kit, PowerPlex ESX 17 and ESI 17 systems

*Kits are kindly provided by **Applied Biosystems, Promega, and Qiagen** for concordance testing performed at NIST*

Forensic Science International: Genetics Supplement Series 3 (2011) e188–e189

Contents lists available at ScienceDirect

Forensic Science International: Genetics Supplement Series

journal homepage: www.elsevier.com/locate/FSIGSS

Concordance testing comparing STR multiplex kits with a standard data set

Carolyn R. Hill *, Margaret C. Kline, David L. Duewer, John M. Butler

U.S. National Institute of Standards and Technology, NIST 100 Bureau Drive, Gaithersburg, MD 20899-8314, USA

- Examined NIST samples across >20 STR kits and in-house assays covering 29 autosomal STR loci
- **99.90% concordance observed to-date**
  - 1,225 total differences due to primer binding site mutations from 1,176,994 allele comparisons (as of Oct 2012)
- Information provided back to kit developers to redesign primers or add extra ones – often prior to kit release

Aiding Improvements with SE33 Primers

Forensic Science International: Genetics Supplement Series 3 (2011) e502–e503

Contents lists available at ScienceDirect

Forensic Science International: Genetics Supplement Series

journal homepage: www.elsevier.com/locate/FSIGSS

SE33 variant alleles: Sequences and implications

John M. Butler[a,*], Carolyn R. (Becky) Hill[a], Margaret C. Kline[a], Ingo Bastisch[b], Volker Weirich[c], Robert S. McLaren[d], Douglas R. Storts[d]

[a] U.S. National Institute of Standards and Technology, Gaithersburg, MD, USA
[b] Bundeskriminalamt (BKA), Wiesbaden, Germany
[c] LKA, Mecklenburg-Vorpommern, Germany
[d] Promega Corporation, Madison, WI, USA

Home » Resources » Articles & Publications » *Profiles in DNA* »

http://www.promega.com/resources/articles/profiles-in-dna/2012/improved-primer-pair-for-the-se33-locus-in-the-powerplex-esi-17-pro-system/

Improved Primer Pair for the SE33 Locus in the PowerPlex® ESI 17 Pro System

Robert S. McLaren[1], Jaynish Patel[1], Douglas R. Storts[1], Carolyn R. Hill[2*], Margaret C. Kline[2] and John M. Butler[2]

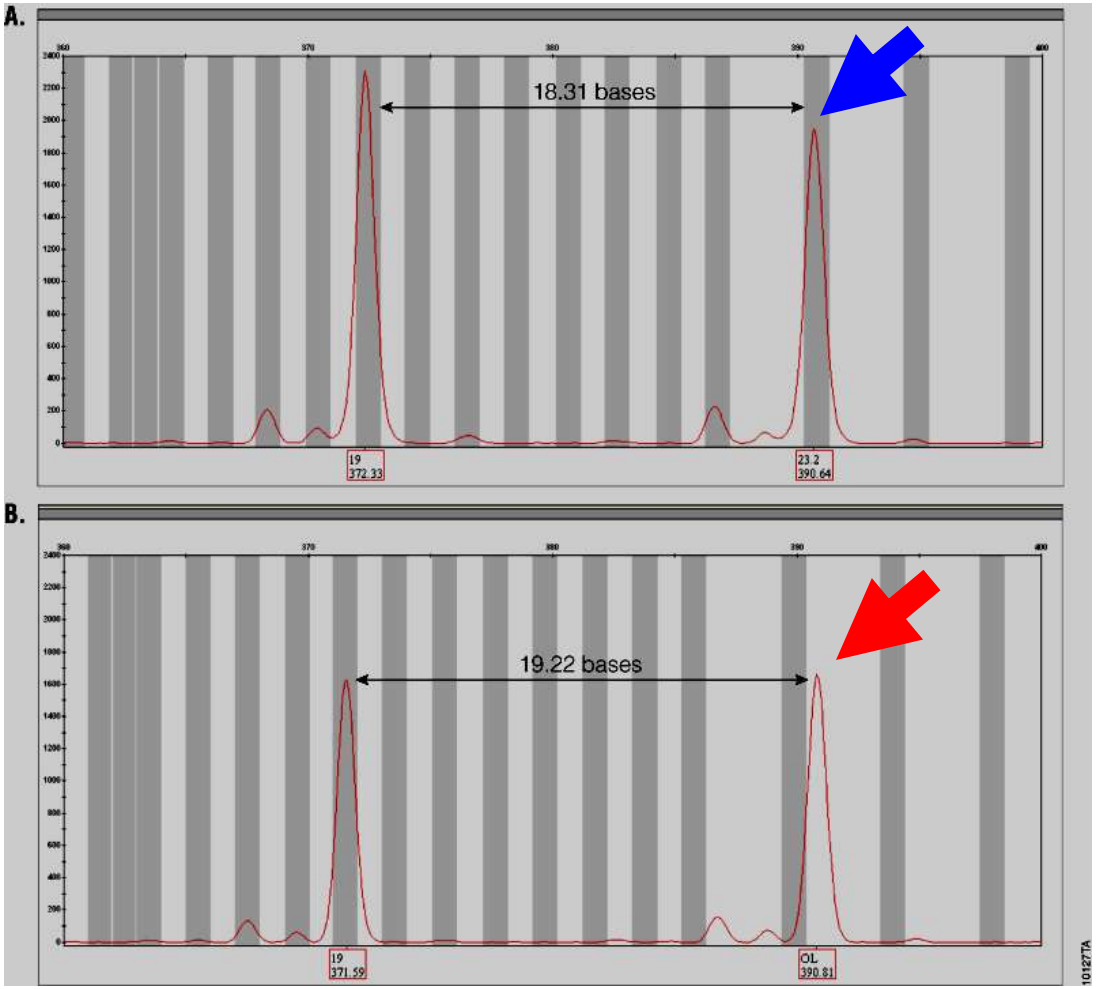
[1]Promega Corporation

[2]Human Identity Project Team, National Institutes of Standards and Technology

Publication Date: 2012

A developmental validation article has also been prepared and submitted

PowerPlex ESI 17 Pro vs ESI 17 **SE33 Results**



PowerPlex **ESI 17 Pro**

SE33 allele 23.2

Reverse primer is **inside** of hairpin region

PowerPlex **ESI 17**

SE33 allele "23.3"

Reverse primer is **outside** of hairpin region

The SE33 locus range is shown for both PowerPlex® ESI 17 Pro (Panel A) and ESI 17 (Panel B) amplifications of DNA sample GT37190. Peak labels show allele calls (top) and sizes in bases (bottom). The off-ladder peak seen with PowerPlex® ESI 17 is correctly called as 23.2 with the PowerPlex® ESI 17 Pro System

http://www.promega.com/resources/articles/profiles-in-dna/2012/improved-primer-pair-for-the-se33-locus-in-the-powerplex-esi-17-pro-system/

Variant STR Allele Sequencing


Margaret Kline

Main Points:

- **STR allele sequencing has been provided free to the community** for the past ten years thanks to NIJ-funding
- Article provides primer sequences (outside of all known kit primers) for 23 autosomal STRs & 17 Y-STRs and full protocol for gel separations and sequencing reactions
  - 111 normal and variant alleles sequenced (at 19 STR & 4 Y-STRs)
  - 17 null alleles sequenced (with impact on various STR kit primers)

