CAC DNA Workshop
06 November 2012 – San Jose, California

NIST Update

NIST Human Identity Project Team

National Institute of Standards and Technology
Gaithersburg, Maryland USA

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 **Fondevila** 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

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)

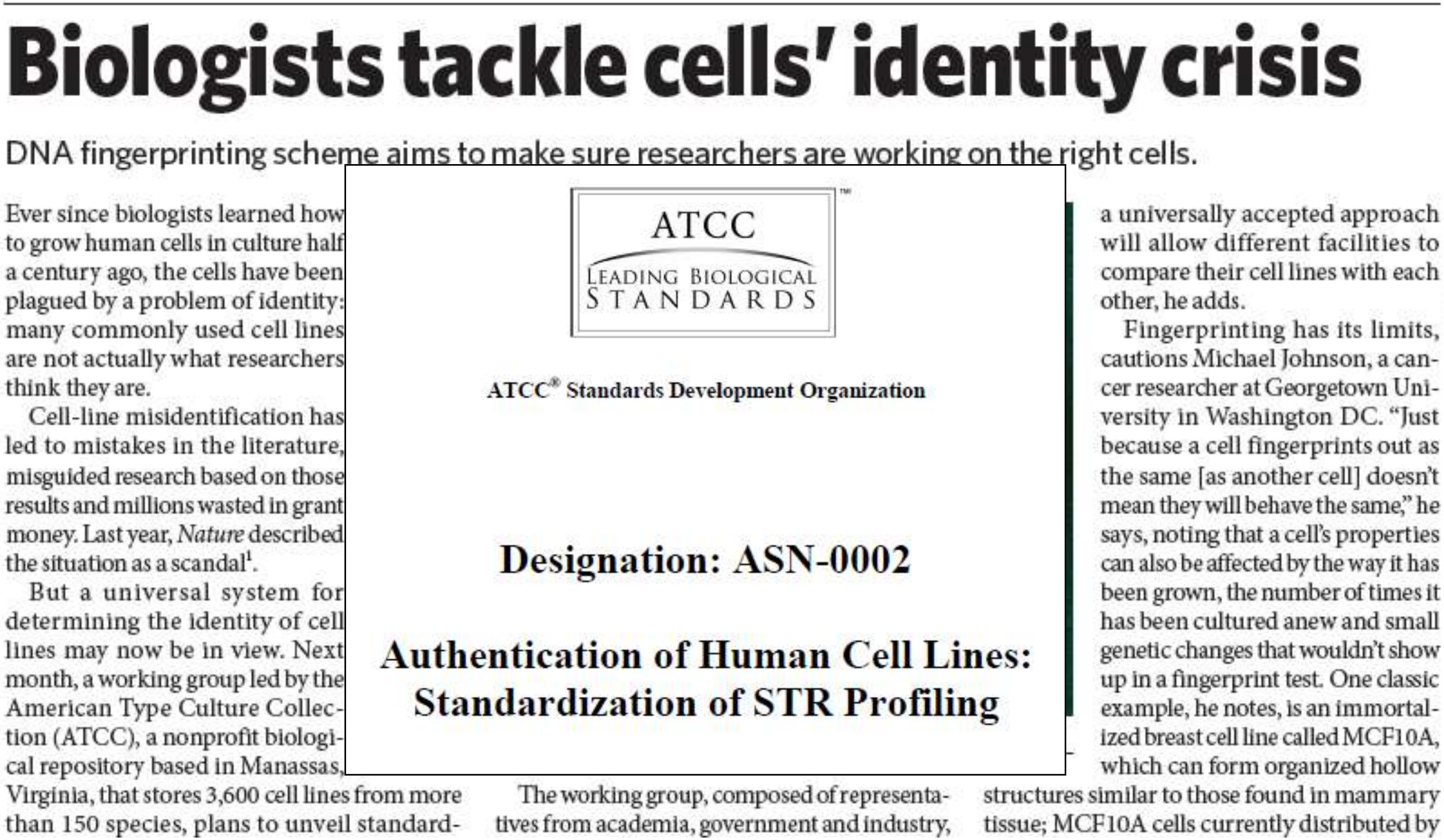
- **Clinical Genetics**
  - Huntington's Disease SRM
  - CMV SRM
  - Exploring future needs

- **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-

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

ATCC
LEADING BIOLOGICAL STANDARDS™

ATCC® Standards Development Organization

Designation: ASN-0002

Authentication of Human Cell Lines: Standardization of STR Profiling

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

2012 Project Update

- InDel work published
- PLEX-ID report available
- TrueAllele evaluation continues…
- New DNA mixture training materials
- 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:

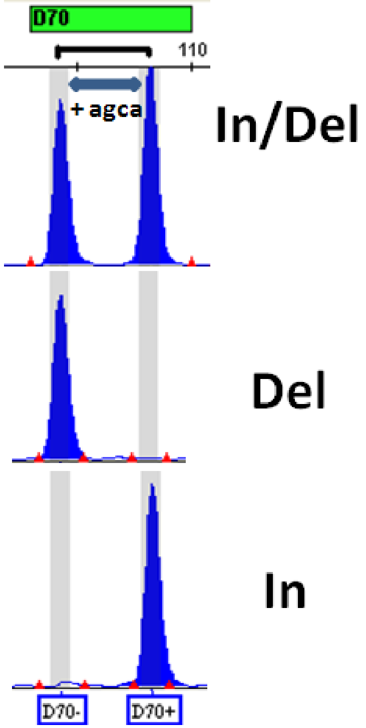
- InDels (insertion-deletion) or DIPs (deletion-insertion polymorphisms) are short length polymorphisms, consisting of the presence or absence of a short (typically 1-50 bp) sequence
- Like SNPs, InDels have low mutation rate (value to kinship analysis), small amplicon target sizes (value with degraded 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

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



Deletion – ggtaactctc____taatcaa

Insertion - ggtaactctc **agca** taatcaa

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)

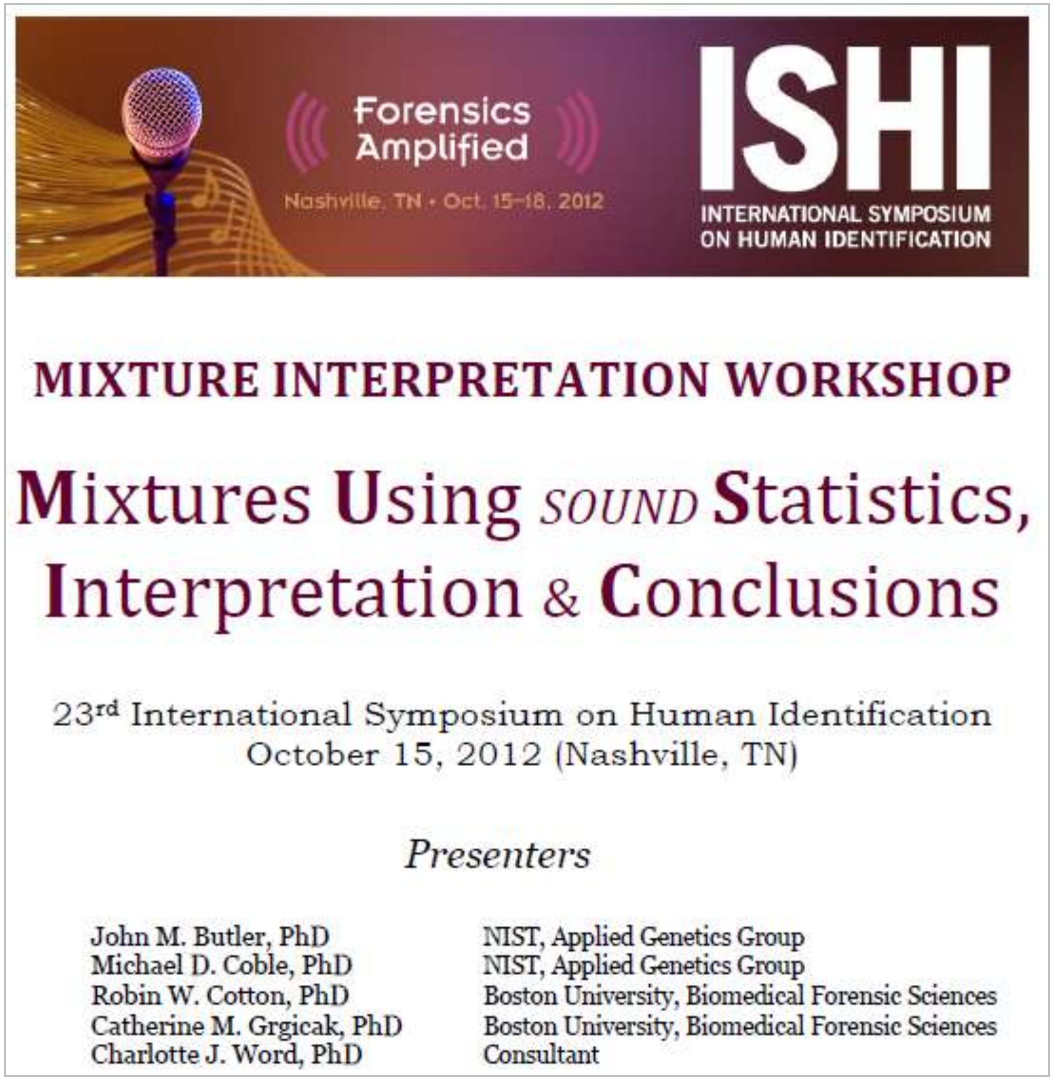
- **In collaboration with FBI**
- **Evaluating ESI-TOF mass spectrometer for mtDNA**
- Base composition of the control region determined from 8 triplex PCRs
- Started running the Plex-ID platform mid-October 2011
- ***136 page NIST report available on STRBase***

