University of North Texas Health Sciences Center (Ft. Worth, TX)
April 12, 2012

NIST Research Projects in Human Identity Testing

John M. Butler

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

Overview of My Career

B.S. Chemistry 1992

UVA Grad Student (Aug 1992- Aug 1995)

Research Conducted at FBI

Armed Forces DNA Identification Laboratory

NIST/NRC Postdoc (Sept 1995- May 1997)

Some Research at AFDIL

Silicon Valley start-up company doing TOF-MS of DNA

Staff Scientist (May 1997 – Sept 1999)

Human Identity Project Leader

Research Chemist (Sept 1999 - present)

Since 2008: NIST Fellow and Group Leader of Applied Genetics

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 **a non-regulatory agency within 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.**

$686 for 3 jars

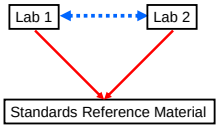
DNA typing standard

Standard Reference Materials (SRMs)

http://www.nist.gov/srm

Traceable standards to ensure accurate and comparable measurements between laboratories

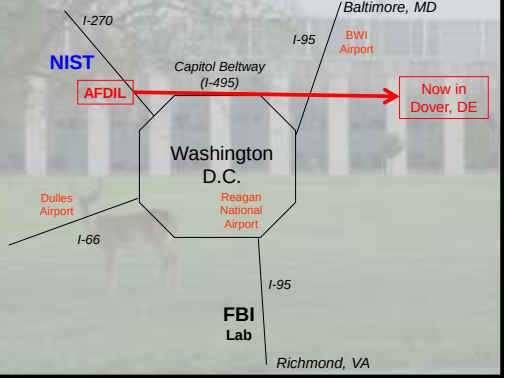
SRM 2391c – autosomal STRs
SRM 2392 &-I – mtDNA sequencing
SRM 2395 – Y-STRs
SRM 2372 – DNA quantitation
SRM 2366 – CMV
SRM 2393 – Huntington's Disease
SRM 2399 – Fragile X



Calibration with SRMs enables confidence in comparisons of results between laboratories

Helps meet ISO 17025 needs for traceability to a national metrology institute

Location of NIST

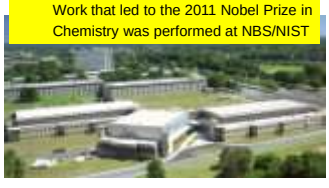


NIST Today

Major Assets

- ~ 2,900 employees
- ~ 2600 associates and facilities users
- ~ 400 NIST staff on about 1,000 national and international standards committees
- **3 Nobel Prizes in Physics in past 15 years**

Work that led to the 2011 Nobel Prize in Chemistry was performed at NBS/NIST



Major Programs

- **NIST Laboratories**
- Baldridge National Quality Program
- Hollings Manufacturing Extension Partnership
- Technology Innovation Program

Joint NIST/University Institutes:

- JILA
- Joint Quantum Institute
- Institute for Bioscience & Biotechnology Research
- Hollings Marine Laboratory

NIST Applied Genetics Group

Group Leader ***Bringing traceability and technology to the scales of justice...***


John Butler


Marcia Holden


Margaret Kline

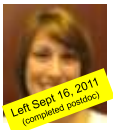

Pete Vallone


Mike Coble


Ross Haynes


Becky Hill


Erica Butts


Left Sept 16, 2011 (completed postdoc)
Kristen O'Connor


Kevin Kiesler

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
- **Ag Biotech**
  - "universal" GMO detection/ quantitation (35S promoter)
- **DNA Biometrics**
  - **Rapid PCR methods**
  - Efforts to standardize testing of future portable DNA systems
  - Kinship analysis
- **Cell Line Authentication**
  - ATCC documentary standard

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.


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

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


Mike Coble


Becky Hill


Margaret Kline

STRBase, Workshops & Textbooks

Mixtures, mtDNA & Y

Concordance & LT-DNA

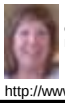
SRM work, variant alleles & Cell Line ID

Guest Researcher


Manuel **Fonde**vila Alvarez

Data Analysis Support


Dave Duewer


Office Manager Patti Rohmiller

DNA Biometrics Team

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


Pete Vallone


Erica Butts


Kevin Kiesler

Rapid PCR, Direct PCR & Biometrics

ABI 3500 & DNA Extraction

PLEX-ID & NGS Exploration

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

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

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 ~~(four)~~ competitions per year
  - **deadlines of February 1 and August 1** ~~(May 1 and November 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