This year we successfully navigated lawyers and legal agreements on both sides to create an MOU with an SDIS lab permitting NIST to sequence supplied variant alleles


ELSEVIER

Short communication

STR sequence analysis for characterizing normal, variant, and null alleles

Margaret C. Kline *, Carolyn R. Hill, Amy E. Decker [1], John M. Butler

National Institute of Standards and Technology, 100 Bureau Drive, M/S 8312, Gaithersburg, MD 20899, USA

Presentations/Publications:

- FSI Genetics article (Aug 2011) and numerous talks

NIST 1036 U.S. Population Samples

- 1032 males + 4 females
  - 361 Caucasians (2 female)
  - 342 African Americans (1 female)
  - 236 Hispanics
  - 97 Asians (1 female)

***Unrelated* samples**
All known or potential related individuals (based on autosomal & lineage marker testing) have been removed from the 1036 data set (e.g., only sons were used from father-son samples)

- Anonymous donors with self-identified ancestry
  - Interstate Blood Bank (Memphis, TN) – obtained in 2002
  - Millennium Biotech, Inc. (Ft. Lauderdale, FL) – obtained in 2001
  - DNA Diagnostics Center (Fairfield, OH) – obtained in 2007

- **Complete profiles with 29 autosomal STRs + PowerPlex Y23**
  - **Examined with multiple kits** and in-house primer sets enabling concordance

- Additional DNA results available on subsets of these samples
  - mtDNA control region/whole genome (AFDIL)
  - >100 SNPs (AIMs), 68 InDel markers, X-STRs (AFDIL)
  - NIST assays: miniSTRs, 26plex, >100 Y-STRs, 50 Y-SNPs

Data available on STRBase: **http://www.cstl.nist.gov/biotech/strbase/NISTpop.htm**

Benefits of NIST 1036 Data Set

- **Elimination of potential null alleles due to primer binding site mutations** through extensive concordance testing performed with different PCR primer sets from all available commercial STR kits

- **Ancestry testing performed** on DNA samples with autosomal SNPs, Y-SNPs, and mtDNA sequencing to verify self-declared ancestry categorization

- **Related individuals removed** based on Y-STR and mtDNA results

Example of Related Individuals in Original NIST Data Set

- **Hispanic samples ZT79994 and ZT79995**

- Out of 24 autosomal STR loci, these samples **share a total of 22 alleles at 22 loci** (only D12S391 and Penta D have non-overlapping heterozygous alleles)
- **Full 23 Y-STR match** with PowerPlex Y23
- **Same mtDNA control region sequences**
- Kinship calculations
  - LR = 0 for parent-child
  - **LR = 56,300 for full-siblings (brothers)**
  - LR = 5,690 for half-siblings (or uncle-nephew, grandfather-grandson)
  - LR = 264 for first cousins

- ***Decision***: **Remove ZT79995 from final data set**
  - ZT79994 represents this individual's family in NIST 1036

Characterizing New STR Loci


John Butler


Becky Hill

Main Points:

- In April 2011, the FBI announced plans to expand the core loci for the U.S. beyond the current 13 CODIS STRs

- Our group is collecting U.S. population data on new loci and characterizing them to aid understanding of various marker combinations

- We are collecting all available information from the literature on the 29 commonly used autosomal STR loci

Presentations/Publications:

- Hill et al (2011) *FSI Genetics* 5(4): 269-275
- Hares (2012) Expanding the U.S. core loci… *FSI Genetics* 6(1): e52-e54
- Butler & Hill (2012) *Forensic Sci Rev* 24(1): 15-26

Determination of Additional CODIS Core Loci

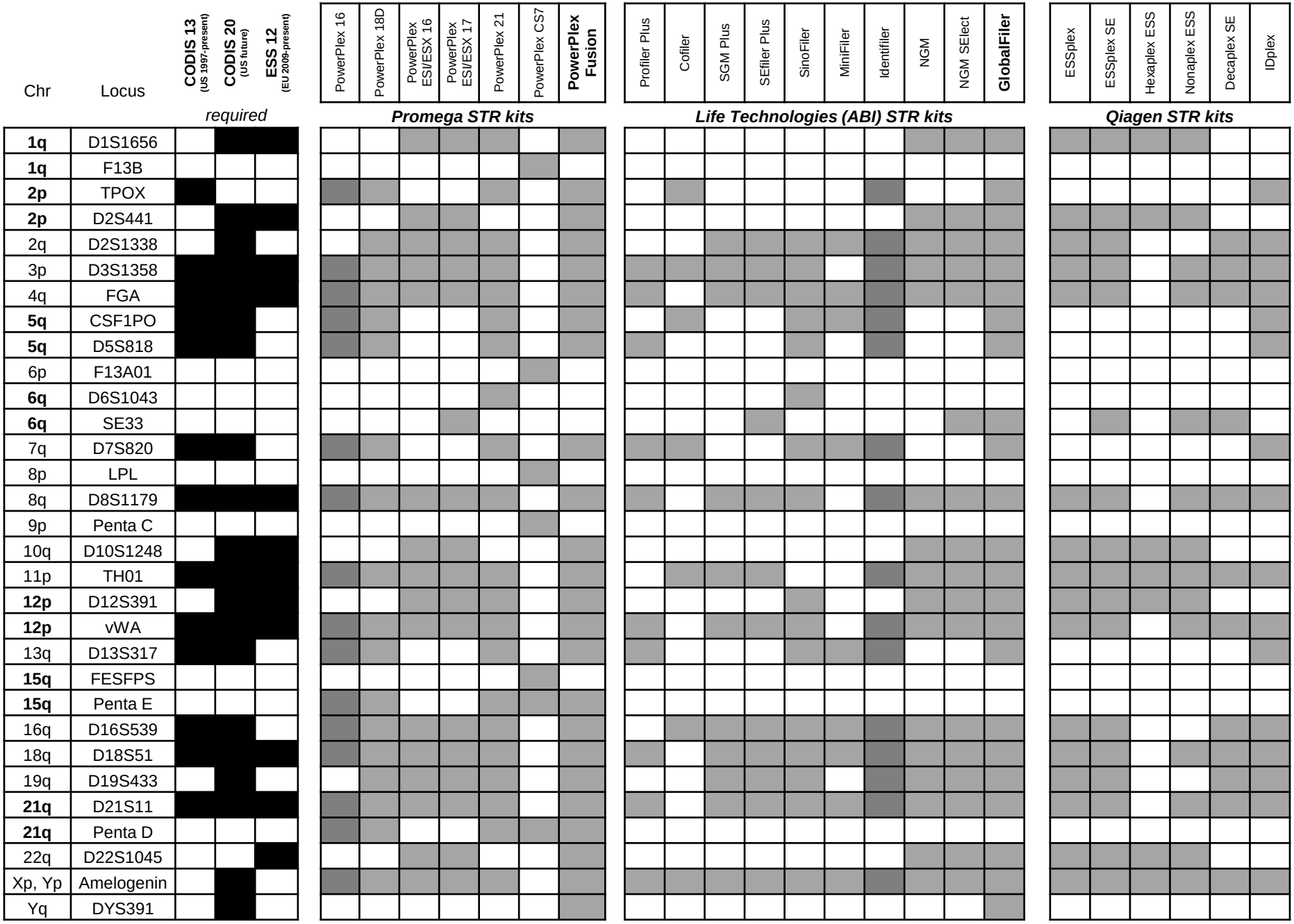
D.R. Hares (2012) Expanding the CODIS Core Loci in the United States. *Forensic Sci. Int. Genet.* 6: e52-e54
Addendum to expanding the CODIS core loci in the United States, Forensic Sci. Int. Genet. (2012) doi:10.1016/j.fsigen.2012.01.003