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

Mixture Training Workshops


John Butler


Mike Coble


Forensics Amplified

Nashville, TN • Oct. 15–18, 2012

ISHI

INTERNATIONAL SYMPOSIUM ON HUMAN IDENTIFICATION

MIXTURE INTERPRETATION WORKSHOP

Mixtures Using *SOUND* Statistics, Interpretation & Conclusions

23rd International Symposium on Human Identification
October 15, 2012 (Nashville, TN)

Presenters

John M. Butler, PhD	NIST, Applied Genetics Group
Michael D. Coble, PhD	NIST, Applied Genetics Group
Robin W. Cotton, PhD	Boston University, Biomedical Forensic Sciences
Catherine M. Grgicak, PhD	Boston University, Biomedical Forensic Sciences
Charlotte J. Word, PhD	Consultant

- 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

Additional Resources

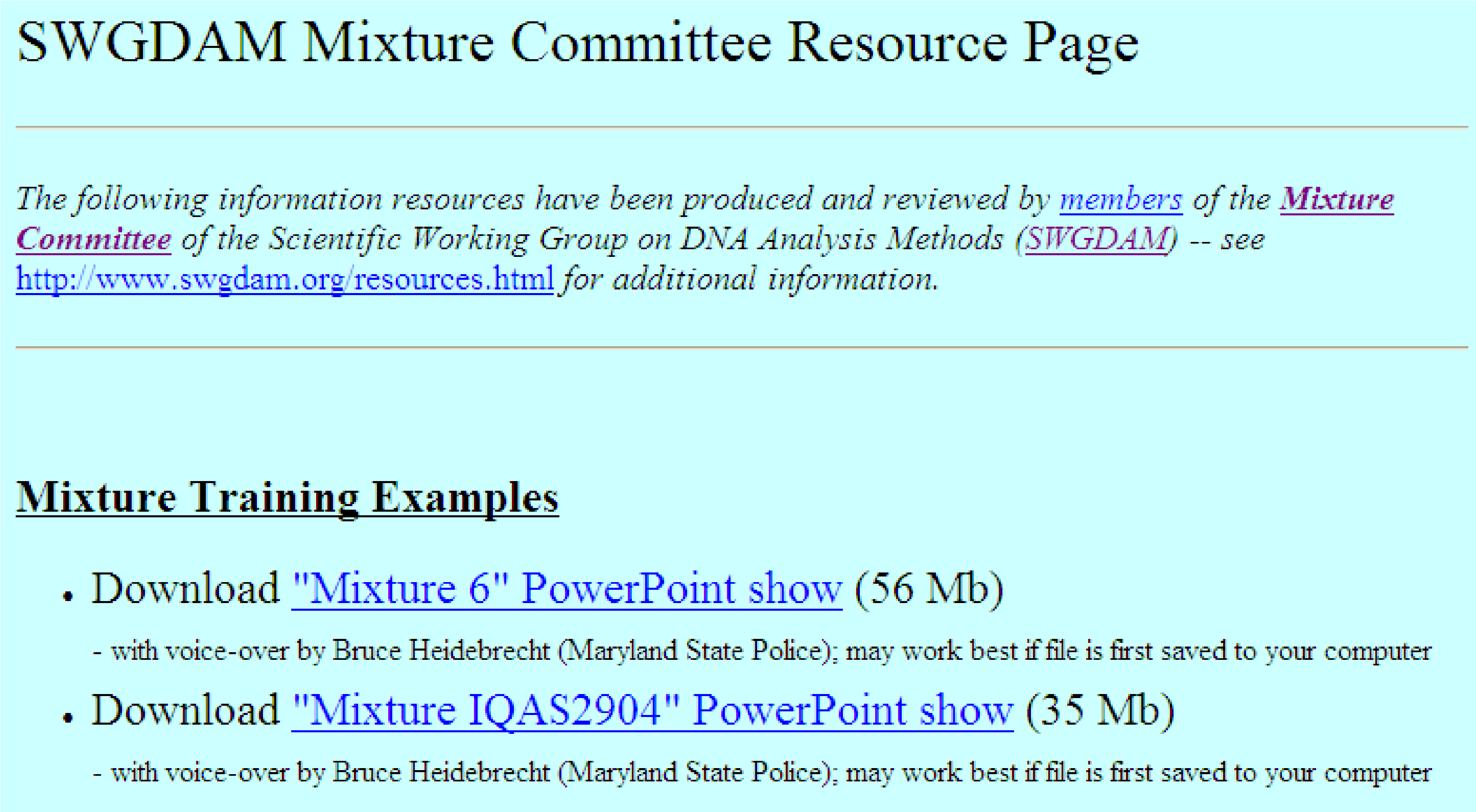
Beginning with the development or/and revision of its next draft guidance document(s), SWGDAM will make a "Draft for Comment" or other work product available for the purpose of receiving comments from the general public. This "Draft for Comment" solicitation will be open for a minimum of 60 days, usually 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

Next Issue of FSI-Genetics

Forensic Science International: Genetics xxx (2012) xxx–xxx

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

Article in press…

Contents lists available at SciVerse ScienceDirect

Forensic Science International: Genetics

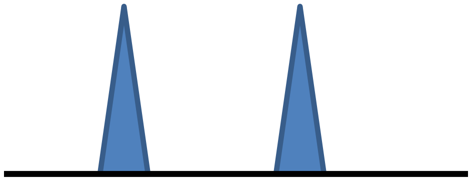
journal homepage: www.elsevier.com/locate/fsig

ELSEVIER

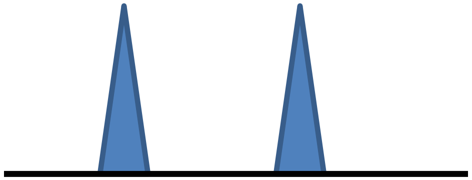
DNA commission of the International Society of Forensic Genetics: Recommendations on the evaluation of STR typing results that may include drop-out and/or drop-in using probabilistic methods

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]

