Bringing traceability and technology to the scales of justice…

NIJ-Funded Research in Forensic DNA at the National Institute of Standards and Technology

John M. Butler

Group Leader, Applied Genetics

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

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

Office Manager
Patti Rohmiller

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

Current Activities at NIST
Enabled by Our NIJ Partnership

- **Standard Reference Materials** **STRBase website**
  - SRM 2372 (DNA quant)
  - **SRM 2391c** (STRs), 2395 (Y-STRs), 2392 (mtDNA)

- **Technology Evaluation and Development**
  - Rapid multiplex PCR protocols* (PCR: 3 hr to <20 min; ***now FBI-funded***)
  - Low-level DNA studies and kinship analysis
  - Mixture interpretation – research and training materials
  - Variant STR allele characterization
  - New STR loci & assays (STR 26plex, SNP testing) and kit concordance
  - U.S. population data (24 autosomal STR loci)
  - Y-chromosome characterization (mutation rates, deletions, nomenclature)

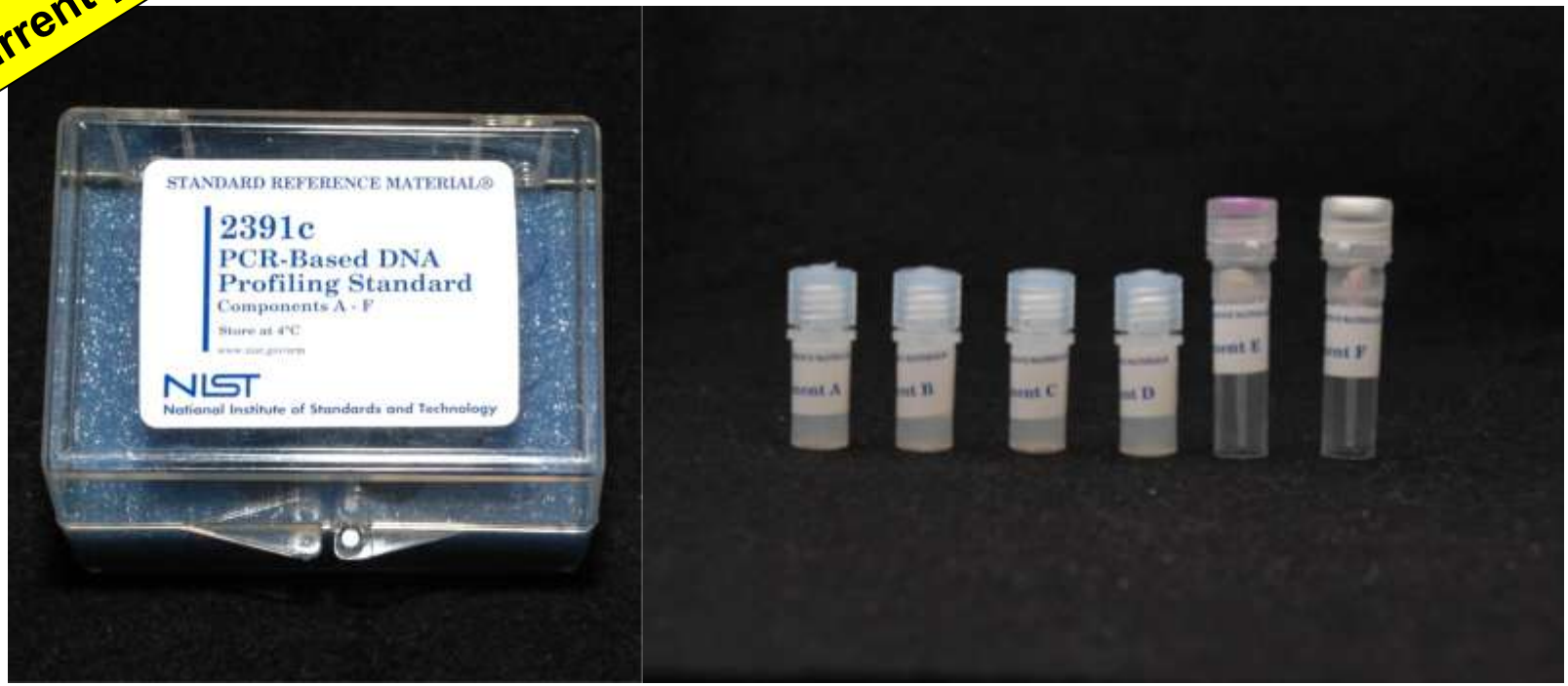
- **Training Materials**
  - AAFS, ISHI, ISFG & lab workshops on mixture interpretation, CE, etc.
  - Third edition (3 volumes) of *Forensic DNA Typing* textbook

