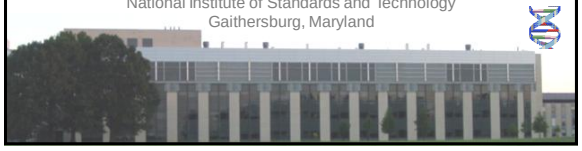
The Copenhagen Forensic Genetic Summer School
Advanced Topics in STR DNA Analysis
June 27-28, 2012

Introduction to NIST

& the Applied Genetics Group

Becky Hill and Mike Coble

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



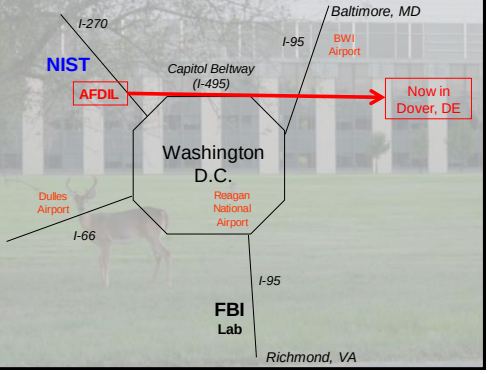
NIST History and Mission

- 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

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

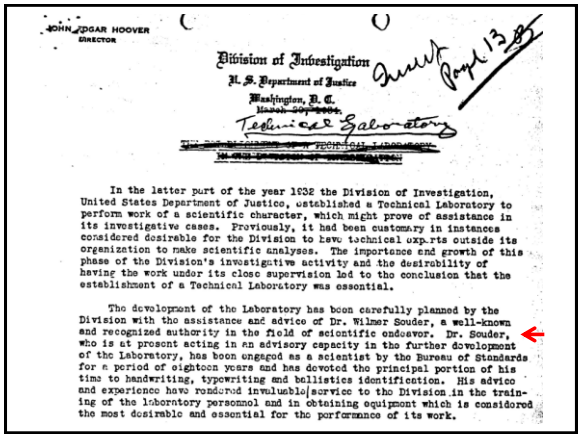
NIST's History of Forensic Science Research

- 1913 - Wilmer Souder was asked to calibrate some precision measuring devices sent to him by famed handwriting expert Albert Osborn.

Wilmer Souder (1934)

NIST's History of Forensic Science Research

- 1913 - Wilmer Souder was asked to calibrate some precision measuring devices sent to him by famed handwriting expert Albert Osborn.
- By the 1930s – Souder was recognized as a pioneer researcher in questioned documents, handwriting, typewriting, ballistics, and firearms.
- Was instrumental in setting up the FBI Technical Laboratory.



JOHN EDGAR HOOVER
DIRECTOR

Division of Investigation
U. S. Department of Justice
Washington, D. C.
Technical Laboratory

In the latter part of the year 1932 the Division of Investigation, United States Department of Justice, established a Technical Laboratory to perform work of a scientific character, which might prove of assistance in its investigative cases. Previously, it had been customary in instances considered desirable for the Division to have technical exp.rts outside its organization to make scientific analyses. The importance and growth of this phase of the Division's investigative activity and the desirability of having the work under its close supervision led to the conclusion that the establishment of a Technical Laboratory was essential.

The development of the Laboratory has been carefully planned by the Division with the assistance and advice of Dr. Wilmer Souder, a well-known and recognized authority in the field of scientific endeavor. Dr. Souder, who is at present acting in an advisory capacity in the further development of the Laboratory, has been engaged as a scientist by the Bureau of Standards for a period of eighteen years and has devoted the principal portion of his time to handwriting, typewriting and ballistics identification. His advice and experience have rendered invaluable service to the Division in the training of the laboratory personnel and in obtaining equipment which is considered the most desirable and essential for the performance of its work.

The Crime of the Century (before OJ Simpson)

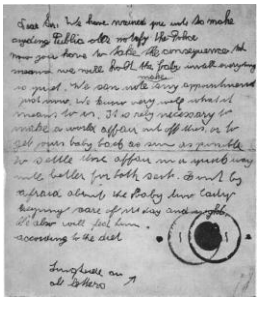
March 1, 1932 – 20 month old Charles Lindbergh, Jr is kidnapped from his home.

Charles Lindbergh, Jr

A handwritten ransom note demanding $50,000 was recovered from the crime scene.

The child's remains were found 10 weeks later

The Investigation



The $50,000 ransom was paid to a man in a cemetery in New York. For the next two years, police searched for the killer.

A break in the case came when a gas station clerk recorded the license plate number of a car that paid for his gas with a marked bill from the ransom.

Bruno Hauptmann was arrested in 1934.

Bruno Hauptmann



Souder analyzed the ransom note used in the kidnapping and murder of Charles Lindbergh's son.

NIST Applied Genetics Group

Group Leader


John Butler


Mike Coble


Margaret Kline


Marcia Holden


Pete Vallone


Patti Rohmiller
Office Manager


Becky Hill


Ross Haynes


Erica Butts


Kevin Kiesler

Bringing calibration to clinical DNA diagnostics, speed to DNA testing, and technology to the scales of justice

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 & direct PCR methods
  - Efforts to standardize testing of future portable DNA systems
  - Kinship analysis
  - PLEX-ID analysis for mtDNA
- **Cell Line Authentication**
  - ATCC documentary standard

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

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


Office Manager Patti Rohmiller

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

Current NIST Projects

Short Overviews…

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

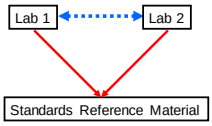
Standard Reference Materials (SRMs)

http://www.nist.gov/srm

Traceable standards to ensure accurate and comparable measurements between laboratories


National Institute of Standards & Technology
Certificate of Analysis
Standard Reference Material® 2391b

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

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**

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.