Suspect

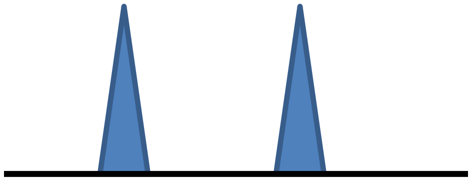


Evidence



$$LR = \frac{1}{2pq}$$

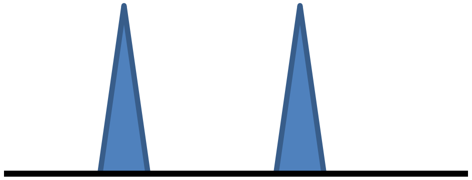
Suspect



Evidence

$$LR = \frac{0}{2pq}$$

Suspect



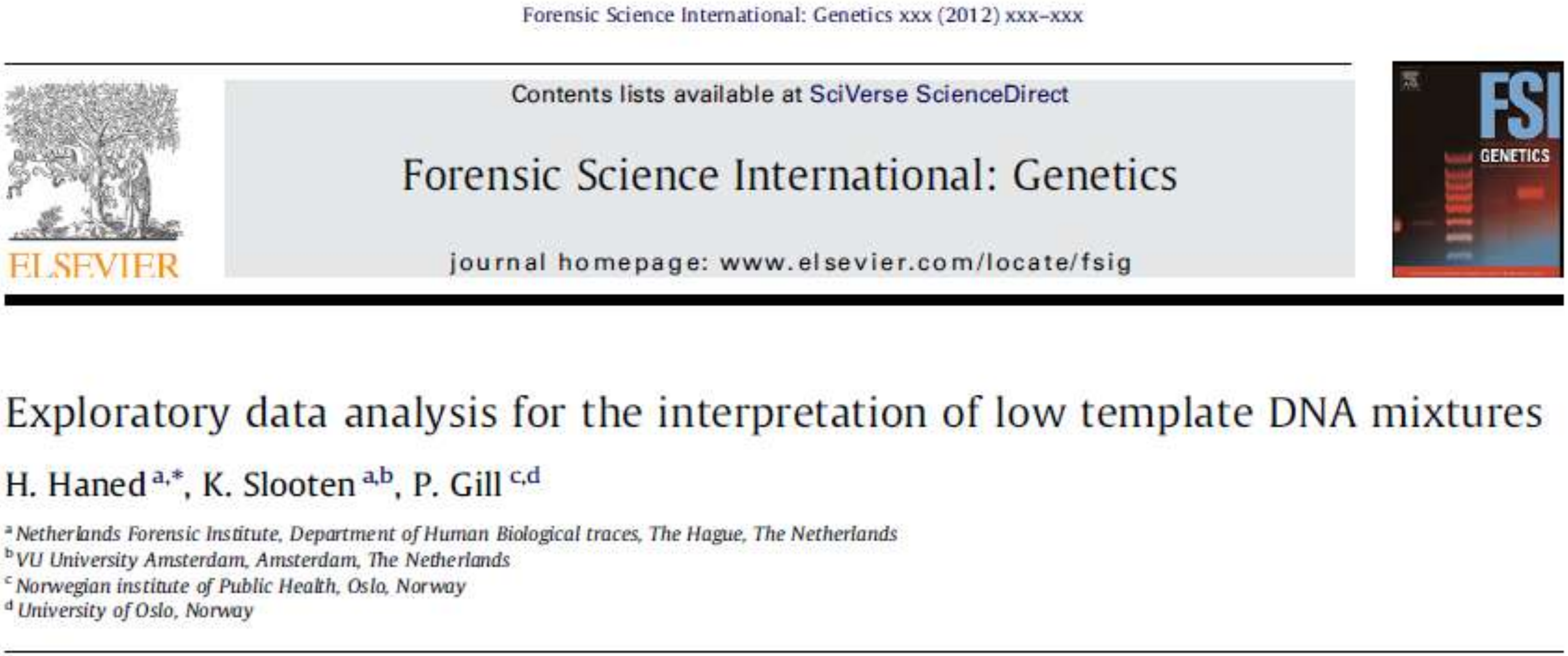
Evidence

$$LR = \frac{?}{2pq}$$

“2p”

$p^2 + 2p(1 - p)$

Haned *et al.*



Forensic Science International: Genetics xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Forensic Science International: Genetics

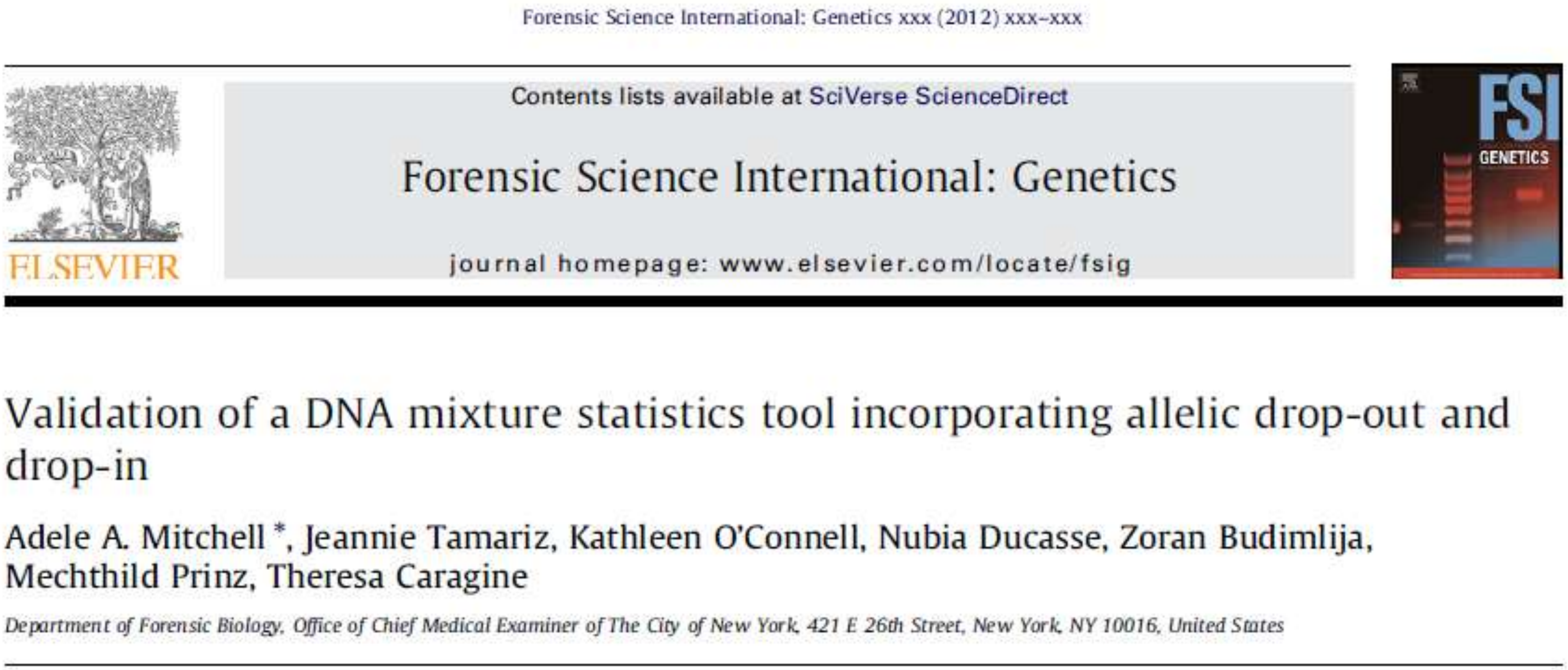
journal homepage: www.elsevier.com/locate/fsig

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*

Mitchell *et al.*



Forensic Science International: Genetics xxx (2012) xxx–xxx

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:

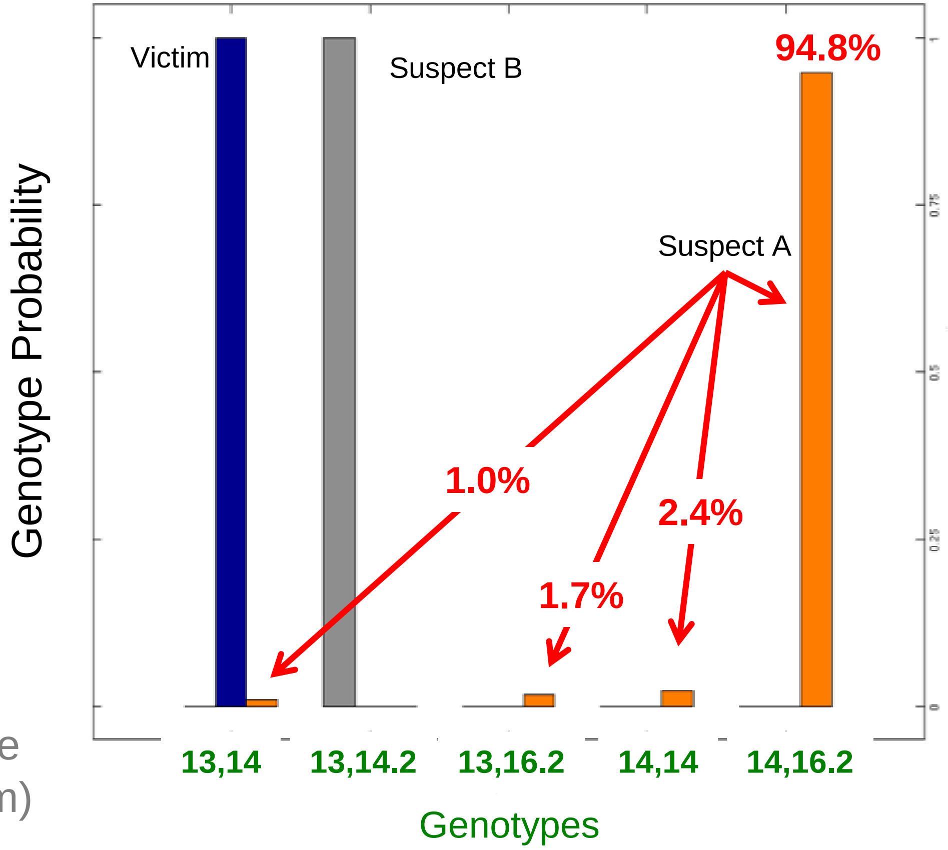
- Exploring the capabilities and limitations of a probabilistic genotyping approach
- Studying TrueAllele software with a number of different types of mixtures (including low-level and 3-4 person mixtures)
- Work being performed at NIST independently of Cybergenetics

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

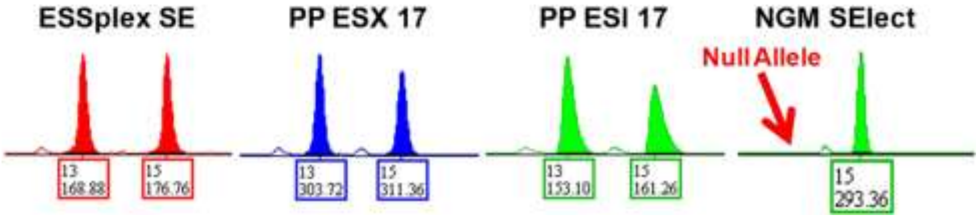


See also Perlin et al. (2011) Validating TrueAllele DNA mixture interpretation. *J. Forensic Sci.* 56(6):1430-1447