What	Why	Who/How	When
Form a Working Group (WG) to discuss initial selection	Establishes target goals	CODIS Core Loci Working Group with FBI Chair and 5 members; Web meetings	May 2010 - present
Announce proposed additional CODIS core loci	Sets desired target goals and informs manufacturers	WG Chair; Publish proposed listing of CODIS core loci	April 2011 online (published Jan 2012)
Ongoing Progress Reports	Provides updates for DNA community	WG Chair; Present updates on status of CODIS Core Loci project at meetings	2010-2012
Implementation Considerations & Strategy	Identify issues for implementation and timeline	WG	June 2011 - present
Manufacturers develop prototype kits	Creates tools to meet target goals	Manufacturers; Provide status reports to WG for timeline	2011-2012
Test and validate prototype kits	Examines if target goals can be met	Validation Laboratories; Follow QAS compliant validation plan	Beginning in 2012
Review and evaluate data from validation	Evaluates if desired performance is obtained	NIST, SWGDAM and FBI; Provide feedback, if any, to Manufacturers	In conjunction with and at the conclusion of validation
Selection of new CODIS core loci	Allows protocols to be established	FBI; seek input from DNA community and stakeholders; Notify Congress	After evaluation of validation data and kit production factors
Implementation of new CODIS core loci at the National DNA Index System	Enables target goals to be met	All NDIS-participating labs	~ 24 months after selection of new CODIS core loci

http://www.fbi.gov/about-us/lab/codis/planned-process-and-timeline-for-implementation-of-additional-codis-core-loci

29 autosomal STRs

STR Loci Present in Current Commercial Kits



Butler, J.M. & Hill, C.R. (2013) *Topics on Forensic DNA Analysis: Current Practices & Emerging Technologies* (CRC Press), Figure 9.1

Locus	Alleles Observed	Genotypes Observed	Het (obs)	P_I Value n=1036
SE33	52	304	0.9353	0.0066
Penta E	23	138	0.8996	0.0147
D2S1338	13	68	0.8793	0.0220
D1S1656	15	93	0.8890	0.0224
D18S51	22	93	0.8687	0.0258
D12S391	24	113	0.8813	0.0271
FGA	27	96	0.8745	0.0308
D6S1043	27	109	0.8494	0.0321
Penta D	16	74	0.8552	0.0382
D21S11	27	86	0.8330	0.0403
D8S1179	11	46	0.7992	0.0558
D19S433	16	78	0.8118	0.0559
vWA	11	39	0.8060	0.0611
F13A01	16	56	0.7809	0.0678
D7S820	11	32	0.7944	0.0726
D16S539	9	28	0.7761	0.0749
D13S317	8	29	0.7674	0.0765
TH01	8	24	0.7471	0.0766
Penta C	12	49	0.7732	0.0769
D2S441	15	43	0.7828	0.0841
D10S1248	12	39	0.7819	0.0845
D3S1358	11	30	0.7519	0.0915
D22S1045	11	44	0.7606	0.0921
F13B	7	20	0.6911	0.0973
CSF1PO	9	31	0.7558	0.1054
D5S818	9	34	0.7297	0.1104
FESFPS	12	36	0.7230	0.1128
LPL	9	27	0.7027	0.1336
TPOX	9	28	0.6902	0.1358

Rank Order of 29 Autosomal STR Loci in Commercial Kits with NIST 1036 U.S. Population Samples

http://www.promega.com/resources/articles/profiles-in-dna/2012/variability-of-new-str-loci-and-kits-in-us-population-groups/

Hill et al. ISHI 2012 poster #84 (see STRBase); Butler et al. (2012) *Profiles in DNA*

Probability of Identity Values
for Various STR Kits or Locus Combinations
based on NIST 1036 U.S. Population Samples

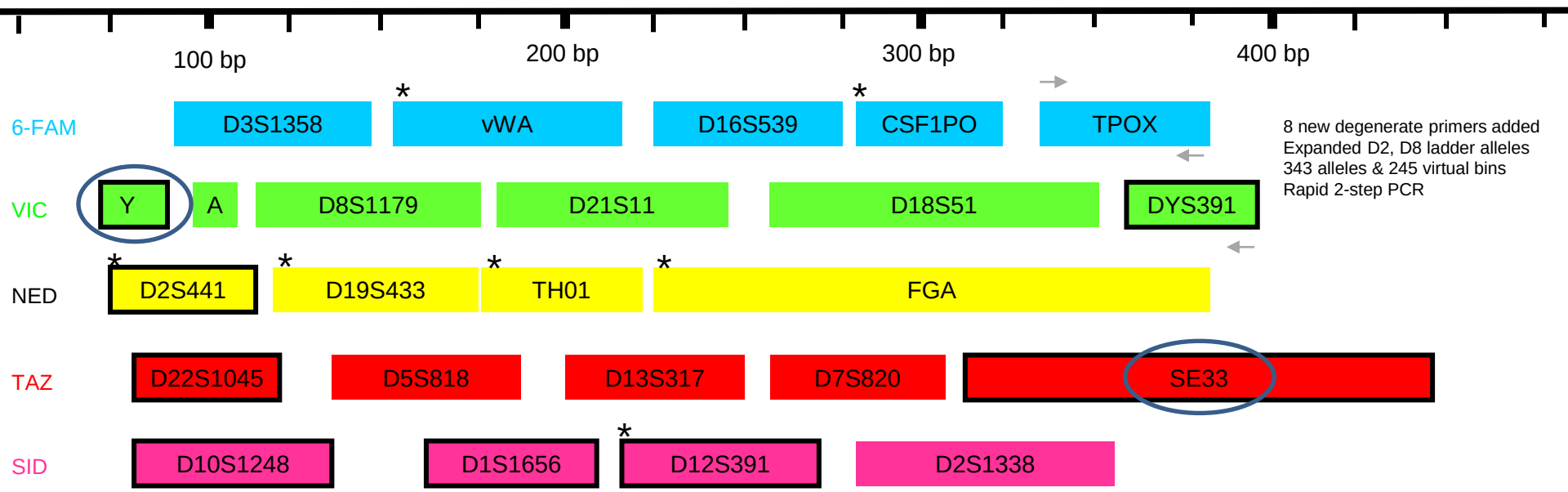
STR Kit or Core Set of Loci	Total N=1036	Caucasians (n=361)	African Am. (n=342)	Hispanics (n=236)	Asians (n=97)
CODIS 13	5.02E-16	2.97E-15	1.14E-15	1.36E-15	1.71E-14
Identifiler	6.18E-19	6.87E-18	1.04E-18	2.73E-18	5.31E-17
PowerPlex 16	2.82E-19	4.24E-18	6.09E-19	1.26E-18	2.55E-17
PowerPlex 18D	3.47E-22	9.82E-21	5.60E-22	2.54E-21	7.92E-20
ESS 12	3.04E-16	9.66E-16	9.25E-16	2.60E-15	3.42E-14
ESI 16 / ESX 16 / NGM	2.80E-20	2.20E-19	6.23E-20	4.03E-19	9.83E-18
ESI 17 / ESX 17 / NGM SElect	1.85E-22	1.74E-21	6.71E-22	3.97E-21	1.87E-19
CODIS 20	9.35E-24	7.32E-23	6.12E-23	8.43E-23	4.22E-21
GlobalFiler	7.73E-28	1.30E-26	3.20E-27	2.27E-26	1.81E-24
PowerPlex Fusion	6.58E-29	2.35E-27	1.59E-28	2.12E-27	1.42E-25
All 29 autosomal STRs	2.24E-37	7.36E-35	3.16E-37	2.93E-35	4.02E-32
29 autoSTRs + DYS391	1.07E-37	3.26E-35	1.77E-37	1.29E-35	2.81E-32