Current NIST Projects

Short Overviews…

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

NIST STRBase Website

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


John Butler

Forensic STR Information

- STRs101: Brief Introduction to STRs
- *Core Loci*: FBI CODIS Core STR Loci and European Core Loci
- STR Fact Sheets (observed alleles and PCR product sizes)
- Multiplex STR kits
- Sequence Information (annotated)
- Variant Allele Reports
- Tri-Allelic Patterns
- Mutation Rates for Common Loci
- Published PCR primers
- Y-chromosome STRs
- Low-template DNA Information — Updated
- Mixture Interpretation — NEW
- Kinship Analysis — NEW
- miniSTRs (short amplicons)
- Null Alleles - discordance observed between STR kits
- STR Reference List - *now 2400 references*

Cataloged as of Mar 2012
632 variant alleles
310 tri-allelic patterns

We invite labs to supply information on variant and tri-alleles observed

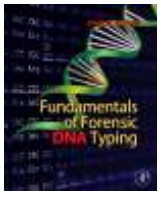
Forensic DNA Typing Textbook

3rd Edition is Three Volumes

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

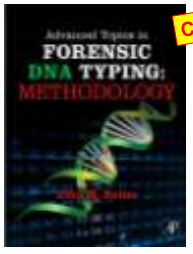

John Butler

For beginning students, general public, & lawyers

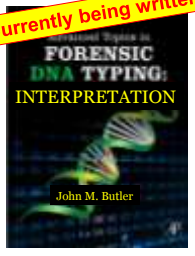


Sept 2009
~500 pages

Currently being written



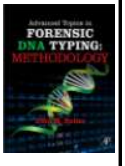
August 2011
~700 pages



Fall 2012
~500 pages

New Material in *Advanced Topics: Methodology*

Released August 2011



>50% new material from previous editions

- Cites >1500 new references (>2800 ref. total)
- **New chapter** on legal aspects (Ch. 18)
  - expert witness prep, perspectives from lawyers
  - App. 4 (interviews): experts, prosecutors, & defense
- **New chapter** on X-chromosome markers (Ch. 15)
- **Extensive updates** on CE (Ch. 6), validation (Ch. 7), database issues (Ch. 8), disaster victim identification (Ch. 9), miniSTRs (Ch. 10), LTDNA (Ch. 11), SNPs (Ch. 12), Y-STRs (Ch. 13), mtDNA (Ch. 14), non-human DNA (Ch. 16), and new technology (Ch. 17)
- Coverage of all the new STR kits (Ch. 5)
- Listing of all known STR alleles for all 23 kit loci (App. 1)
- Most detail to-date on the Grim Sleeper case (D.N.A. Box 8.5)

NIST SRM 2391c


Margaret Kline


Becky Hill

Main Points:

- Traceable physical reference materials to ensure accurate and comparable measurements between laboratories
- Helps meet ISO 17025 needs for traceability to a national metrology institute
- **http://www.nist.gov/srm**
- **SRM 2391c released Aug 2011**


http://www.promega.com/resources/articles/profiles-in-dna
The Latest and Greatest NIST PCR-Based DNA Profiling Standard: Updates and Status of…

Presentations/Publications:

- *Profiles in DNA* article (Sept 2011)
- ISFG 2011 and ISHI 2011 posters
- Forensic Sci. Int. Genet. Suppl. Ser. (2011)

NIST Standard Reference Material (SRM) for Forensic DNA Testing

SRM 2391b (2003-2011)	**SRM 2391c** (2011-future)
• **48 autosomal STR loci** with certified values	• **23 autosomal STR loci and 17 Y-STRs** certified
• **10 liquid genomic DNA** components + **2 punches** (cells on 903 paper)	• **4 liquid genomic DNA** components + **2 punches** (cells on **FTA** & 903 paper)
• All single source samples	• 5 single source + 1 mixture
• 4 males + 6 females	• 3 males + 2 females (unique)
• 9947A & 9948 included	• All new samples – no 9947A or 9948

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

NIST SRM 2391c

Selling since Aug 16, 2011
Current price: $626

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

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			

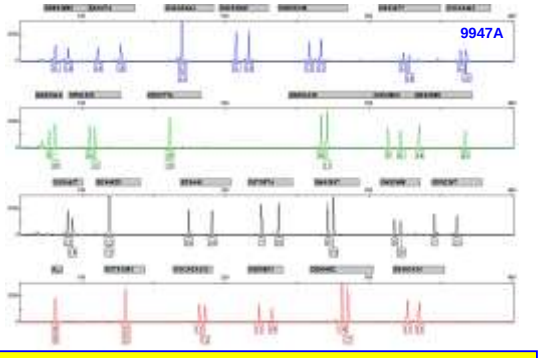
All results are concordant across all kits.