Selling since
Aug 16, 2011
Current price: $626

NIST SRM 2391c


Margaret Kline


Becky Hill



Produced with an entirely new set of genomic DNA samples

9947A & 9948 are NOT included.

https://www-s.nist.gov/srmors/view_detail.cfm?srm=2391C

NIST Standard Reference Material (SRM) for Forensic DNA Testing

SRM 2391b (2003-2011)

- **48 autosomal STR loci** with certified values
- **10 liquid genomic DNA** components + **2 punches** (cells on 903 paper)
- All single source samples
- 4 males + 6 females
- 9947A & 9948 included

SRM 2391c (2011-future)

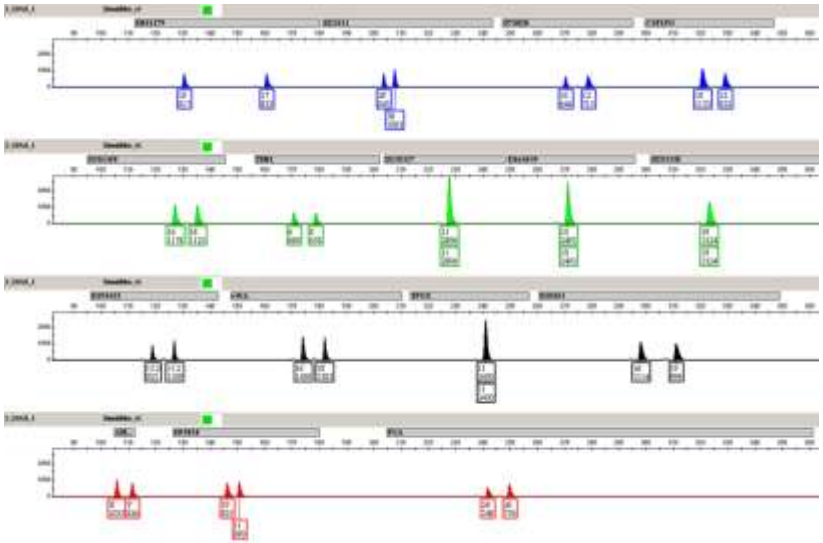
- **23 autosomal STR loci and 17 Y-STRs** certified
- **4 liquid genomic DNA** components + **2 punches** (cells on **FTA** & 903 paper)
- 5 single source + 1 mixture
- 3 males + 2 females (unique)
- All new samples
  - no 9947A or 9948

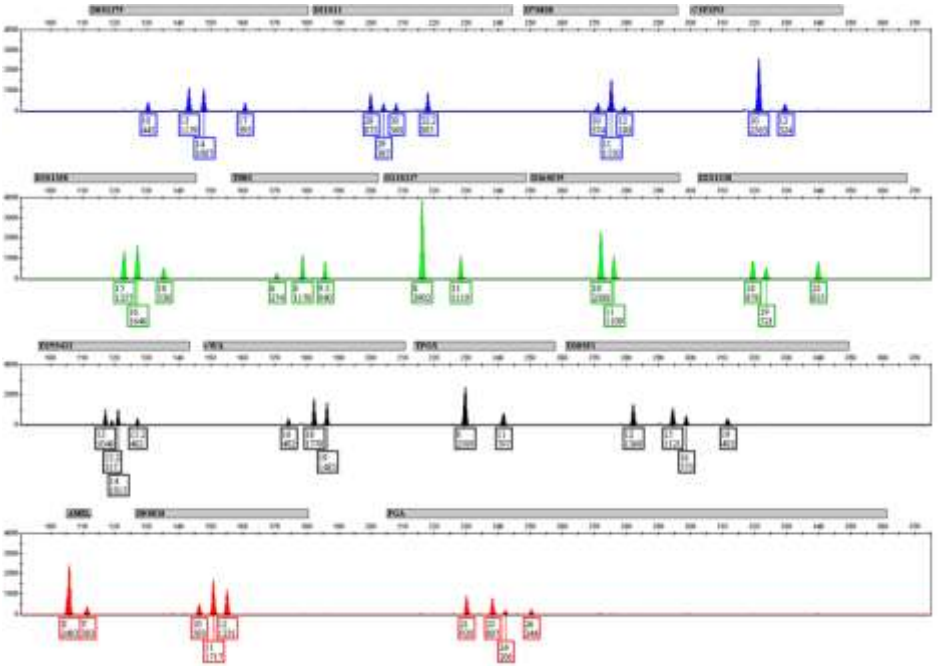
SRM 2391c to replace SRM 2391b and SRM 2395 (for Y-STRs)