Hill et al. ISHI 2012 poster #84 (see STRBase); Butler et al. (2012) *Profiles in DNA*

STR Kit Layouts by Dye Label and PCR Product Size

Life Technologies/Applied Biosystems **GlobalFiler** (6-dye – LIZ600 size standard)

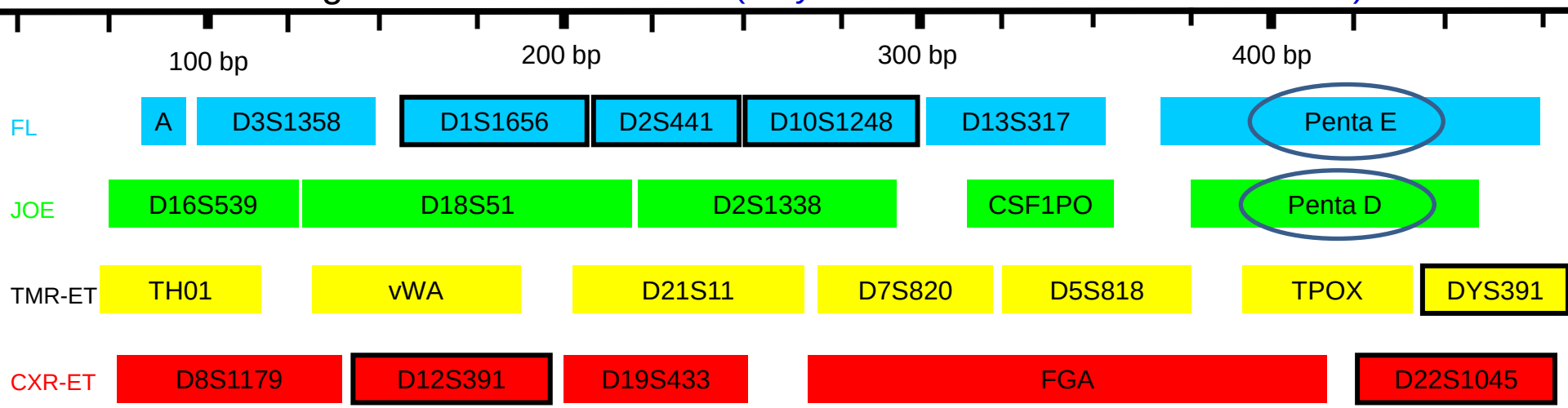
24plex



8 new degenerate primers added
Expanded D2, D8 ladder alleles
343 alleles & 245 virtual bins
Rapid 2-step PCR

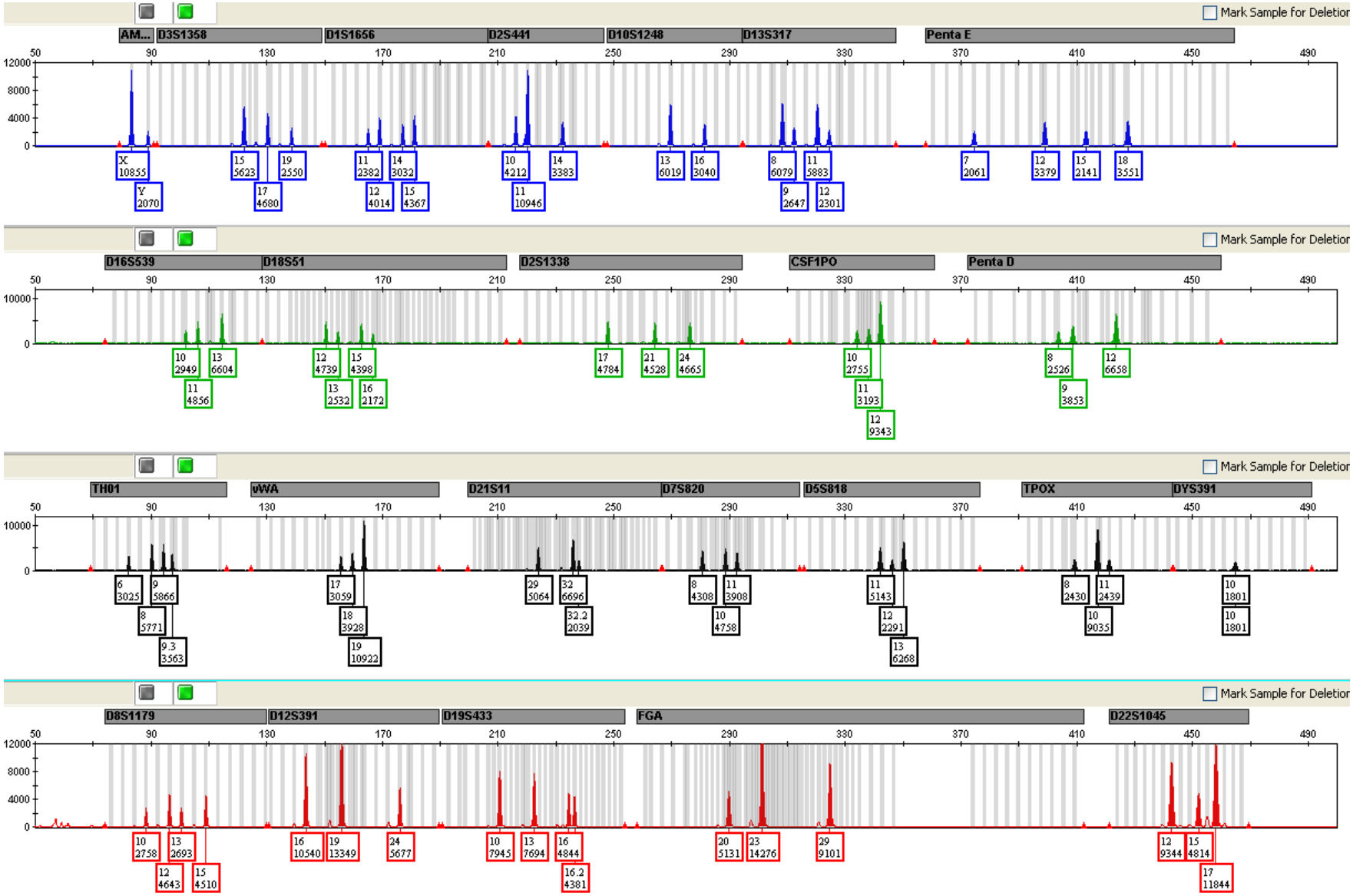
Promega PowerPlex **FUSION** (5-dye – CC5 internal lane standard 500)

