The Future of Forensic DNA

John M. Butler, PhD
National Institute of Standards and Technology
Gaithersburg, Maryland
United States of America
# Checks and Controls on DNA Results

<table>
<thead>
<tr>
<th>Community</th>
<th>FBI Quality Assurance Standards <em>(and interlaboratory studies)</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory</td>
<td>ASCLD/LAB Accreditation and Audits</td>
</tr>
<tr>
<td>Analyst</td>
<td>Proficiency Tests &amp; Continuing Education</td>
</tr>
<tr>
<td>Method/Instrument</td>
<td><strong>Validation of Performance</strong> <em>(along with traceable standard sample)</em></td>
</tr>
<tr>
<td>Protocol</td>
<td>Standard Operating Procedure is followed</td>
</tr>
<tr>
<td>Data Sets</td>
<td>Allelic ladders, positive and negative amplification controls, and reagent blanks are used</td>
</tr>
<tr>
<td>Individual Sample</td>
<td>Internal size standard present in every sample</td>
</tr>
<tr>
<td>Interpretation of Result</td>
<td>Second review by qualified analyst/supervisor</td>
</tr>
<tr>
<td>Court Presentation of Evidence</td>
<td>Defense attorneys and experts with power of discovery requests</td>
</tr>
</tbody>
</table>
Presentation Outline

• Introduction to NIST
  – Our role with forensic DNA in the United States
  – Some current projects

• Near-term future
  – New autosomal STR loci for expanded core loci
  – Expanded use of databases (e.g., familial searching)
  – Rapid DNA testing

• More distant future
  – Loci besides STRs for identity testing?
  – Phenotyping capabilities?
  – Next-generation DNA sequencing?
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 part of 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.
NIST Today

Major Assets

- ~ 2,900 employees
- ~ 2,600 associates and facilities users
- ~ 400 NIST staff on about 1,000 national and international standards committees
- 4 Nobel Prizes in Physics in past 15 years (including 2012 to David Wineland for quantum physics)

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
Current Activities at NIST

Standard Reference Materials
- SRM 2372 (DNA quantitation standard)
- SRM 2391c (STR typing)

Technology Evaluation and Development
- Rapid multiplex PCR protocols (multiplex STR amplification in <35 min)
- Low-level DNA studies
- Mixture interpretation – research and training materials
- Unusual STR allele characterization
- New STR loci and assays (STR 26plex, kit concordance, InDels & SNPs)

Training Materials
- Workshops on mixture interpretation and CE troubleshooting
NIST Reference Materials for Forensic DNA Measurement Assurance

Margaret Kline

DNA quantity measurement calibration

Autosomal and Y-chromosome short tandem repeat (STR) measurement calibration
Standard Reference Materials (SRMs)

http://www.nist.gov/srm/

Traceable standards to ensure accurate measurements in crime laboratories

Helps meet FBI QAS and ISO 17025 requirements

SRM 2391c – Autosomal and Y-STRs
SRM 2392-I – mtDNA
SRM 2372 – DNA quantitation

Calibration with SRMs enables confidence in comparisons of results between laboratories
Forensic DNA Typing Textbooks Have Set the Standard for the Field

1st Edition

2nd Edition

3rd Edition (3 volumes)

Jan 2001
335 pages

Feb 2005
688 pages

Sept 2009
520 pages

Aug 2011
704 pages

Fall 2014
(being written)
~500 pages

Language Editions

Chinese (2007)

Japanese (2009)
NIST STRBase Website
Serving the Forensic DNA Community for >15 Years

Short Tandem Repeat DNA
Internet Database

NIST Standard Reference Database SRD 130

Serving the forensic DNA and human identity testing communities for over 15 years... These data are intended to benefit research and application of short tandem repeat DNA markers to human identity testing. The authors are solely responsible for the information herein.

Please Rate Our Products and Services: http://tsapps.nist.gov/MSDSurvey/default.aspx?ID=5&DB=130

This database has been accessed 458551 times since 10/02/97. (Court courtesy www.digits.com - see disclaimer)

Created by John M. Butler
and Dennis J. Reeder (NIST Biochemical Science Division),
with invaluable help from Jan Redman, Christian Raitberg and Michael Ting
Site creators' curriculum vitae available using links above.