Component D

3 part A

1 part C





The certified ratio for Component D, the mass of Component A relative to that of Component C, is **3.1 ± 0.1 Component A / Component C.**

STR Genotyping kits and primer mixes used at NIST to certify SRM 2391c

Kit Provider			Primer Mixes
Life Technologies	*Promega*	*Qiagen*	*NIST*
Identifiler	Powerplex 16	ESSplex	26plex
Identifiler Plus	Powerplex 16 HS	IDplex	miniSTRs
NGM	Powerplex ESX 17		
NGM SElect	Powerplex ESI 17		
COfiler	Powerplex ES		
Profiler	Powerplex S5		
Profiler Plus	**Powerplex Y**		
Profiler Plus ID	FFFL		
SGM Plus			
SEfiler			
MiniFiler			
Yfiler			

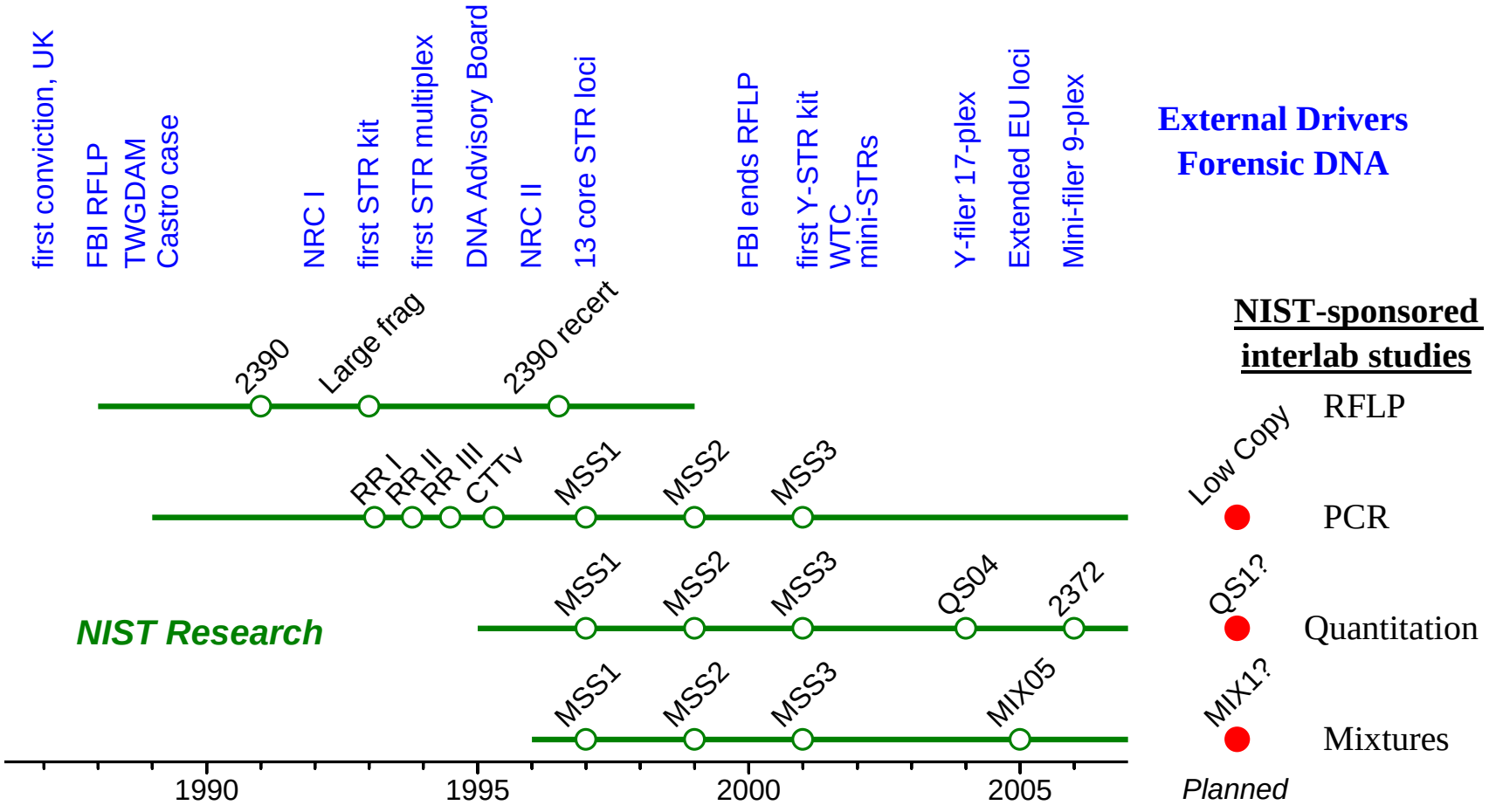
All results are concordant across all kits.