24plex



DNA Mixture with **PowerPlex Fusion** (Promega)

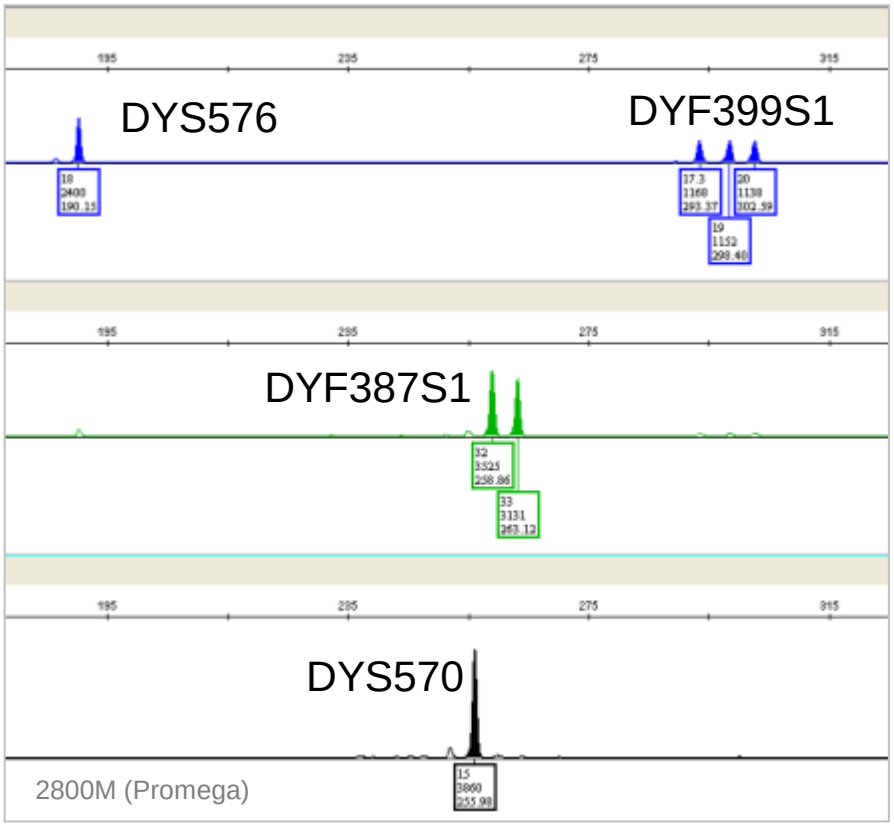
24plex assay



Rapidly Mutating Y-STR Loci

Mike Coble

Becky Hill

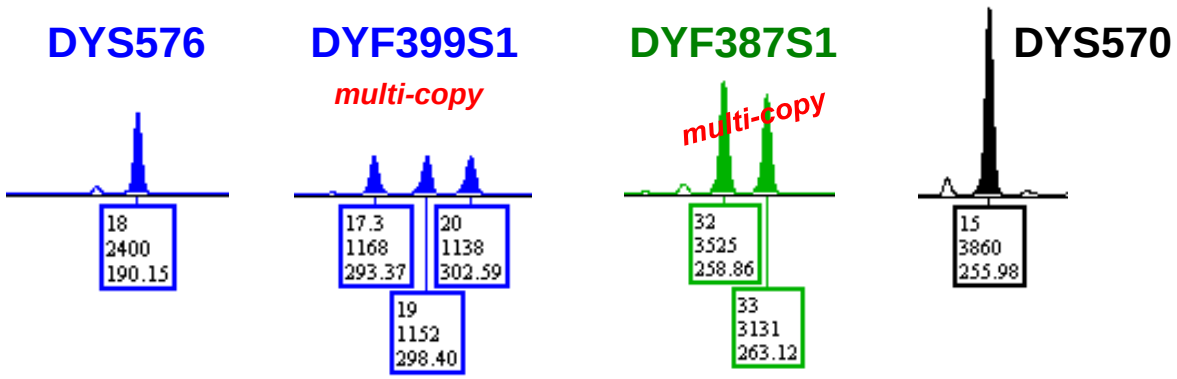


- Part of RM Y-STR Study Group organized by Manfred Kayser (Erasmus University, The Netherlands)
- Supplied data from 1,296 U.S. samples (634 population + 331 father/son pairs)
- Publication with RM Y-STR Study Group is forthcoming

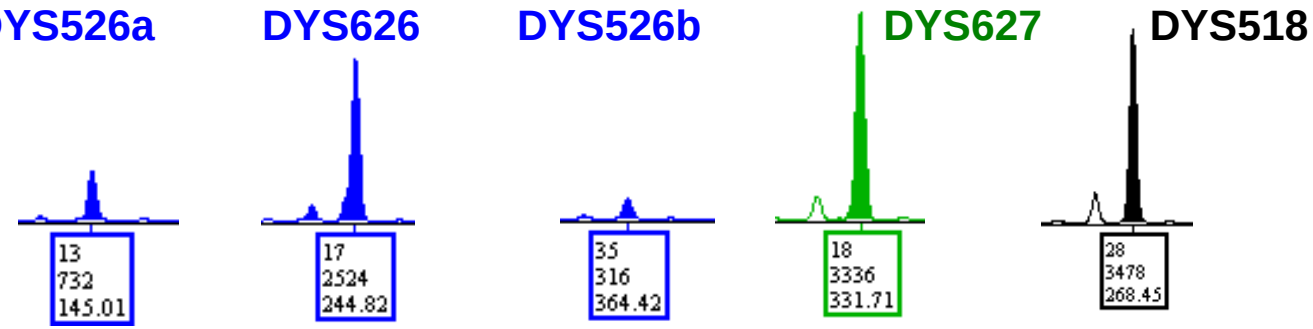
Rapidly Mutating (RM) Y-STRs

NIST supplied data from 1,296 U.S. samples (634 population + 331 father/son pairs)
to RM Y-STR Study Group led by Manfred Kayser (11,978 samples from 169 worldwide populations)