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

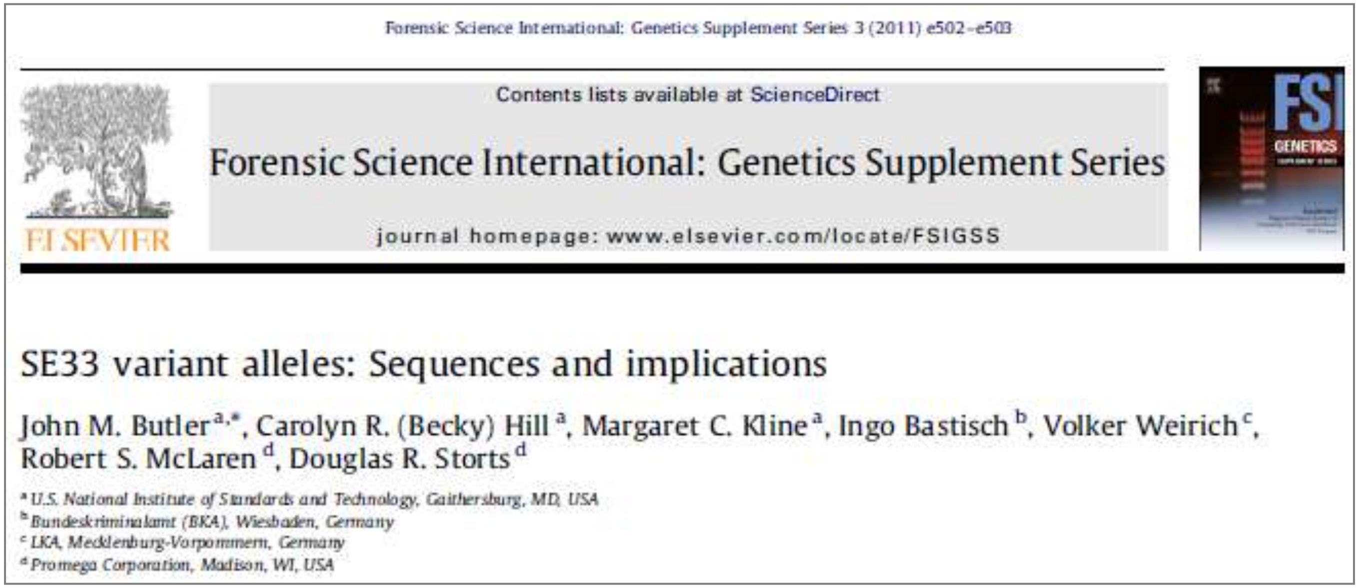
Concordance testing comparing STR multiplex kits with a standard data set

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

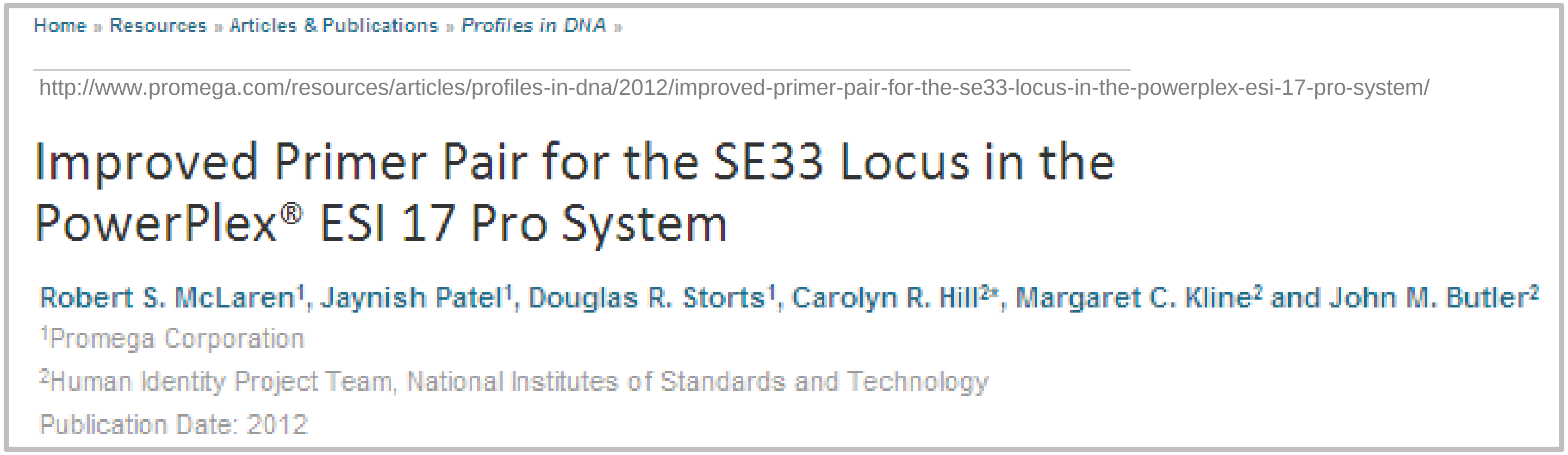
Forensic Science International: Genetics Supplement Series

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]