In total there is data for 51 autosomal STRs and 17 Y-STRs

NIST-Sponsored Interlab Studies


Margaret Kline


Dave Duewer



13 interlaboratory studies conducted over the past 20 years

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)

Contents lists available at ScienceDirect

Forensic Science International: Genetics

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

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

STR Kit Concordance Testing

Becky Hill

Main Points:

- When different primer sets are utilized, there is a concern that allele dropout may occur due to primer binding site mutations that impact one set of primers but not another
- To test SRM 2391b/2391c (PCR-based DNA Profiling Standard) components with all new STR multiplex kits and verify results against certified reference values
- To gain a better understanding of primer binding site mutations that cause null alleles

If no primer binding site mutations

Set 1 Amplicons Set 2 Amplicons

If a primer binding site mutation exists

Set 1 Amplicons Set 2 Amplicons

Presentations/Publications:

- *Profiles in DNA* article (Hill et al. 2010)
- ISFG 2011 and ISHI 2011 posters (Hill et al.)

Commercially Available STR Kits

Applied Biosystems (17)

- ~~AmpFlSTR Blue (1996)~~
- ~~AmpFlSTR Green I (1997)~~
- Profiler (1997)
- Profiler Plus (1997)
- COfiler (1998)
- SGM Plus (1999)
- **Identifiler** (2001)
- Profiler Plus ID (2001)
- ~~SEfiler (2002)~~
- Yfiler (2004)
- MiniFiler (2007)
- SEfiler Plus (2007)
- Sinofiler (2008) – China only
- **Identifiler Direct** (2009)
- NGM (2009)
- **Identifiler Plus** (2010)
- NGM SElect (2010)

Promega Corporation (15)

- PowerPlex 1.1 (1997)
- PowerPlex 1.2 (1998)
- PowerPlex 2.1 (1999)
- **PowerPlex 16** (2000)
- PowerPlex ES (2002)
- PowerPlex Y (2003)
- PowerPlex S5 (2007)
- **PowerPlex 16 HS** (2009)
- PowerPlex ESX 16 (2009)
- PowerPlex ESX 17 (2009)
- PowerPlex ESI 16 (2009)
- PowerPlex ESI 17 (2009)
- PowerPlex 18D (2011)
- PowerPlex 21 (2012)
- PowerPlex ESI 17 Pro (2012)

Qiagen (2010)

Primarily selling kits in Europe

Due to patent restrictions cannot sell in U.S.

- ESSplex
- ESSplex SE
- Decaplex SE
- IDplex
- Nonaplex ESS
- Hexaplex ESS
- HD (Chimera)
- Argus X-12
- Argus Y-12
- DIPlex (30 InDels)

~1/3 of all STR kits were released in the last three years

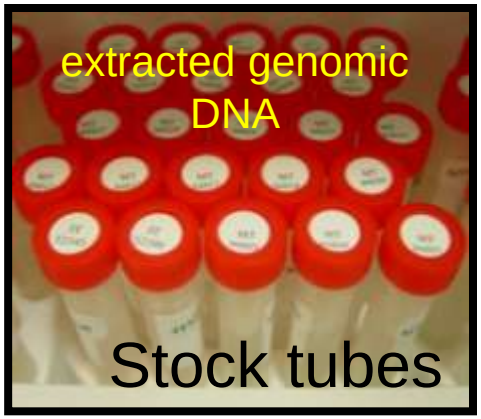
NIST Standard Sample Sets

- **U.S. Population Samples** (**663 samples**)
  - Previously studied with Identifiler, MiniFiler, Yfiler, PP16, PP ESX/ESI 17, NGM, miniSTRs, and 23plex *(>200,000 allele calls)*
  - 260 African Americans, 260 Caucasians, 140 Hispanics, and 3 Asians