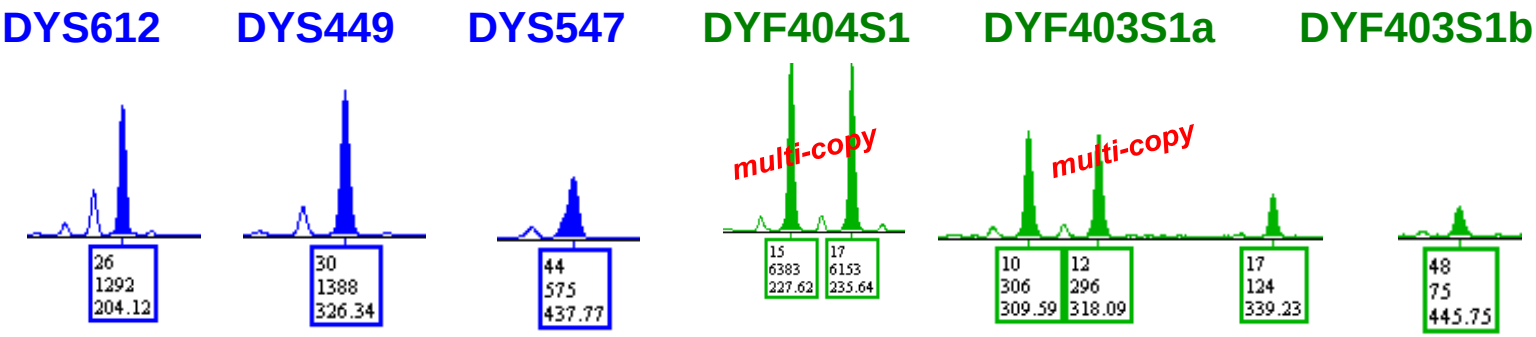
RM Y-STR
Multiplex 1



RM Y-STR
Multiplex 2



RM Y-STR
Multiplex 3

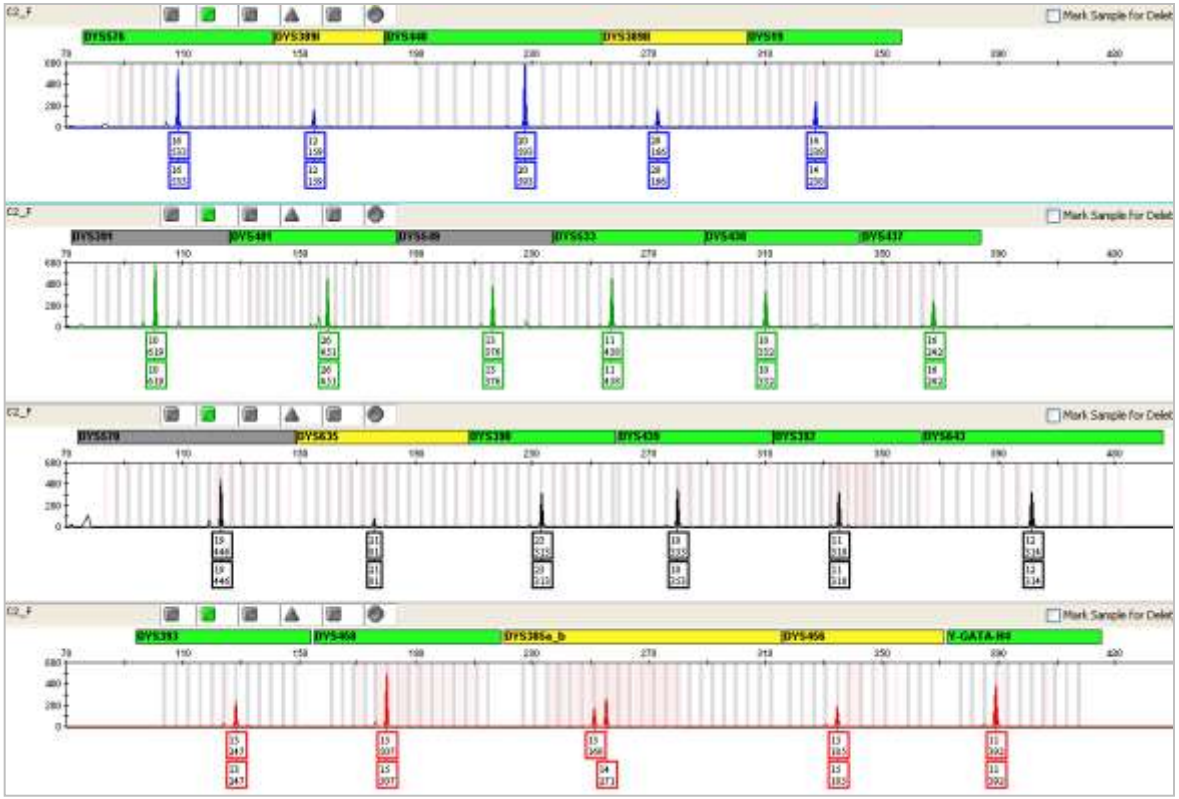


PowerPlex Y23 Kit


Mike Coble


Becky Hill

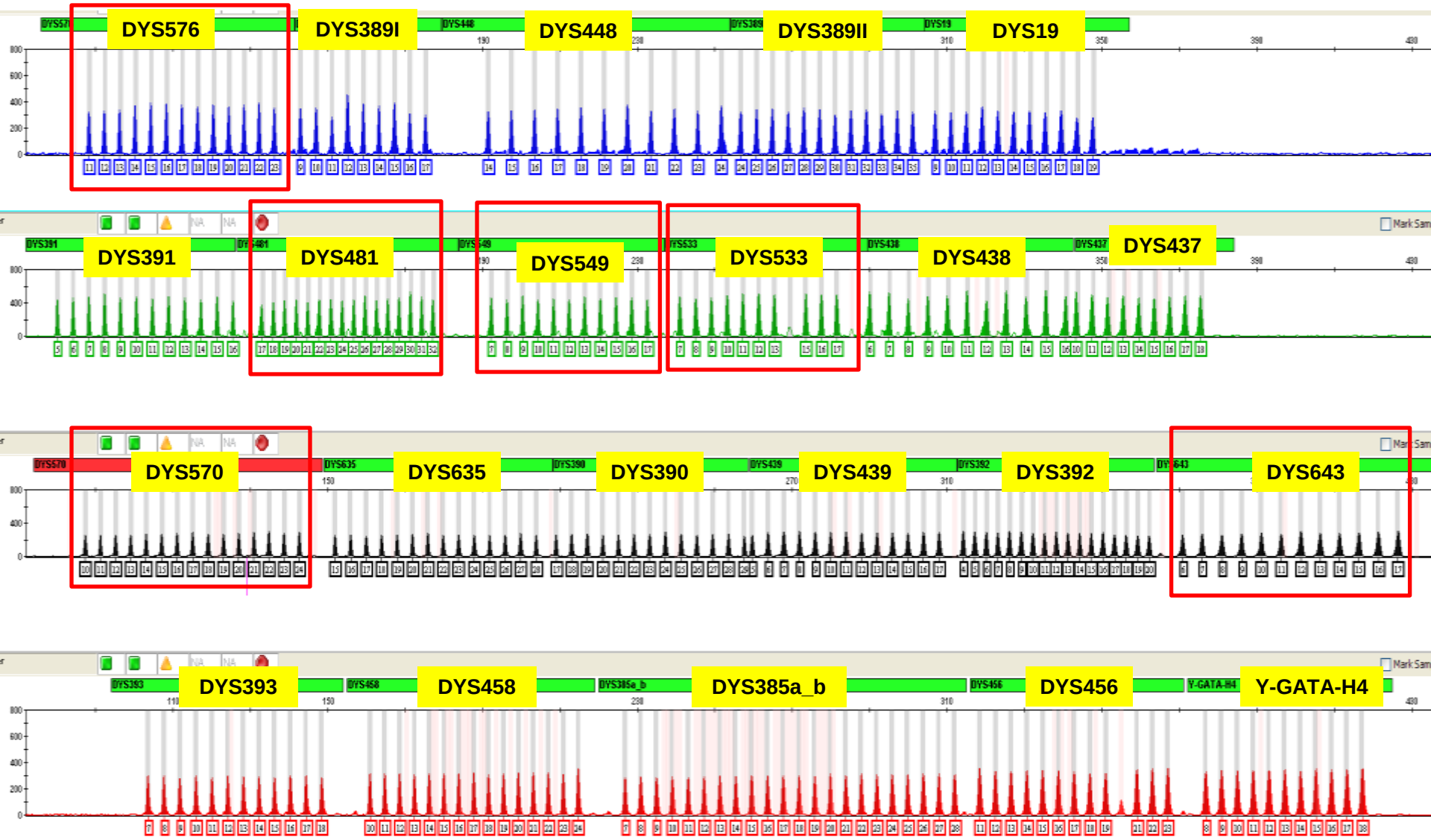
125pg male + 400ng female **(3200x female)**