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

NIST STR 26plex

Hill et al. (2009) *Journal of Forensic Sciences*, 54(5):1008-1015

http://www.cstl.nist.gov/biotech/strbase/str26plex.htm



Gender identification + 25 autosomal STR loci in a single amplification

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

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

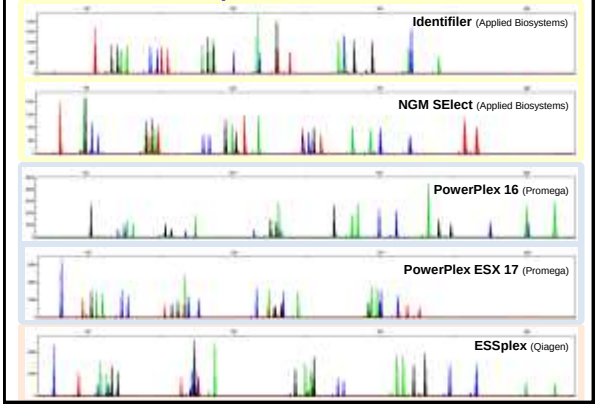
Set 1 Amplicons Set 2 Amplicons

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.)

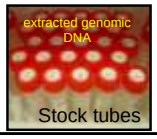
Same DNA Sample Tested with Five STR Kits



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

Kit Concordance Comparisons

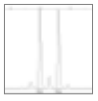

Becky Hill

Kits compared	Samples	Loci compared	Comparisons	# Differences	Concordance (%)
SGM-ID	1436	11	15,796	1	99.994
ID-ProPlus	1427	10	14,270	1	99.993
ID-IDplex	669	16	10,704	19	99.822
ID-PP16	662	14	9,268	4	99.957
ID-MiniFiler	1308	9	11,772	27	99.771
SGM-NGM	1436	11	15,796	4	99.975
ID-NGM	1449				
ProPlus-NGM	1427				
SGM-ESI	1436				
ProPlus-ESX	1427				
ESI-ESX	1455				
ESI-ESSplex	1445				
ESX-ESSplex	1445				
ESI-NGMSElect	715				

128 kit-to-kit comparisons
1,104,031 allele comparisons
1224 differences observed
~99.9% concordance
(many corrected now)

*Kits (except Identifiler) were kindly provided by **Applied Biosystems, Promega, and Qiagen** for concordance testing performed at NIST*

Extra (Degenerate) Primers Added with NGM SElect

	NGM (original)	NGM SElect and NGM'
D2S441 9.1 allele missing in 7 Asians	 11,11	 9.1,11
D22S1045 15 allele missing in 4 samples	 17,17	 15,17
Amelogenin X allele missing in 3 samples	 Y,Y	 X,Y

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)

Presentations/Publications:

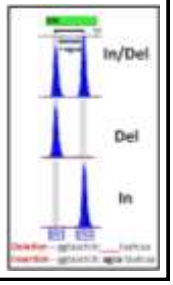
- FSI Genetics article (Aug 2011) and numerous talks

Insertion/Deletion (InDel) Markers


Manuel Fondevila Alvarez
Guest Researcher from Spain

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



Presentations/Publications:

- FSI Genetics Suppl. Series 2011 article
- ISFG 2011 poster and ISHI 2011 presentation

SWGDAM Website and Resources Available

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

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

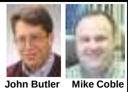
The following information resources have been produced and reviewed by members of the Mixture Committee of the Scientific Working Group on DNA Analysis Methods (SWGDAM) -- see http://www.swgdam.org/resources.html for additional information.