- **U.S. Father/Son pairs** (**800 samples**)
  - Previously studied with Identifiler, MiniFiler, Yfiler, PP ESX/ESI 17, NGM, 23plex
  - ~**100 fathers/100 sons for each group**: African Americans, Caucasians, Hispanics, and Asians

- **NIST SRM 2391b** PCR DNA Profiling Standard (**12 samples**)
  - Components 1-10 (includes 9947A and 9948): *well characterized*
  - ABI 007 and K562

>1450 total samples



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

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 24 commonly used autosomal STR loci

Presentations/Publications:

- AAFS 2011 presentation
- 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

Loci sorted on Probability of Identity (P_I) values

STR Locus	Alleles Observed	Genotypes Observed	Het. (obs)	P_I value N = 938
SE33	53	292	0.9360	0.0069
Penta E*	20	114	0.8799	0.0177
D2S1338	13	68	0.8785	0.0219
D1S1656	15	92	0.8934	0.0220
D18S51	21	91	0.8689	0.0256
D12S391	23	110	0.8795	0.0257
FGA	26	93	0.8742	0.0299
D6S1043*	25	91	0.8627	0.0343
Penta D*	16	71	0.8754	0.0356
D21S11	25	81	0.8358	0.0410
D19S433	16	76	0.8124	0.0561
D8S1179	11	45	0.7878	0.0582
vWA	11	38	0.8060	0.0622
D7S820	11	32	0.8070	0.0734
TH01	8	24	0.7580	0.0784
D16S539	9	28	0.7825	0.0784
D13S317	8	29	0.7655	0.0812
D10S1248	12	39	0.7825	0.0837
D2S441	14	41	0.7772	0.0855
D3S1358	11	30	0.7569	0.0873
D22S1045	11	42	0.7697	0.0933
CSF1PO	9	30	0.7537	0.1071
D5S818	9	34	0.7164	0.1192
TPOX	9	28	0.6983	0.1283

24 STR Loci in STR kits rank ordered by their variability

Better for mixtures (more alleles seen)

There are several loci more polymorphic than the current CODIS 13 STRs

Better for kinship (low mutation rate)

TrueAllele Mixture Software Evaluation

Mike Coble

Main Points:

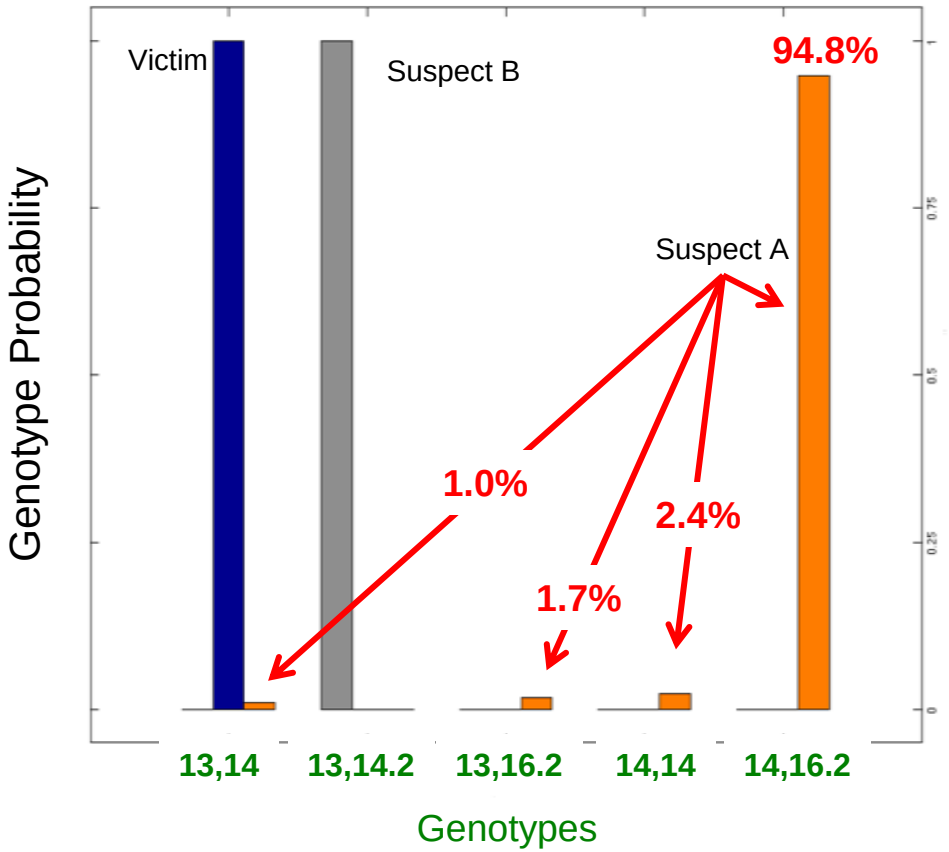
- Exploring the capabilities and limitations of a probabilistic genotyping approach
- Studying TrueAllele software with a number of different types of mixtures (including low-level and 3-4 person mixtures)
- Work being performed at NIST independently of Cybergenetics