Kit found to be *sensitive* and *specific* to male DNA

- Typed 1032 males from 4 U.S. population groups
- Data supplied to YHRD and USYSTR databases
- Publications are forthcoming
- Full dataset to be released on STRBase

PowerPlex Y23 Allelic Ladders



23 Y-STRs = 17 Yfiler + 6 additional loci

Number of unique and shared haplotypes observed with various combinations of Y-STR loci across 1032 U.S. population samples

N = 1032 males	PowerPlex Y	Yfiler	PowerPlex Y23
# haplotypes	**891**	**1013**	**1029**
discrimination capacity	0.863	0.982	0.997
# times haplotype observed	PPY (12 loci)	Yfiler (17 loci)	PPY23 (23 loci)
1	821	998	1026
2	41	12	3
3	16	2	.
4	6	1	.
5	2	.	.
6	2	.	.
7	1	.	.
8	.	.	.
9	1	.	.
10	.	.	.
11	.	.	.
12	.	.	.
13	.	.	.
14	.	.	.
15	.	.	.
16	.	.	.
17	.	.	.
18	.	.	.
19	1	.	.

1026 PPY23 haplotypes occur once;
and
3 sets of sample pairs cannot be resolved from one another

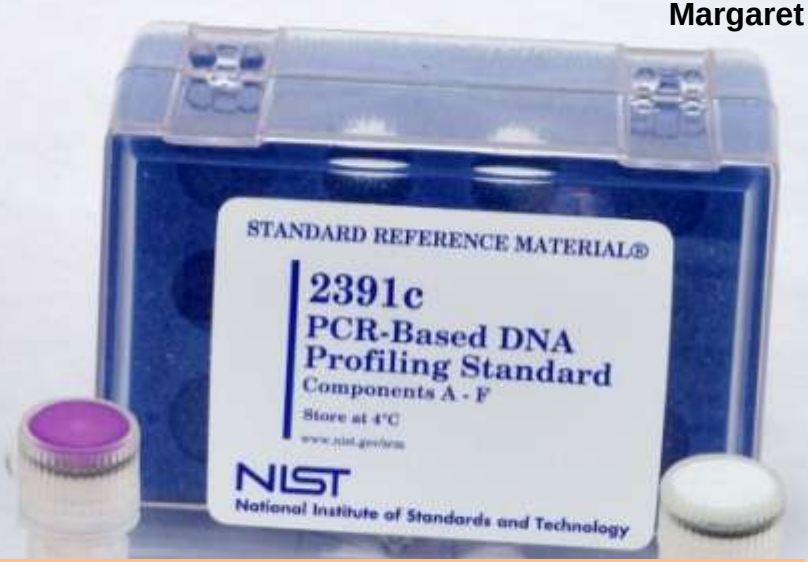
From Butler et al. (2012) *Profiles in DNA* article

NIST Reference Materials for Forensic DNA Measurement Assurance

Margaret Kline

SRM 2372 is currently not available because the dsDNA has unraveled, which impacts absorbance certification values. We are re-certifying the samples with aid of digital PCR measurements. **We hope to have it available again by early 2013.**

DNA quantity measurement calibration



SRM 2391c currently does not cover the six additional Y-STR markers in PowerPlex Y23. We plan to certify values for these markers by mid-2013.

Autosomal and Y-chromosome short tandem repeat (STR) measurement calibration

ABI 3500 Validation Studies

Erica Butts

Main Points:

- The 3500 has proven to be reliable, reproducible and robust in our hands – we have provided feedback to ABI to improve use
- Produces excellent DNA sequencing results
- Signal strength is different compared to ABI 3130xl and requires studies to set analytical and stochastic thresholds
- **Dye-specific analytical thresholds** resulted in less allelic and full locus dropout than applying one analytical threshold to all dyes
- RFID tracking decreases flexibility in our research experience

Presentations/Publications:

- MAAFS talk (May 2011)
- ABI road show talks (July & Aug 2011)
- ISFG presentation (Sept 2011)
- *Forensic News* (Spring 2012)

http://marketing.appliedbiosystems.com/mk/get/FORENSICNEWS_HIDINACTION#article5

Rapid DNA Efforts

Pete Vallone **Erica Butts**

Accelerated Nuclear DNA Equipment (ANDE) developed by **NetBIo**

http://ishinews.com/wp-content/uploads/2012/10/Rapid-DNA-Miles-1.58MB.pdf

RapidHIT 200 developed by **IntegenX**

http://integenx.com/wp-content/uploads/2010/06/RapidHIT-200.png

- Evaluating ANDE (NetBio) and IntegenX rapid DNA instruments
  - both instruments are capable of swab in → STR profile out in less than 90 minutes without user intervention
- Exploring rapid DNA techniques including direct PCR and rapid PCR
  - STR profiles generated in <2 hours with standard lab equipment and rapid protocols
  - See ISHI 2012 poster available on STRBase "Rapid DNA Testing Approaches for Reference Samples"

Fastest results swab-to-profile (Identifiler): 57 minutes

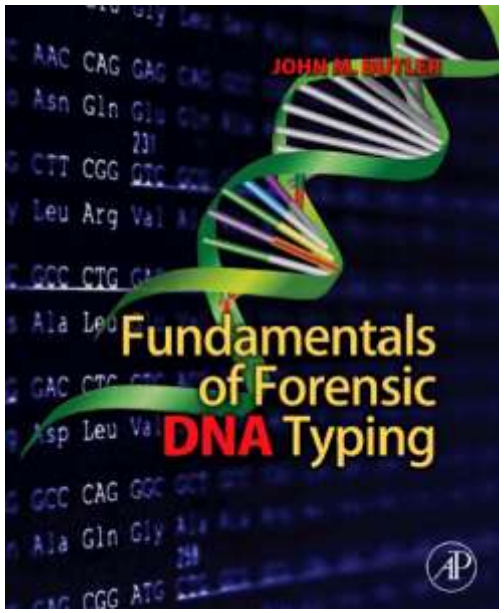
Forensic DNA Typing Textbook

3rd Edition is Three Volumes

Now part of job at NIST (no royalties are received)