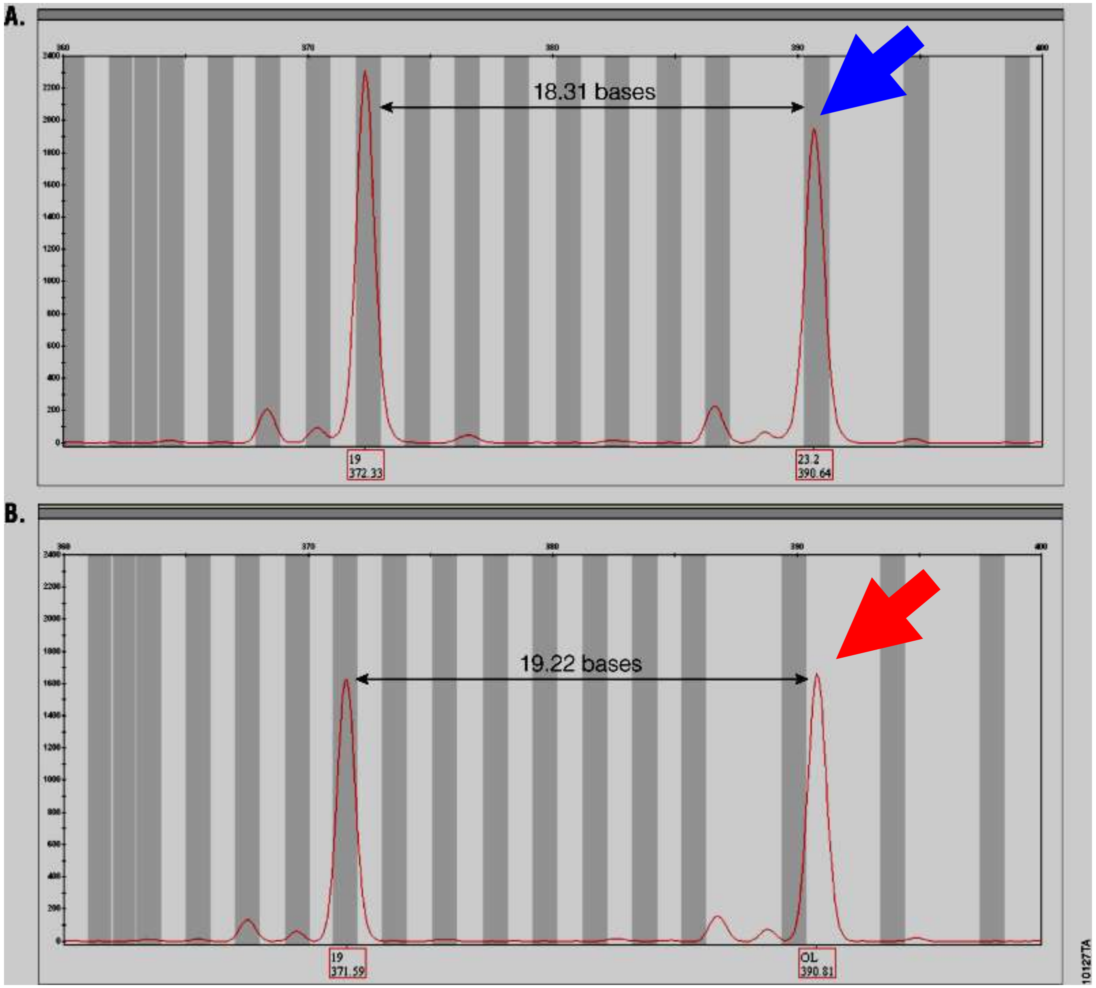
[1]Promega Corporation

[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/

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

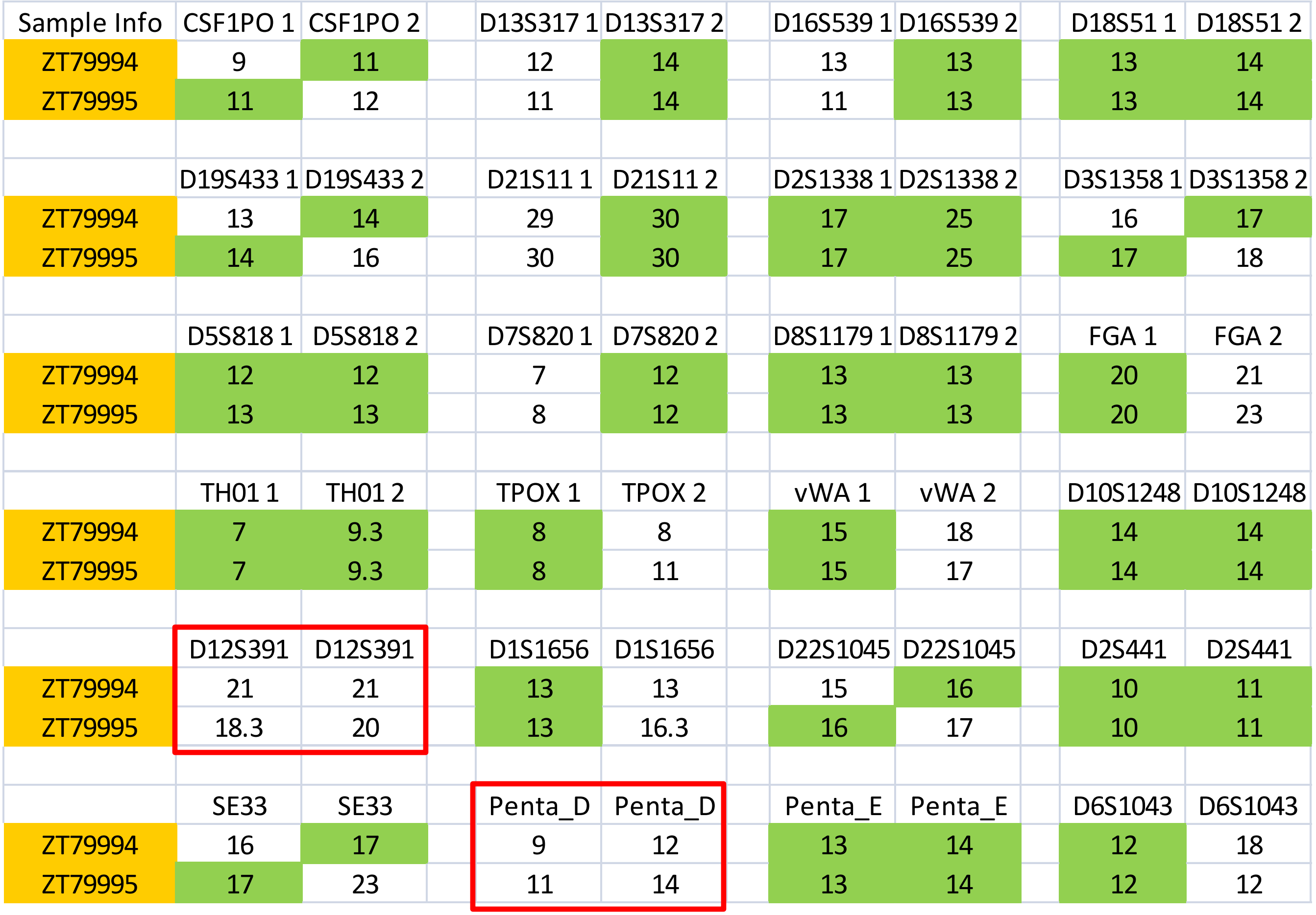
Two Samples from the Population Plates ZT79994 and ZT79995

Match exactly over 23 Y-STRs

ZT79994	**Pos**	**Seq**	**ZT79995**	**Pos**	**Seq**
	16172	**T - C**		**16172**	**T - C**
	16219	**A - G**		**16219**	**A - G**
	16311	**T - C**		**16311**	**T - C**
	16344	**C -T**		**16344**	**C -T**
	73	**A -G**		**73**	**A -G**
	263	**A- G**		**263**	**A- G**
	315.1	**C**		**315.1**	**C**
	469	**C - T**		**469**	**C - T**
	523	**A-del**		**523**	**A-del**
	524	**C-del**		**524**	**C-del**

Same mtDNA haplotype (U6a)

Autosomal STR Analysis



Sample Info	CSF1PO 1	CSF1PO 2		D13S317 1	D13S317 2		D16S539 1	D16S539 2		D18S51 1	D18S51 2
ZT79994	9	11		12	14		13	13		13	14
ZT79995	11	12		11	14		11	13		13	14
	D19S433 1	D19S433 2		D21S11 1	D21S11 2		D2S1338 1	D2S1338 2		D3S1358 1	D3S1358 2
ZT79994	13	14		29	30		17	25		16	17
ZT79995	14	16		30	30		17	25		17	18
	D5S818 1	D5S818 2		D7S820 1	D7S820 2		D8S1179 1	D8S1179 2		FGA 1	FGA 2
ZT79994	12	12		7	12		13	13		20	21
ZT79995	13	13		8	12		13	13		20	23
	TH01 1	TH01 2		TPOX 1	TPOX 2		vWA 1	vWA 2		D10S1248	D10S1248
ZT79994	7	9.3		8	8		15	18		14	14
ZT79995	7	9.3		8	11		15	17		14	14
	D12S391	D12S391		D1S1656	D1S1656		D22S1045	D22S1045		D2S441	D2S441
ZT79994	21	21		13	13		15	16		10	11
ZT79995	18.3	20		13	16.3		16	17		10	11
	SE33	SE33		Penta_D	Penta_D		Penta_E	Penta_E		D6S1043	D6S1043
ZT79994	16	17		9	12		13	14		12	18
ZT79995	17	23		11	14		13	14		12	12

24 STR Markers

Kinship Analysis

	Combined
Parent-Child	0
Full Sib	**56327**
Half-Sib*	5692
1st Cousin	264

*Is also the same stat for Aunt/Uncle/Niece/Nephew and Grandparent/Grandchild relationships

Removed ZT79995 from final data set

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 has collected and characterized U.S. population data on new loci 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

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

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

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

Probability of Identity Values

for Various STR Kits or Locus Combinations

based on NIST 1036 U.S. Population Samples

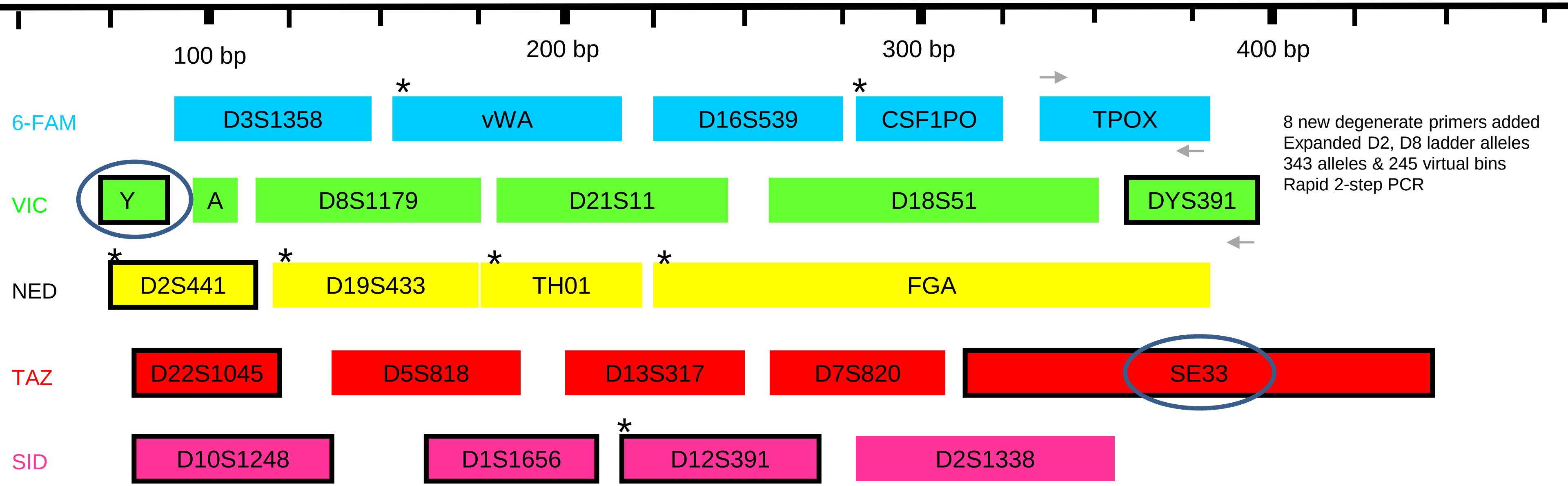
STR Kit or Core Set of Loci	Total N=1036	Caucasians (n=361)	African Am. (n=342)	Hispanics (n=236)	Asians (n=97)
CODIS 13	5.02E-16	2.97E-15	1.14E-15	1.36E-15	1.71E-14
Identifiler	6.18E-19	6.87E-18	1.04E-18	2.73E-18	5.31E-17
PowerPlex 16	2.82E-19	4.24E-18	6.09E-19	1.26E-18	2.55E-17
PowerPlex 18D	3.47E-22	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 (in press)*