Presentations/Publications:

- ISFG 2011 presentation
- ISHI 2011 mixture workshop

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

2011 Training Workshops


John Butler


Mike Coble

- AAFS (February 22, 2011)
  - **Mixture Interpretation (with 6 other speakers)**
- ISFG (August 30, 2011)
  - **CE Fundamentals and Troubleshooting**
- Int. Symp. Human Ident. (October 3, 2011)
  - **Mixture Interpretation (with Boston University)**
- Int. Symp. Human Ident. (October 6, 2011)
  - **Troubleshooting Laboratory Systems (with Bruce McCord, FIU)**

Slide handouts available at
http://www.cstl.nist.gov/strbase/training.htm

2012 Training Workshops


John Butler


Mike Coble

- New York/New Jersey DNA analysts (April 18, 2012)
  - **Mixture Interpretation, STRs & CE, Y-STRs & mtDNA**
- Canadian Society of Forensic Science (May 8, 2012)
  - **Mixture Interpretation & Statistics**
- FBI Laboratory DNA Units (May 24, 2012)
  - **NIST Research Projects**
- Taiwan DNA analysts (June 6-7, 2012)
  - **Mixture Interpretation, STRs & CE, Y-STRs & mtDNA**

Slide handouts available at
http://www.cstl.nist.gov/strbase/training.htm

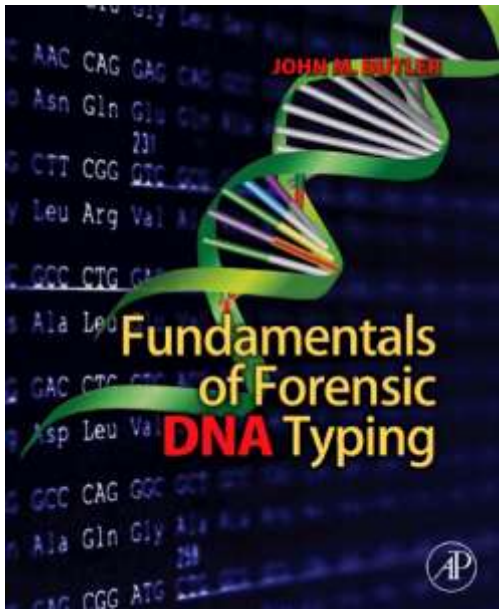
Forensic DNA Typing Textbook

3rd Edition is Three Volumes

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

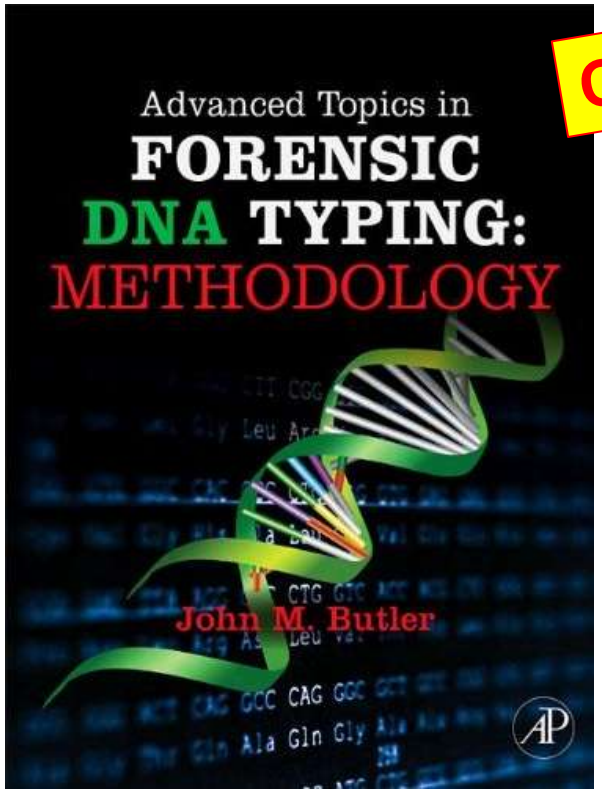
John Butler

For beginning students, general public, & lawyers



Sept 2009

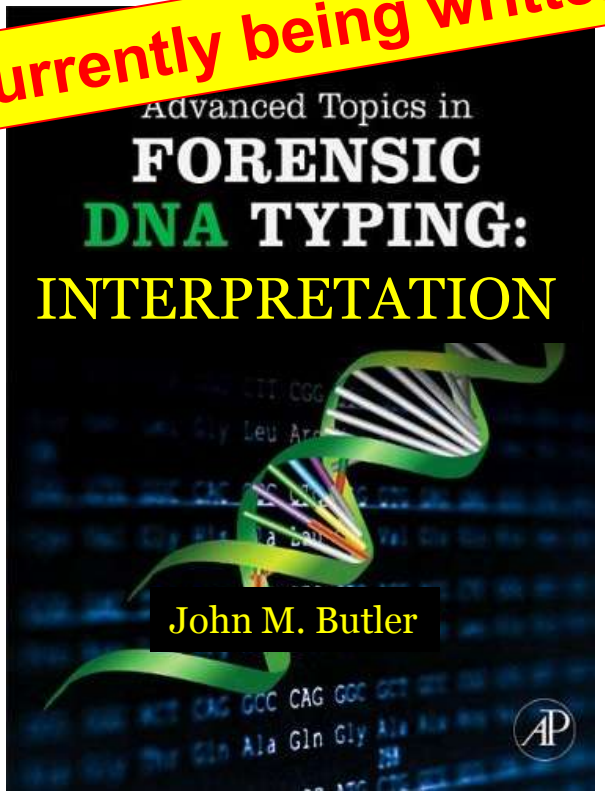
~500 pages



August 2011

~700 pages

Currently being written



2013

~500 pages

Our FY2011 Group Productivity

(Oct 2010 to Sept 2011)

- **21 publications**