*Partial support for the design and maintenance of this website is being provided by The National Institute of Justice through the NIST Office of Law Enforcement Standards.*

General Information
- Purpose of STRBase/NAR 2001 Paper describing STRBase/Overview Presentation
- Publications and Presentations from NIST Human Identity Project Team
- NIJ-Funded Projects
- Training Materials
- Links to other web sites
- Glossary of commonly used terms

http://www.cstl.nist.gov/strbase/
Publications on Forensic DNA
from the NIST Applied Genetics Group

- **144 publications since 2002**
  - 40 in the past 2 years
  - Includes journal articles, book chapters, and textbooks
- References are all listed on STRBase
  - [http://www.cstl.nist.gov/strbase/NISTpub.htm](http://www.cstl.nist.gov/strbase/NISTpub.htm)
  - Many are available directly from STRBase

Most of our articles are published in *Forensic Sci. Int. Genetics* – currently the highest impact journal in the field

136 page report written by Kevin Kiesler

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)
<table>
<thead>
<tr>
<th>Stages</th>
<th>Time Frame</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exploration</td>
<td>1985-1995</td>
<td>Beginnings, different methods tried (RFLP and early PCR)</td>
</tr>
<tr>
<td>Stabilization</td>
<td>1995-2005</td>
<td>Standardization to STRs, selection of core loci, implementation of Quality Assurance Standards</td>
</tr>
<tr>
<td>Growth</td>
<td>2005-2013</td>
<td>Rapid growth of DNA databases, extended applications pursued</td>
</tr>
<tr>
<td>Sophistication</td>
<td>The Future</td>
<td>Expanding tools available, confronting privacy concerns</td>
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More than Half of the U.S. Permits Arrestee DNA Testing

U.S. Supreme Court case *Maryland v King* (5-4 ruling June 3, 2013)

+ Federal & DoD

27 states as of Sept 2013

Alabama
Alaska
Arizona
Arkansas
California
Colorado
Illinois
Florida
Kansas
Louisiana
Maryland
Michigan
Missouri
New Jersey
New Mexico
Nevada
North Carolina
North Dakota
Ohio
South Carolina
South Dakota
Tennessee
Texas
Utah
Virginia
Vermont
Wisconsin

http://www.dnasaves.org/states.php
311 exonerated as of September 10, 2013
New Handbook on Biological Evidence Preservation


73 page handbook that makes recommendations for evidence retention, safe handling, packaging and storage, chain-of-custody and tracking, and appropriate disposal once evidence retention is no longer required by law.

Table III-2: Long-Term Storage Conditions Matrix

<table>
<thead>
<tr>
<th>Type of Evidence</th>
<th>Frozen</th>
<th>Refrigerated</th>
<th>Temperature Controlled</th>
<th>Room Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid Blood</td>
<td>Never</td>
<td>Best</td>
<td>Best</td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
<td></td>
<td>Best</td>
</tr>
<tr>
<td>Dry Biological Stained Items</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bones</td>
<td>Best</td>
<td></td>
<td>Best</td>
<td></td>
</tr>
<tr>
<td>Hair</td>
<td>Best</td>
<td></td>
<td>Best</td>
<td></td>
</tr>
<tr>
<td>Swabs with Biological Material</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal Smears</td>
<td>Best</td>
<td></td>
<td>Best</td>
<td></td>
</tr>
<tr>
<td>Feces</td>
<td>Best</td>
<td></td>
<td>Best</td>
<td></td>
</tr>
<tr>
<td>Buccal Swabs</td>
<td>Best</td>
<td></td>
<td>Best</td>
<td></td>
</tr>
<tr>
<td>DNA Extracts</td>
<td>Best (liquid)</td>
<td>Acceptable (liquid)</td>
<td>Acceptable (dried)</td>
<td></td>
</tr>
</tbody>
</table>

Released April 2013
Number of Offender DNA Profiles
in the U.S. National DNA Database

Growth due in part to federal funding from the Debbie Smith Act and new DNA collection laws

Source: FBI Laboratory’s CODIS Unit
Number of Investigations Aided in the U.S. National DNA Database

Source: FBI Laboratory’s CODIS Unit
Growth of DNA Databases

• Within the U.S., we have benefited from significant federal funding over the past decade

• Expanded laws now enable more offenders to be included (currently 26 states and federal government have laws to collect DNA from arrestees)

• Have effectively locked technology with core STR markers used to generate DNA profiles that now number greater than 10 million profiles
Position of Forensic STR Markers on Human Chromosomes