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

Recent Training Workshops



- 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

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

Mixture Workshop (Promega ISHI 2010)

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

Handout >200 pages

Literature list of >100 articles

13 Modules Prese

Introductio

SWGD (John)

olds (Catherine)

e)

astic effects (Robin)

eak height ratios (Charlotte)

Number of contributors (John)

Mixture ratios (John)

Mixture principles (Charlotte)

Statistics (Mike)

Case Example 1 (Robin)

Case Example 2 (Charlotte)

Case Example 3 (John)

Catherine Grgicak	Mike Coble	Robin Cotton	John Butler	Charlotte Word
Boston U.	NIST	Boston U.	NIST	Consultant

NIJ Grant to Boston University **funded ~150 state & local lab analysts to attend**

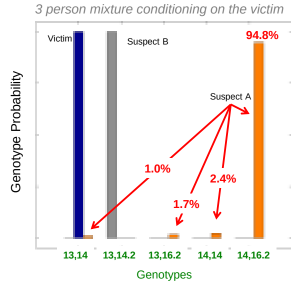
TrueAllele Mixture Software Evaluation

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

D19S433 result from one replicate of 50,000 simulations

3 person mixture conditioning on the victim



Presentations/Publications:

- ISFG 2011 presentation
- ISHI 2011 mixture workshop

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

Rapid PCR and Rapid DNA Testing

Main Points:

- **Performing research on reducing the total time required** for STR typing
  - Focusing on the multiplex amplification of commercial STR kits with faster polymerases and thermal cyclers
  - Single-source reference samples (sensitivity > 200 pg)
- **Designing testing plans for rapid DNA typing devices**
  - NIST will be examining rapid DNA instruments with FBI collaboration
- **Exploring direct PCR protocols** with FTA and 903 papers

Presentations/Publications:

- Vallone et al. (2008) FSI Genetics - on rapid PCR
- ISFG 2011 and ISHI 2011 presentations by Tom Callaghan (FBI)
- ISFG 2011 presentation and poster on direct PCR

Common Thermal Cycling Times

Can we reduce PCR cycling times? What are the effects or limitations?

Thermal Cycling Times for Current STR Typing Kits

Year	Run on a 9700 thermal cycler	Hot start	Time per cycle	Cycles	Post soak	Total time
1997/98	Profiler Plus/Cofiler	11 min	3 min	28	60 min	2:52
1999	SGM Plus	11 min	3 min	28	45 min	2:53
2000	PowerPlex 16	12 min	1 min 45 s	32	30 min	3:00
2001	Identifiler	11 min	3 min	28	60 min	2:58
2003	PowerPlex Y	12 min	1 min 45 s	32	30 min	3:18
2004	Yfiler	11 min	3 min	30	80 min	2:45
2007	PowerPlex S5	2 min	4 min	30	45 min	3:21
2007	minifiler	11 min	3 min 20 s	30	45 min	3:16
2009	ESI 16, 17 ESX 16,17	2 min	4 min	30	45 min	3:22
2009	PowerPlex 16 HS	2 min	1 min 45 s	32	30 min	2:42
2009	NGM	11 min	3 min 20 s	29	10 min	2:33
2009	Identifler Direct	11 min	3 min	26	25 min	2:34
2010	Idenfiler Plus	11 min	3 min 20 s	28	10 min	2:18
2011	PowerPlex 18D	2 min	1 min 10s	27	20 min	1:25

Thermal Cyclers

1. GeneAmp 9700 (Applied Biosystems)
2. Mastercycler Pro S (Eppendorf)
   - Peltier based
3. Rotor-Gene Q (Qiagen)
   - Air heated and cooled
4. SmartCycler (Cepheid)
   - Hot plates for heating, fans for cooling

Intended for real-time PCR

- Cycling for most STR kits is run in the
- '9600 emulation mode' (1°C/s)

PCR Thermal Cycling Profile

Identifiler STR kit

28 cycles of PCR

95°C	95°C	59°C	72°C	60°C
10 min	1 min	1 min	1 min	60 min

95°C	95°C	58°C	72°C	72°C
1 min	5 s	10 s	10 s	1 min

Sub 36 min run time

Maximum heating/cooling rate of ~2 to 6°C/s (cycler dependent)