STR Kit Layouts by Dye Label and PCR Product Size

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

24plex



100 bp 200 bp 300 bp 400 bp

6-FAM: D3S1358, *vWA, D16S539, *CSF1PO, TPOX

VIC: Y, A, D8S1179, D21S11, D18S51, DYS391

NED: *D2S441, *D19S433, *TH01, *FGA

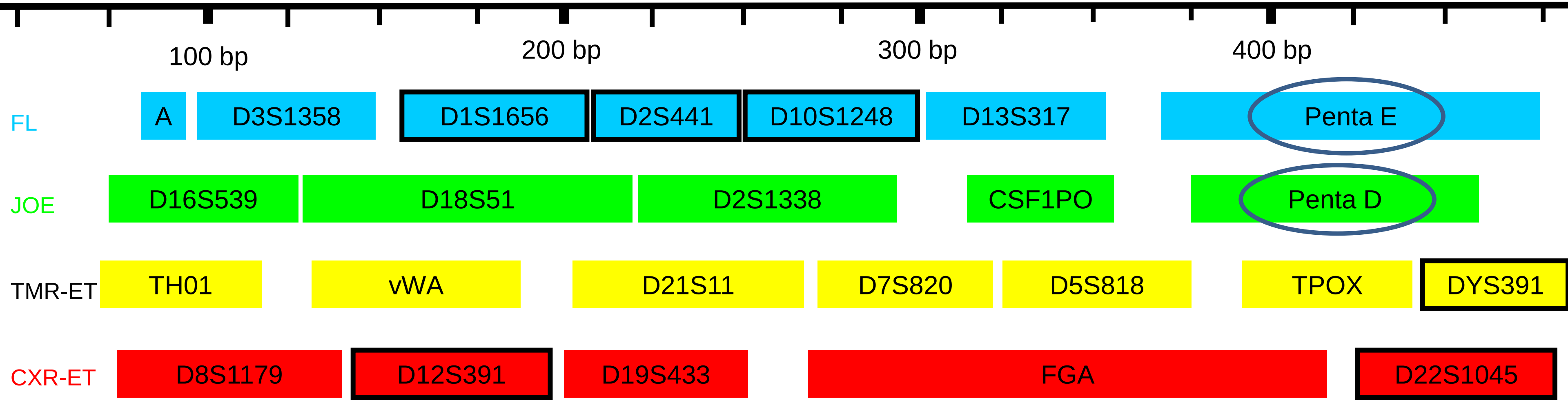
TAZ: D22S1045, D5S818, D13S317, D7S820, SE33

SID: D10S1248, D1S1656, *D12S391, D2S1338

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



100 bp 200 bp 300 bp 400 bp

FL: A, D3S1358, D1S1656, D2S441, D10S1248, D13S317, Penta E

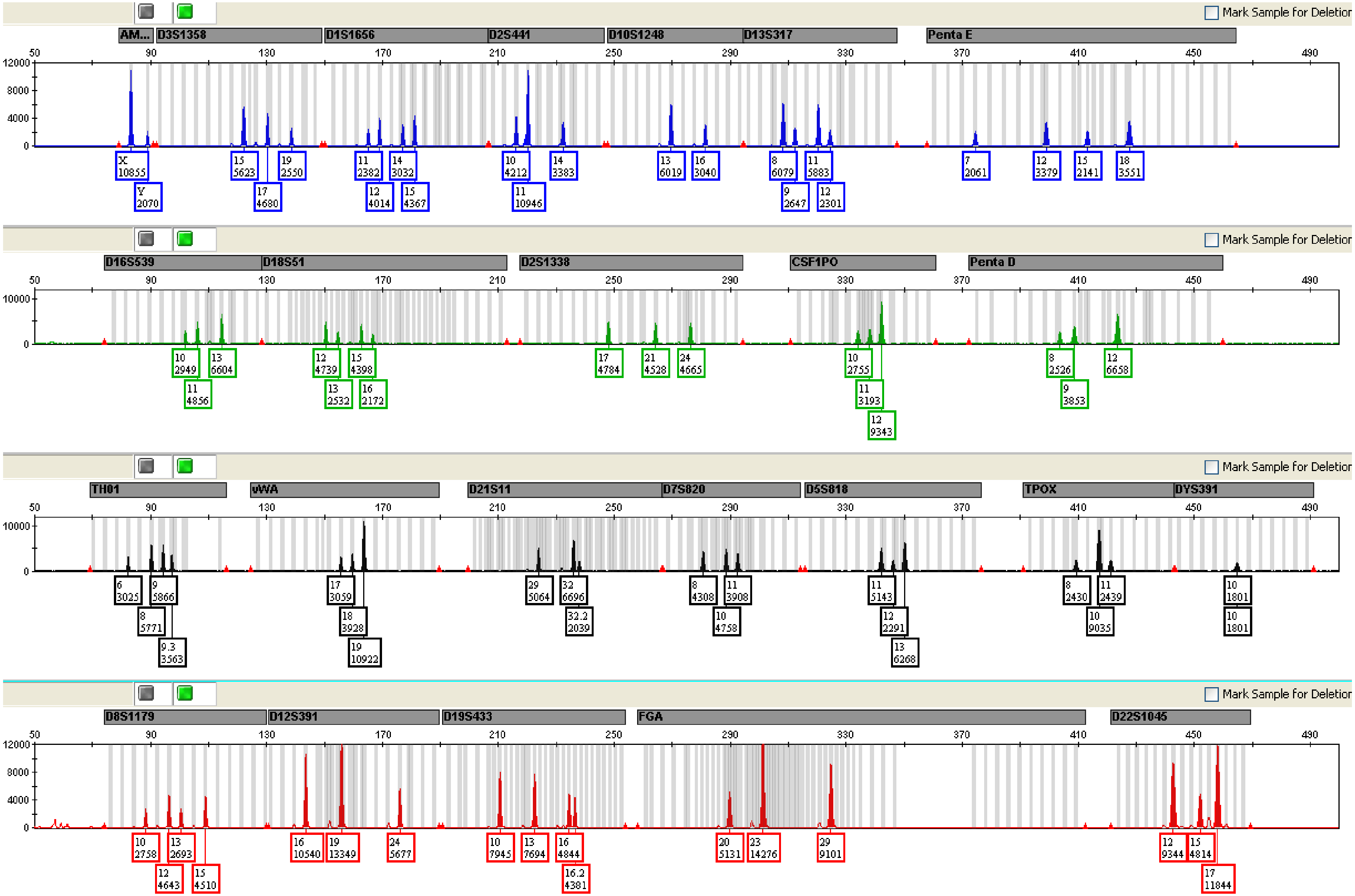
JOE: D16S539, D18S51, D2S1338, CSF1PO, Penta D