13 CODIS Core STR Loci

- TPOX
- D3S1358
- D5S818
- FGA
- CSF1PO
- D8S1179
- D7S820
- TH01
- VWA

8 STR loci overlap between U.S. and Europe

- D13S317
- D16S539
- D18S51
- D21S11

Sex-typing

Core STR Loci for the United States

1997
Expanding the U.S. CODIS Core Loci


Letter to the Editor

Expanding the CODIS core loci in the United States

**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**

major reasons for expanding the CODIS core loci in the United States:

1. To reduce the likelihood of adventitious matches [7] as the number of profiles stored at NDIS continues to increase each year (expected to total over 10 million profiles by the time of this publication). There are no signs that this trend will slow down as States expand the coverage of their DNA database programs and increase laboratory efficiency and capacity.

2. To increase international compatibility to assist law enforcement data sharing efforts.

3. To increase discrimination power to aid missing persons cases.
Three major reasons for expanding the CODIS core loci in the United States


- To reduce the likelihood of adventitious matches as the number of profiles stored at NDIS continues to increase each year

- To increase international compatibility to assist law enforcement data sharing efforts

- To increase discrimination power to aid missing persons cases
International Comparability

Currently there are 24 autosomal STR markers present in commercial kits

ESS = European Standard Set

13 CODIS loci

U.S.
- TPOX
- CSF1PO
- D5S818
- D7S820
- D13S317

Europe
- FGA
- vWA
- D3S1358
- D8S1179
- D18S51
- D21S11
- TH01
- D16S539
- D2S1338
- D19S433
- Penta D
- Penta E

7 ESS loci

- D12S391
- D1S1656
- D2S441
- D10S1248
- D22S1045

5 loci adopted in 2009 to expand to 12 ESS loci

3 miniSTR loci developed at NIST

Locus used in China

D6S1043

Core locus for Germany

SE33
Promega PowerPlex FUSION (5-dye – CC5 internal lane standard 500)

**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

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

- FGA
- A
- vWA
- D18S51
- D19S433
- D8S1179
- D21S11
- D3S1358
- TH01
- D16S539
- D2S1338
- D22S1045
- D2S441
- SE33

- Y±
- D8S1179
- D21S11
- D18S51
- D19S433
- TH01
- FGA

- D2S441
- D19S433
- TH01
- FGA

- D2S441
- D19S433
- TH01
- FGA

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

**STR Kit Layouts by Dye Label and PCR Product Size**
GlobalFiler Allelic Ladder

343 alleles across these 24 loci
LOS ANGELES -- A one-time police mechanic was arrested and charged Wednesday in the serial killing of 10 people over 25 years after a DNA sample from his son was found to bear a close resemblance to DNA found on the victims.

Lonnie Franklin Jr., 57, was charged with 10 counts of murder, one count of attempted murder and special circumstance allegations of multiple murders that could make him eligible for the death penalty if convicted, District Attorney Steve Cooley said.

He is charged with 10 counts of murder and one count of attempted murder for a series of killings that date back to 1985.
Putative Relative Is Found

• June 30, 2010: Second familial search of the California database yielded one likely relative

• Database profile belonged to Christopher Franklin (31 years old)
  – Profile added to the database in 2009 after a felony weapons possession charge

• Grim Sleeper profile matched C. Franklin’s profile with one allele at all 15 loci

• Both individuals shared the same Y-STR profile, indicating a possible paternal relationship
Identifying the Grim Sleeper

- Given that the murders spanned at least 25 years, the paternal relationship was likely father-son

- Undercover police shadowed C. Franklin’s father, Lonnie David Franklin, Jr., who lived in the vicinity of the murders

- Police collected a DNA sample from Lonnie Franklin
  - Direct match between L. Franklin and the Grim Sleeper
Rapid DNA Efforts

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

- Evaluating ANDE (NetBio) and IntegenX rapid DNA instruments
  - both instruments are capable of swab in \(\rightarrow\) STR profile out in less than 90 minutes without user intervention

- Exploring rapid DNA techniques including direct PCR and rapid PCR
  - STR profiles generated in <2 hours with standard lab equipment and rapid protocols
  - See ISHI 2012 poster available on STRBase “Rapid DNA Testing Approaches for Reference Samples”

**Fastest results swab-to-profile (Identifiler): 57 minutes**
Rapid 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
1 min 5 s 10 s 1 min

Sub 36 min run time

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

Slide from Peter Vallone (NIST)
Full Identifier STR Profile with 19 min PCR

Slide from Peter Vallone (NIST)
Potential Applications with Rapid PCR Capabilities