  - 20 articles + 1 book
- **77 presentations**
  - 65 talks (58 invited) + 12 posters (all available on STRBase)
- **10 training workshops**
  - Mixture interpretation (ISHI, AAFS, NFSTC, IN, HI, AZ, MI, Palm Beach, Houston)
  - Capillary electrophoresis (ISFG)
- **3 Standard Reference Materials (SRMs) completed**
  - 2391c (forensic STRs), 2393 (HD), 2366 (CMV)
- **10 committee assignments**
  - VA SAC, DOD DNA oversight, FBI new CODIS core loci, SWGDAM (mixture interpretation, rapid DNA, enhanced detection methods), NIST/NIJ evidence preservation TWG, JCTLM, NIJ DNA TWG, ATCC cell line authentication

Some Recent Publications from Our Group

See full listing at **http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm**

- Butler, J.M. (2012) *Advanced Topics in Forensic DNA Typing: Methodology*. Elsevier Academic Press: San Diego.

- Butler, J.M. and Hill, C.R. (2012) Biology and genetics of new autosomal STR loci useful for forensic DNA analysis. *Forensic Sci. Rev.* 24(1): 15-26.

- Coble, M.D., Kline, M.C., Butler, J.M. (2011) Metrology needs and NIST resources for the forensic DNA community. *Accred. Qual. Assur.* 16: 293-297.

- Coble, M.D. (2011) The identification of the Romanovs: can we (finally) put the controversies to rest? *Investigative Genetics* 2: 20.

- Hill, C.R., Duewer, D.L., Kline, M.C., Sprecher, C.J., McLaren, R.S., Rabbach, D.R., Krenke, B.E., Ensenberger, M.G., Fulmer, P.M., Stort, D.R., Butler, J.M. (2011) Concordance and population studies along with stutter and peak height ratio analysis for the PowerPlex® ESX 17 and ESI 17 Systems. *Forensic Sci. Int. Genet.* 5(4): 269-275.