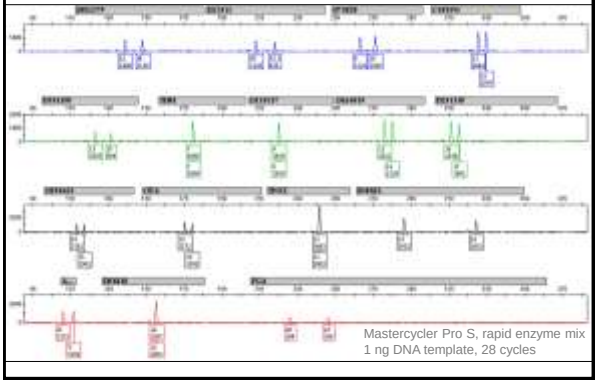
Rapid PCR Conditions

- 1 X Takara PCR mastermix, 1 U SpeedStar polymerase
  - *Premix Ex Taq*™ (Perfect Real Time)
- 10 μL total reaction in a thin walled tube (8-strip)
- 2 μL of Identifiler PCR primer mix
- ~1 ng of template DNA

- Utilize maximum ramp rate on thermal cyclers
  - GeneAmp 9700 = 1.6°C/s (36 min)
  - Rotor-Gene Q = 1.6°C/s (36 min)
  - SmartCycler = 5.8°C/s (20 min)
  - Mastercycler Pro S = 6.8°C/s (19 min)

Effective heating/cooling rates

Full Identifiler STR Profile with **19 min PCR**



Mastercycler Pro S, rapid enzyme mix
1 ng DNA template, 28 cycles

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

ABI 3500 Open Letter Update

John Butler

Concerns Expressed in 3/31/11 Open Letter

1. RFID tags
2. New .hid file structure requires new software
3. Short shelf life of reagents – would like to see data for expiration times

At the Promega ISHI meeting (Oct 2011), ABI described data for studies around reagent expiration through a poster at their booth. Sailus, Wheaton, Fisher, Calandro. "Understanding the Consumables on the 3500 Genetic Analyzers in the context of a Human Identification (HID) Laboratory"

They have promised that **polymer and buffer expiration dates will no longer be a hard stop** but only a warning with the future Windows 7 software upgrade (3500 Data Collection v1.3).

Performance Assessment of PlexID


Kevin Kiesler


Pete Vallone

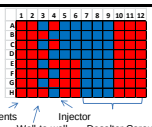
Abbott Ibis Biosciences
PLEX-ID System

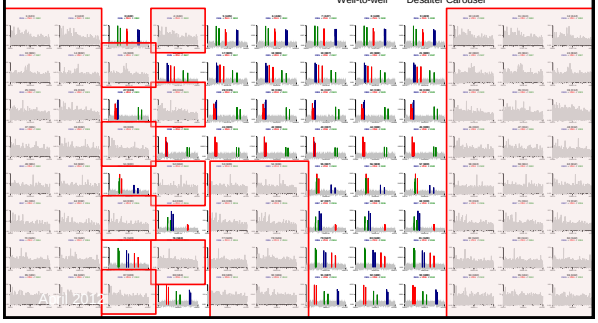
- **In collaboration with FBI**
- **Evaluating ESI-TOF mass spectrometer for mtDNA**
- Base composition of the control region determined from 8 triplex PCRs
- Started running the PlexID platform mid-October 2011
- **Have examined >100 plates of data → report for FBI**

Contamination Check

Checks run weekly on the PlexID to monitor baseline noise and potential contamination

No signal detected in 'red' wells





Recent Next-Generation Sequencing (NGS) Meeting Held at NIST

- **Interagency Workshop on the use of Next-Generation DNA Sequencing for Human Identification and Characterization**
- Held January 31, 2012 at NIST
- Presentations by MIT/Lincoln Labs and NIST scientists to government agency representatives
- Minutes of meeting and presentations available at http://www.nist.gov/mml/biochemical/genetics/ngs_hid_workshop.cfm

Characterizing New STR Loci


John Butler


Becky Hill

Main Points:

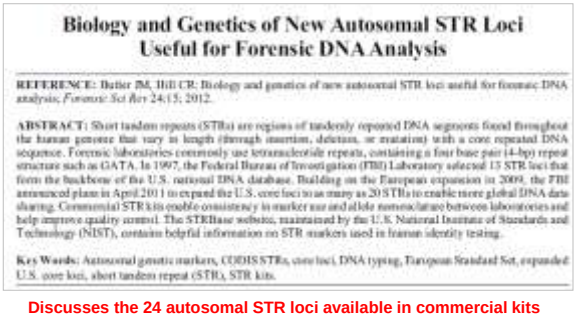
- In April 2011, the FBI announced plans to expand the core loci for the U.S. beyond the current 13 CODIS STRs
- Our group is collecting U.S. population data on new loci and characterizing them to aid understanding of various marker combinations
- We are collecting all available information from the 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

Article in the January 2012 issue of *Forensic Science Review*

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



Biology and Genetics of New Autosomal STR Loci Useful for Forensic DNA Analysis

REFERENCE: Butler JM, Hill CR: Biology and genetics of new autosomal STR loci useful for forensic DNA analysis. *Forensic Sci Rev* 24(1): 2012.