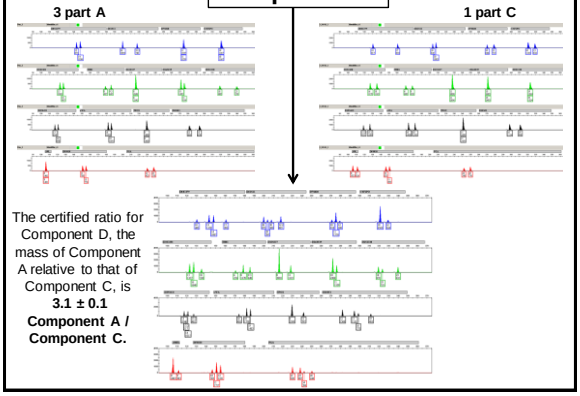
9947A & 9948 are NOT included.

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

Description of Components in SRM 2391c

Component	Description	Quantity [a]
A	50 µL of anonymous **female** genomic DNA	1.4 – 1.9 ng DNA/µL
B	50 µL of anonymous **male** genomic DNA	1.3 – 1.5 ng DNA/µL
C	50 µL of anonymous **male** genomic DNA	1.3 – 2.0 ng DNA/µL
D	50 µL of **mixed-source** (Components A and C)	1.4 – 2.0 ng DNA/µL
E	Two 6 mm punches of CRL-1486	~75,000 cells per punch

Component D



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

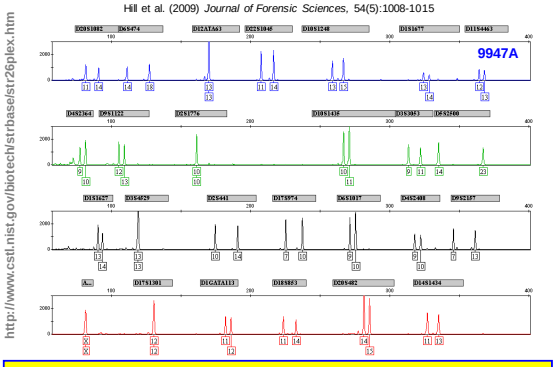
Kit Provider			Primer Mixes
Life Technologies	*Promega*	*Qiagen*	*NIST*
Identifiler	Powerplex 16	ESSplex	26plex
Identifiler Plus	Powerplex 16 HS	IDplex	miniSTRs
NGM	Powerplex ESX 17		
NGM SElect	Powerplex ESI 17		
COfiler	Powerplex ES		
Profiler	Powerplex S5		

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



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

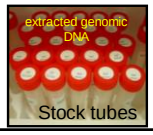
If a primer binding site mutation exists

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

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

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

Presentations/Publications:

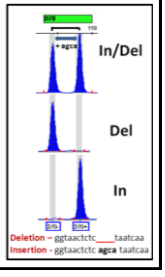
- FSI Genetics article (Aug 2011) and numerous talks

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 & IJLM (in press) articles
- ISFG 2011 poster and ISHI 2011 presentation

Recent Training Workshops


John Butler


Mike Coble

- Int. Symp. Human Ident. (October 3, 2011)
  - **Mixture Interpretation (with Boston University)**
- Int. Symp. Human Ident. (October 6, 2011)
  - **Troubleshooting Laboratory Systems**
- NYC OCME & NY/NJ Labs (April 18, 2012)
  - **Statistics, Mixtures, STRs & CE, Y-STRs, mtDNA, and the Romanov case**

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

TrueAllele Mixture Software Evaluation

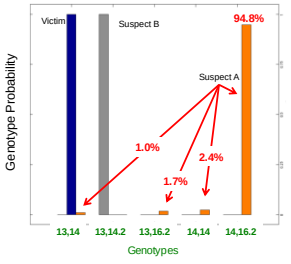

Mike Coble

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

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


Pete Vallone

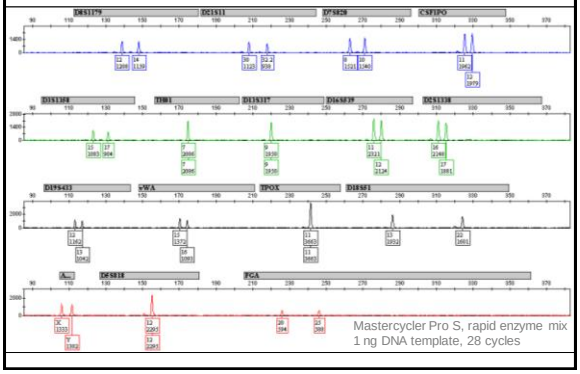
Main Points:

- **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

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

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**

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* 24:15; 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 use 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

NIST STRBase Website


John Butler

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

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 3400 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

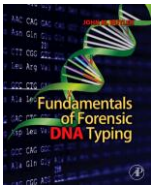
Forensic DNA Typing Textbook

3rd Edition is Three Volumes

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


John Butler

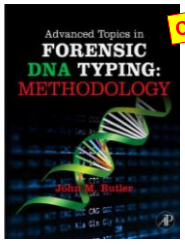
For beginning students, general public, & lawyers



Sept 2009

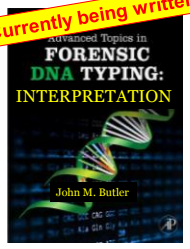
~500 pages

Currently being written



August 2011

~700 pages



Fall 2012

~500 pages

Thank you for your attention

Acknowledgments: NIJ & FBI Funding

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