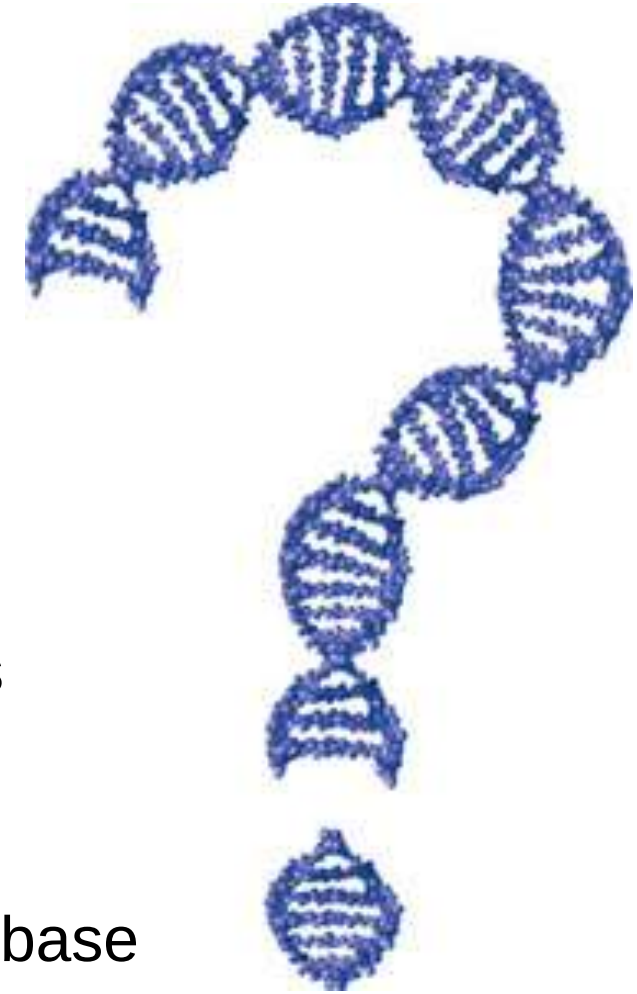
- Hill, C.R., Kline, M.C., Duewer, D.L., Butler, J.M. (2011) Concordance testing comparing STR multiplex kits with a standard data set. *Forensic Sci. Int. Genet.: Suppl. Ser.* 3: e188-e189.

- Kline, M.C., Hill, C.R., Decker, A.E., Butler, J.M. (2011) STR sequence analysis for characterizing normal, variant, and null alleles. *Forensic Sci. Int. Genet.* 5(4): 329-332.

- Kline, M.C., Butts, E.L.R., Hill, C.R., Coble, M.D., Duewer, D.L., Butler, J.M. (2011) The new Standard Reference Material 2391c: PCR-based DNA profiling standard. *Forensic Sci. Int. Genet.: Suppl. Ser.* 3: e355-e356.

Acknowledgments and Contact Information

Acknowledgments: NIJ & FBI Funding;
Applied Biosystems, Promega, and Qiagen
for STR kits used in concordance studies



Contact Information

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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

SAVE THE DATE

Forensics@NIST 2012

Three day symposium on cutting edge forensic science research at NIST

Date: November 28-30th, 2012
Time: 9:00 am to 5:00 pm
Location: NIST (Gaithersburg, Maryland)

For more information:
www.nist.gov/oles/forensics-2012.cfm

Note: registration is required

FORENSIC SCIENCES

NIST/NRC Postdoc Program

- Current stipend (2011-2012) is **$65,600 per year**
  - Currently a limit of 120 slots per year
  - Congressionally-mandated program for NIST
  - Maximum 2-year appointments
- Awardees **must be U.S. citizens**
- Awardees are chosen through a **national competition** administered by the National Research Council of the National Academy of Sciences.
  - Research opportunities include those in chemistry, physics, materials science, mathematics, computer sciences, and engineering.
- Two competitions per year
  - **deadlines of February 1 and August 1**

Any of the projects our group is doing are open to possibilities

Multiplex PCR Assay Development
RO#: 50.63.11.B5906
Adviser: Peter Vallone

http://www.nist.gov/iaao/postdoc.cfm

http://nrc58.nas.edu/RAPLab10/Opportunity/Program.aspx?LabCode=50