ABSTRACT: Short tandem repeats (STRs) are regions of tandemly repeated DNA segments found throughout the human genome that vary in length (through insertion, deletion, or mutation) with a core repeated DNA sequence. Forensic laboratories commonly use tetranucleotide repeats, containing a four base pair (4-bp) repeat structure such as GATA. In 1997, the Federal Bureau of Investigation (FBI) Laboratory selected 13 STR loci that form the backbone of the U.S. national DNA database. Building on the European expansion in 2009, the FBI announced plans in April 2011 to expand the U.S. core loci to as many as 20 STRs to enable more global DNA data sharing. Commercial STR kits enable consistency in marker size and allele nomenclature between laboratories and help improve quality control. The STRBase website, maintained by the U.S. National Institute of Standards and Technology (NIST), contains helpful information on STR markers used in human identity testing.

Key Words: Autosomal genetic markers, CODIS STRs, core loci, DNA typing, European Standard Set, expanded U.S. core loci, short tandem repeat (STR), STR kits.

Discusses the 24 autosomal STR loci available in commercial kits

International Comparability

Currently there are 24 autosomal STR markers present in commercial kits

U.S.	Europe
TPOX	
CSF1PO	
D5S818	
D7S820	
D13S317	
FGA	FGA
vWA	vWA
D3S1358	D3S1358
D8S1179	D8S1179
D18S51	D18S51
D21S11	D21S11
TH01	TH01
D16S539	D16S539
D2S1338	D2S1338
D19S433	D19S433
Penta D	
Penta E	
	D12S391
	D1S1656
	D2S441
	D10S1248
	D22S1045
	SE33

13 CODIS loci

ESS = European Standard Set

7 ESS loci

3 miniSTR loci developed at NIST

5 loci adopted in 2009 to expand to 12 ESS loci

Locus used in China ← **D6S1043**

→ Core locus for Germany

The 11 STR Loci Beyond the CODIS 13

	STR Locus	Location	Repeat Motif	Allele Range*	# Alleles*
5 new European loci	**D2S1338**	2q35	TGCC/TTCC	10 to 31	40
	D19S433	19q12	AAGG/TAGG	5.2 to 20	36
	Penta D	21q22.3	AAAGA	1.1 to 19	50
	Penta E	15q26.2	AAAGA	5 to 32	53
	D1S1656	1q42	TAGA	8 to 20.3	25
	D12S391	12p13.2	AGAT/AGAC	13 to 27.2	52
	D2S441	2p14	TCTA/TCAA	8 to 17	22
	D10S1248	10q26.3	GGAA	7 to 19	13
	D22S1045	22q12.3	ATT	7 to 20	14
	SE33	6q14	AAAG‡	3 to 49	**178**
	D6S1043	6q15	AGAT/AGAC	8 to 25	25

**Allele range and number of observed alleles from Appendix 1, J.M. Butler (2012) Advanced Topics in Forensic DNA Typing: Methodology;* ‡*SE33 alleles have complex repeat structure*

Loci sorted on Probability of Identity (P_I) values

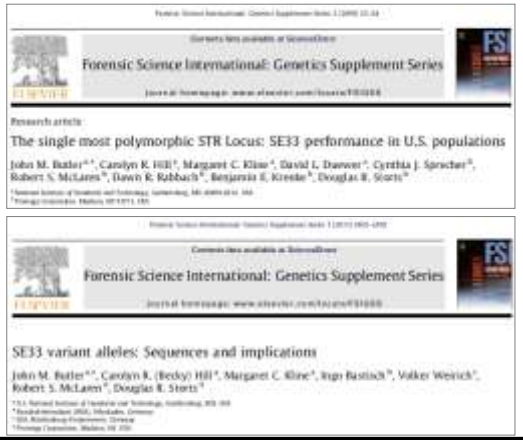
STR Locus	Alleles Observed	Genotypes Observed	Het. (obs)	P_I value N = 938
SE33	53	292	0.9360	0.0069
Penta E*	20	114	0.8799	0.0177
D2S1338	13	68	0.8785	0.0219
D1S1656	15	92	0.8934	0.0220
D18S51	21	91	0.8689	0.0256
D12S391	23	110	0.8795	0.0257
FGA	26	93	0.8742	0.0299
D6S1043*	25	91	0.8627	0.0343
Penta 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)