TMR-ET: TH01, vWA, D21S11, D7S820, D5S818, TPOX, DYS391

CXR-ET: D8S1179, D12S391, D19S433, FGA, D22S1045

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

24plex assay

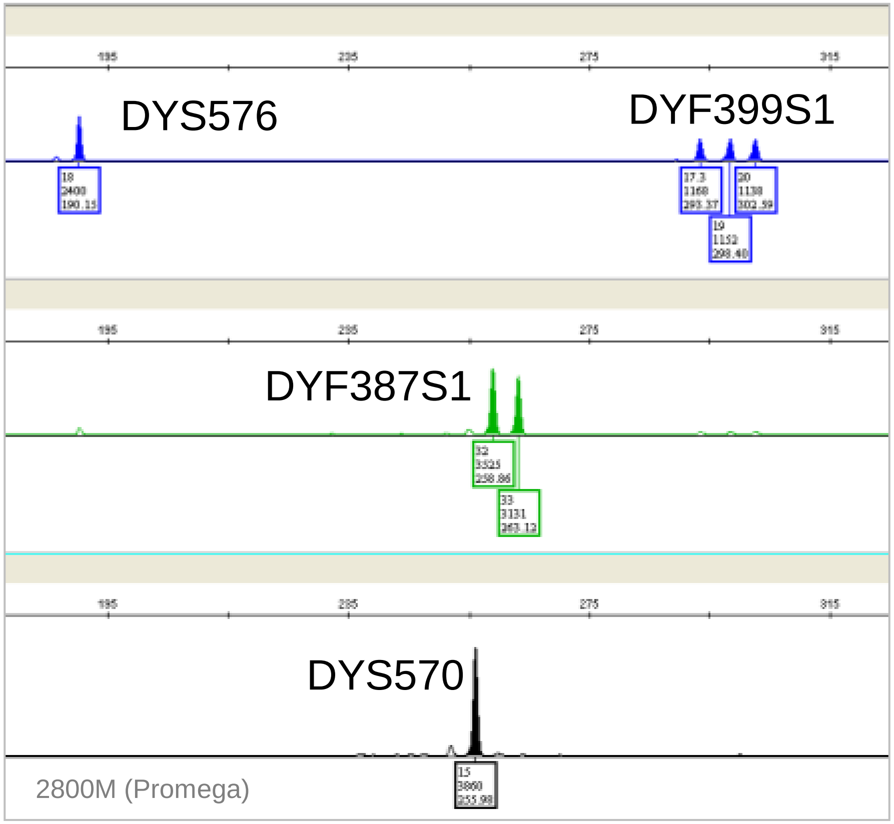


Rapidly Mutating Y-STR Loci


Mike Coble


Becky Hill

RM Y-STR multiplex 1



- 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

K. Ballantyne et al. 2010; K. Ballantyne et al. 2012

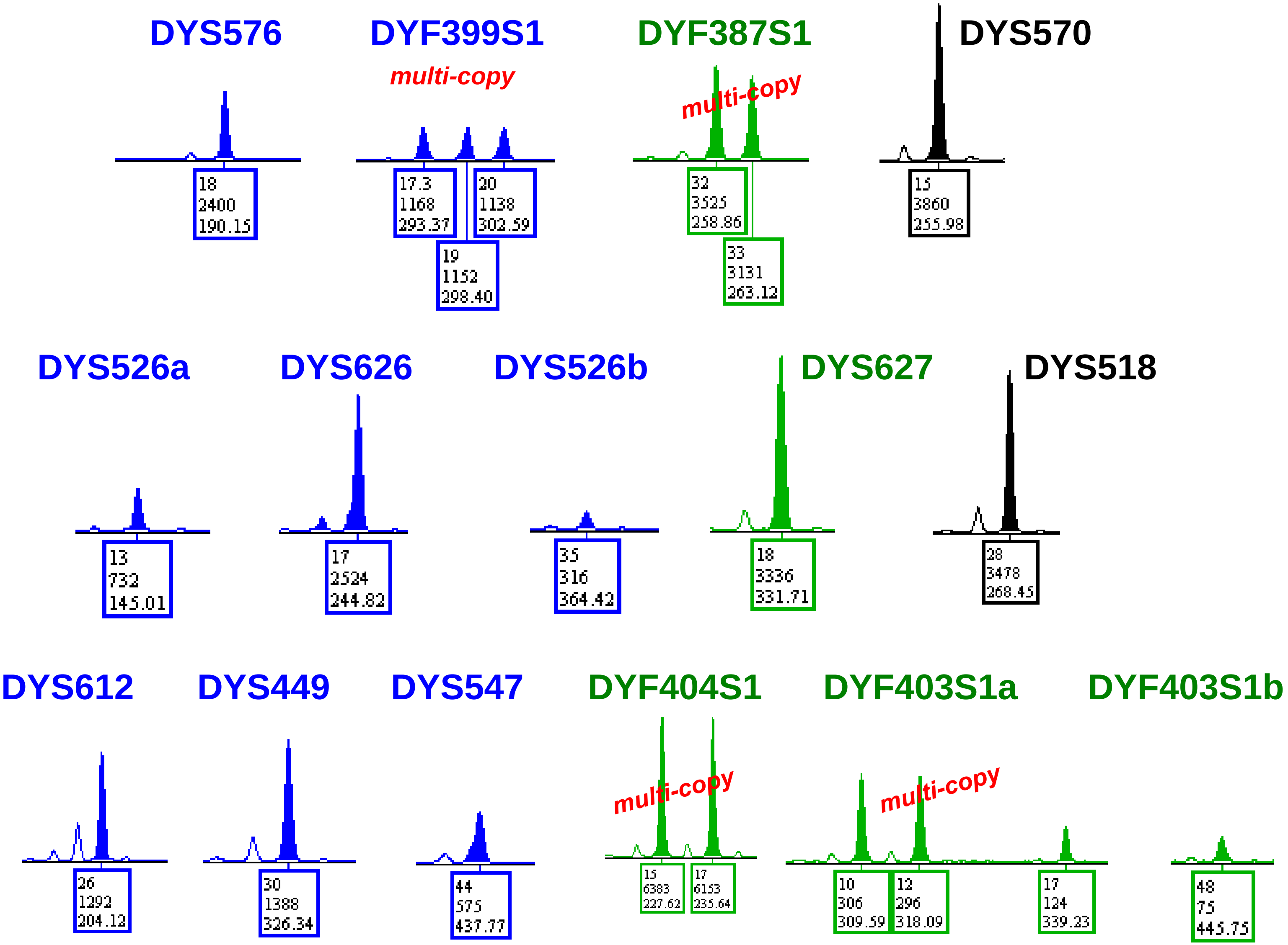
Rapidly Mutating (RM) Y-STRs

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

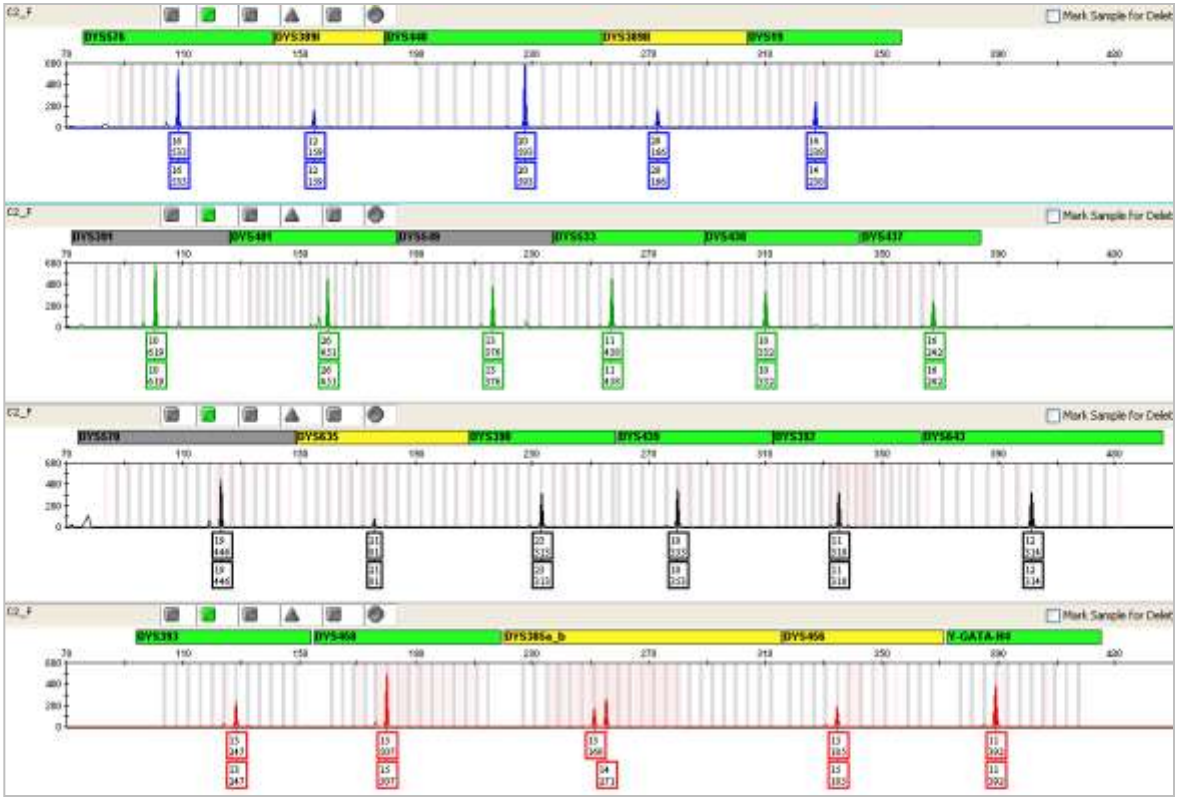


PowerPlex Y23 Kit

Mike Coble

Becky Hill

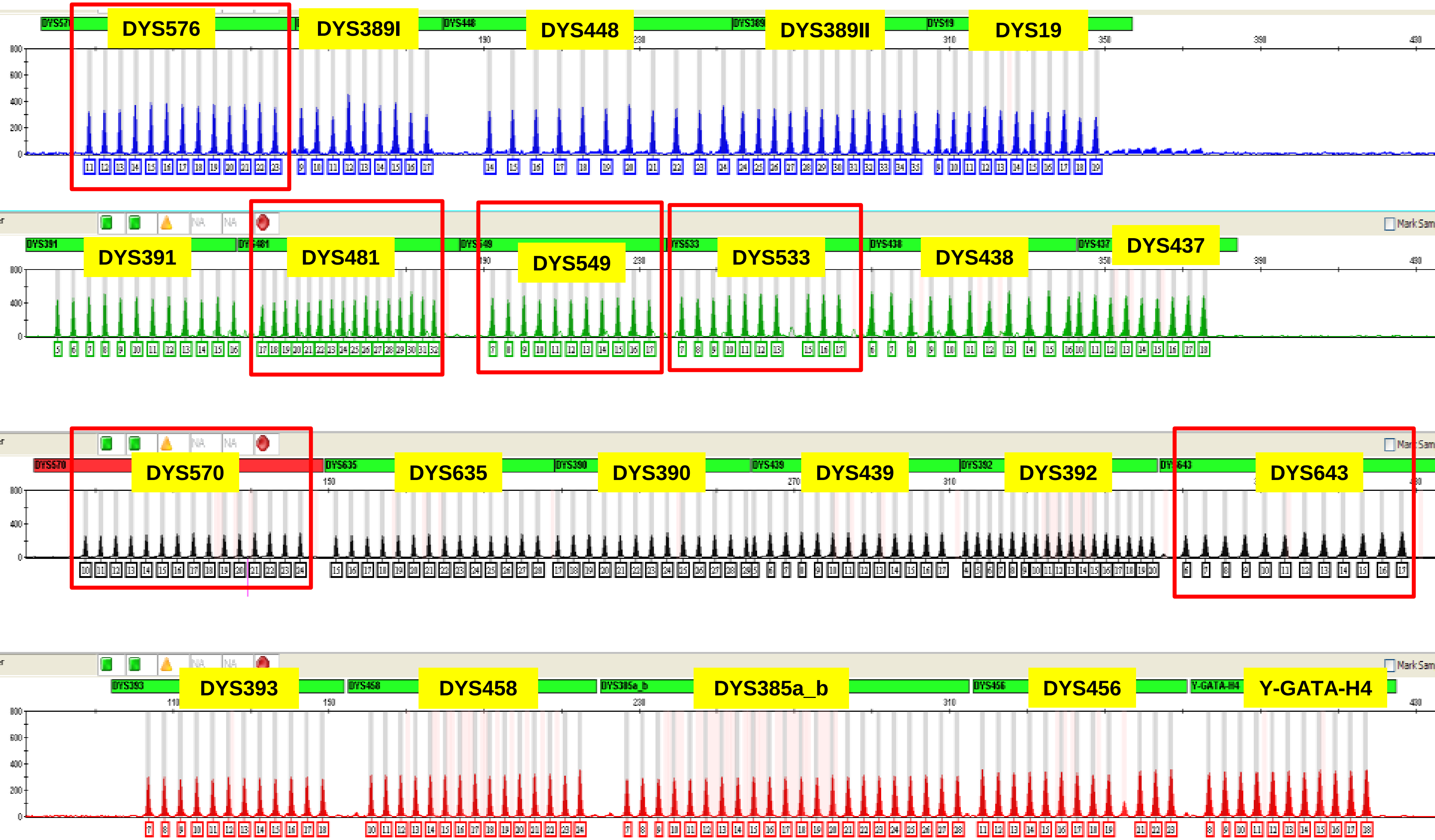
125pg male + 400ng female **(3200x female)**