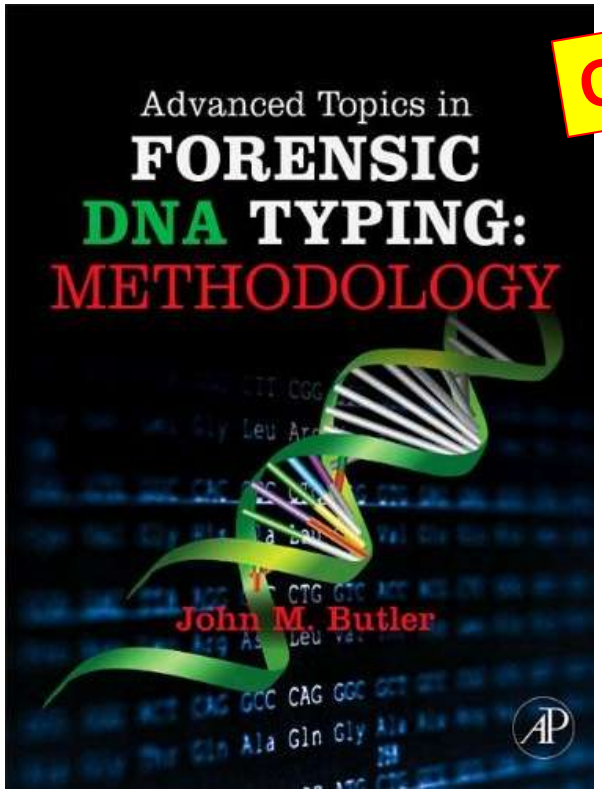
John Butler

For beginning students, general public, & lawyers



Fall 2009

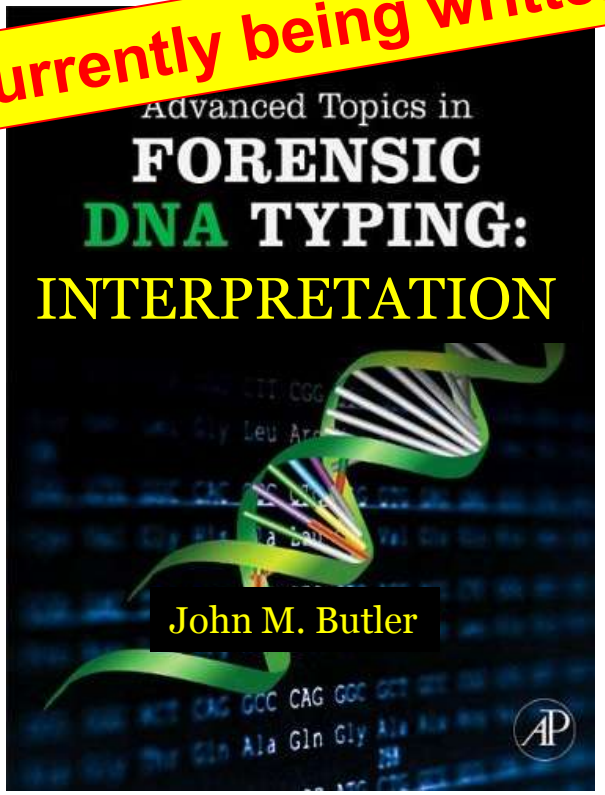
~500 pages



Fall 2011

~700 pages

Currently being written



Fall 2013

~500 pages

Advanced Topics in Forensic DNA Typing: INTERPRETATION

Chapter	Topic (current planned chapters)
	Introduction
1	Data interpretation overview
2	Thresholds
3	STR alleles & artifacts
4	STR genotypes & dropout
5	STR profiles
6	**Mixture interpretation**
7	Low-level DNA and complex mixtures
8	CE troubleshooting
9	Statistical interpretation overview
10	STR population data analysis
11	Profile frequency estimates
12	**Mixture statistics**
13	Coping with potential missing alleles
14	**Kinship and parentage analysis**
15	Lineage marker statistics
16	Drawing conclusions & **report writing**
	Glossary
App 1	U.S. Population Data (29 loci with N=1036)
App 2	NRC I and II Recommendations (1992/1996)
App 3	DAB Recommendations on Stats (Feb 2000)
App 4	SWGDAM Guidelines (Jan 2010)
App 5	Worked Example for Mixture Interpretation

Features in New Book

(planned for Fall 2013 release)

- Numerous D.N.A. Boxes (**D**ata, **N**otes, & **A**pplications)
  - Worked examples to show relevance of equations
  - "Better know a statistician"
- Interviews on report writing from multiple perspectives
- Explanations of SWGDAM interpretation guidelines
- Mixture interpretation
- Kinship analysis
- CE troubleshooting
- Standard U.S. pop data

Upcoming Events Sponsored by NIST

November 28-30, 2012

Forensics@NIST 2012

- **Forensic research at NIST highlighted**
  - **November 28 AM devoted to DNA**

    www.nist.gov/oles/forensics-2012.cfm

April 12, 2013

- **Webcast mixture workshop**
- Agenda to be similar to ISHI workshops

Fall 2013

- **U.S. DNA Technical Leaders Summit**
- In partnership with the FBI CODIS Unit

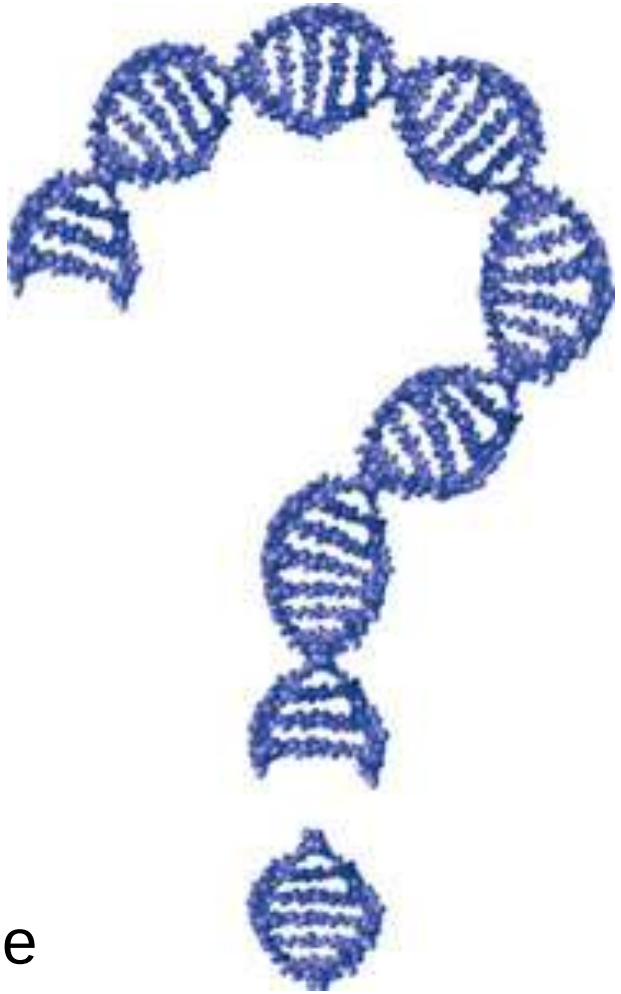
More details will be announced on STRBase and NIST OLES web pages in the near future

FORENSIC SCIENCES

OLES

Thank you for your attention

Acknowledgments: Applied Biosystems, Promega, and Qiagen for STR kits used in concordance studies



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

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