Forensic Science International: Genetics Supplement Series

The single most polymorphic STR Locus: SE33 performance in U.S. populations

Forensic Science International: Genetics Supplement Series

SE33 variant alleles: Sequences and implications

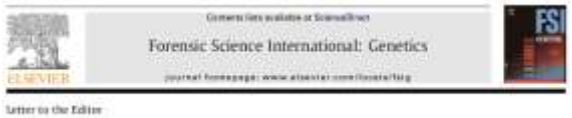

Forensic Science International: Genetics

Concordance and population studies along with stutter and peak height ratio analysis for the PowerPlex® ESX 17 and ESI 17 Systems

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

Expanding the 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

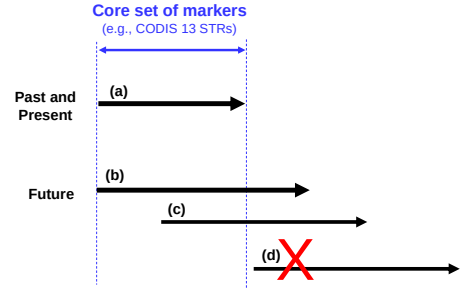

Forensic Science International: Genetics

Letter to the Editor

CODIS Core Loci Working Group
Formed in May 2010 to make recommendations to FBI CODIS Unit

Douglas Hares (Chair) – FBI
John Butler – NIST
Cecelia Crouse – FL PBSO
Brad Jenkins – VA DFS
Ken Konzak – CA DOJ
Taylor Scott – IL SP

Possible scenarios for extending sets of genetic markers to be used in national DNA databases



Maintaining connection to legacy data is essential

Figure 8.4 in Butler (2012) Advanced Topics in Forensic DNA Typing: Methodology, p. 243

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

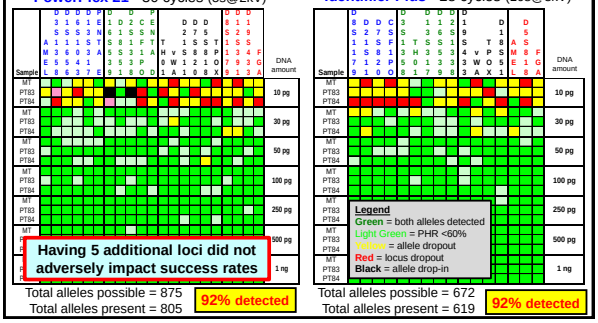
What	Why	Who/How	When
Form a Working Group (WG) to discuss initial selection	Establishes target goals	CODIS Core Loci Working Group with FBI Chair and 5 members; Web meetings	May 2010 - present
Announce proposed additional CODIS core loci	Sets desired target goals and informs manufacturers	WG Chair; 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

Data courtesy of Becky Hill (NIST)

Measurement of Allele Dropout and Extreme Peak Height Imbalance for 2 STR Kits

Three fully heterozygous (except PT83 at Penta D) pristine DNA samples were examined in a dilution series with PowerPlex 21 and Identifiler Plus. Results are ordered by amplicon size and dye color.



Future Projects Planned

- New book in progress on interpretation issues
- Additional mixture software evaluation
- Rapidly mutating Y-STR loci (European collaboration)
- More concordance testing with new STR kits
- Complete PLEX-ID mass spec assessment with mtDNA base composition (FBI collaboration)
- Rapid DNA test device evaluation (FBI collaboration)
- Exploration of Next-Generation Sequencing
- Digital PCR for human DNA quantitation

The Future of Forensic DNA

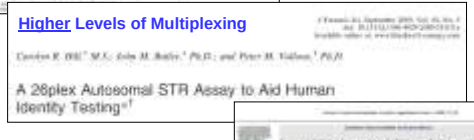
is Similar to the Olympic Motto of
"Swifter, Higher, Stronger"

Resources **Training** **Action**

Recent NIST Publications Demonstrating "Swifter, Higher, Stronger" DNA Analysis

Swifter PCR Amplification

Higher Levels of Multiplexing



Stronger Powers of Discrimination

Thank you for your attention

Acknowledgments: NIJ & FBI Funding;
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