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 release on STRBase

PowerPlex Y23 Allelic Ladders



23 Y-STRs = 17 Yfiler + 6 additional loci

	N = 1032 Combined Y-STR Haplotypes				
Number of times haplotype observed	**Minimal Haplotype** 9 Y-STRs	**SWGDAM** 11 Y-STRs	**PowerPlex Y** 12 Y-STRs	**Yfiler** 17 Y-STRs	**PowerPlex Y23** 23 Y-STRs
n=1 (singletons)	671	787	821	998	1026
n=2	56	47	41	12	3
n=3	20	13	16	2	·
n=4	9	6	6	1	·
n=5	3	3	2	·	·
n=6	2	1	2	·	·
n=7	·	3	1	·	·
n=8	3	1	·	·	·
n=9	1	·	1	·	·
n=12	1	·	·	·	·
n=13	1	·	·	·	·
n=14	1	·	·	·	·
n=15	·	1	·	·	·
n=19	·	·	1	·	·
n=20	1	·	·	·	·
n=23	·	1	·	·	·
n=34	1	·	·	·	·
Haplotype Diversity	0.99749	0.99884	0.99924	0.99995	0.99999
Match Probability	0.00348	0.00213	0.00173	0.00101	0.00097
MP as 1 in…	288	470	579	986	1026
Discrimination Capacity	0.74612	0.83624	0.86337	0.98159	0.99709

Coble et al poster at ISHI 2012

NIST Reference Materials for Forensic DNA Measurement Assurance

Margaret Kline

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.

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

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

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”

Forensic DNA Typing Textbook

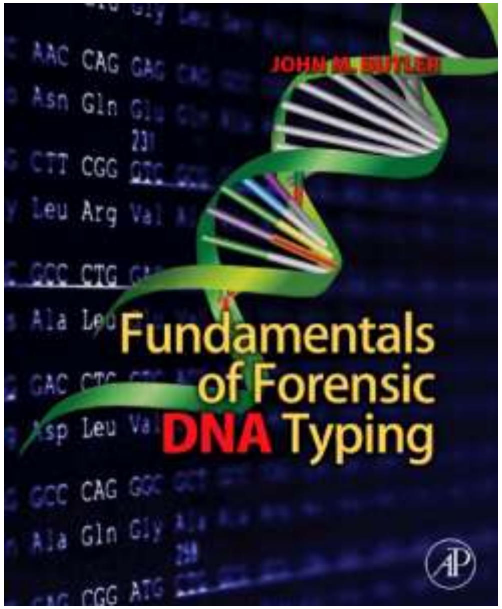
3rd Edition is Three Volumes

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

John Butler

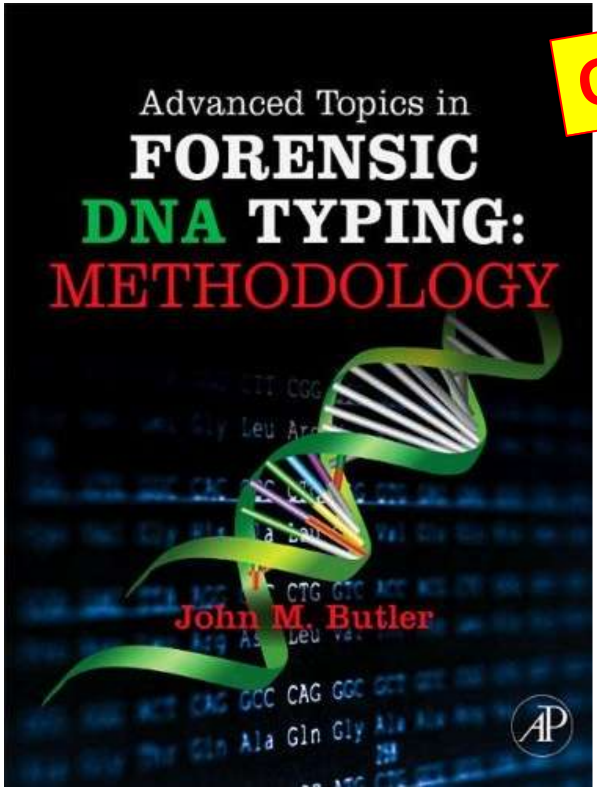
Currently being written

For beginning students, general public, & lawyers



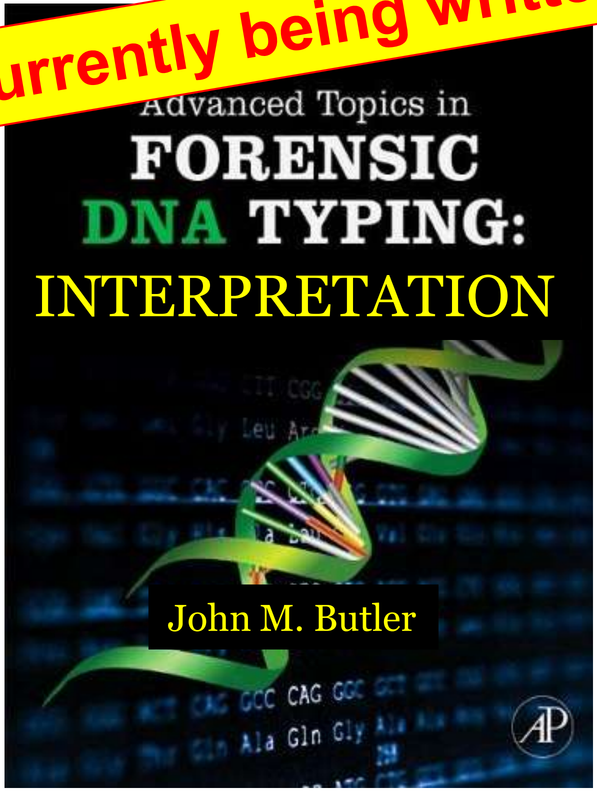
Fall 2009

~500 pages



Fall 2011

~700 pages



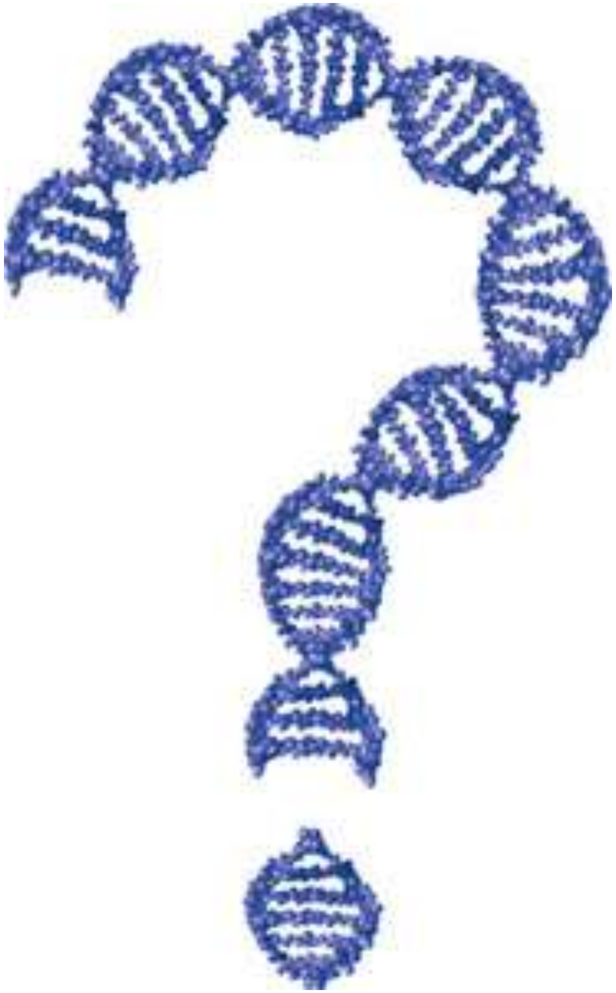
Fall 2013

~500 pages

Thank you for your attention

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

CAC Organizing Committee
Dr. Steve Lee (SJSU)



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