- **Improve overall laboratory throughput**
  - Multiplex PCR amplification is already in many situations the longest part of the DNA analysis process (depending on DNA extraction and DNA quantitation methods)
  - With increased use of robotic sample preparation and expert system data analysis, bottleneck for sample processing will shift to time for PCR amplification…

- **Enable new potential DNA biometric applications** (because the overall DNA analysis process is faster)
  - Permit analysis of individuals at a point of interest such as an embassy, an airport, or a country border
A “Crystal Ball” to the Future?
Possible scenarios for extending sets of genetic markers to be used in national DNA databases

- **Past and Present**
  - Core set of markers (e.g., CODIS 13 STRs)
  - Highly unlikely to start over with new loci
  - Extra loci would be included (due to large PCR multiplexes)
  - Some loci may be dropped to enable replacement with better loci

- **Future**
  - Maintaining connection to legacy data is essential

Figure 18.1 in forthcoming Butler (2009), Fundamentals of Forensic DNA Typing
SNPs are unlikely to replace STRs for routine forensic DNA testing due to challenges with high-level multiplexing and mixture detection/interpretation.

Most likely use of SNPs will be as ancestry-informative markers (AIMs) for sample ethnicity estimation.
Geographical Origin Prediction


Phenotypic Trait Prediction

Traits of interest

• Traits whose variation may be classified on discreet categories.

• Regulated by a relatively low number of genes.

• Fine example: Iris and hair pigmentation.
Next Generation Sequencing
Forensic Applications

• Going in depth into STR loci and beyond
  – STRs are useful for legacy (databases)
  – SNPs within STRs identify ‘sub-alleles’
  – Millions of bases of sequence variants (SNPs)

• Opens up new human identity applications: biogeographical ancestry, externally visible traits, complex kinship, degraded samples, mixtures, other applications

Applications are currently being addressed by the forensic genetics community (Kayser and deKnijff 2011)
Next Generation Sequencing Workshop

• Interagency Workshop on the use of Next-Generation DNA Sequencing for Human Identification and Characterization (Jan 31 2012)

• Discussion of forensic applications of NGS (NIST, DoD, FBI, DHS) – materials can be found at:

• We are in the process of looking at platforms to characterize forensic markers (mitochondrial, STRs, SNPs)

• Evaluate accuracy, reproducibility, identify initial requirements for a NGS forensic reference material
Some Thoughts on the Future…

- **PCR amplification**
  - Faster enzymes to enable rapid PCR
  - More robust enzymes and master mixes to overcome inhibition
- **Instrumentation**
  - More dye colors to aid higher levels of multiplexing
  - Rapid, integrated devices
  - Alternatives to capillary electrophoresis: PLEX-ID and NGS
- **Quantitative information**
  - qPCR and digital PCR
- **Marker systems**
  - Expanding sets of STR loci for growing DNA databases
  - Other marker systems: SNPs, InDels, X-STRs, RM Y-STRs
  - Body fluid identification with mRNA, miRNA, and DNA methylation
  - Phenotyping for external visible characteristics
  - Challenges with potential whole genome information
- **Data interpretation**
  - Probabilistic genotyping for low-level DNA and mixture interpretation
  - Probability of dropout
We Need Continued Efforts to Improve DNA Interpretation (especially low-level DNA and mixtures)

December 2012 – Forensic Science International: Genetics, volume 6, issue 6
DNA Mixture Interpretation
April 12, 2013 Webcast


• 8-hours of DNA mixture interpretation training

• 11 presentations from five different presenters
  – John Butler, Mike Coble, Robin Cotton, Bruce Heidebrecht, Charlotte Word

• 20 poll questions asked via SurveyMonkey (>600 participated)
  – Addressed additional questions sent via email or Twitter

• >1000 participants (almost entire U.S. represented and >10 countries)

• Available for viewing or download for at least six months (storage costs may limit longer-term storage)

Left to right:
Gladys Arrisueno (NIST, Twitter feed monitor & poll questions)
John Paul Jones (NIST, webcast organizer)
Mike Coble (NIST, presenter)
John Butler (NIST, presenter & organizer)
Charlotte Word (Consultant, presenter)
Robin Cotton (Boston University, presenter)
Bruce Heidebrecht (Maryland State Police Lab, presenter)
DNA Interpretation Training Workshops

September 2-3, 2013
Two days of basic and advanced workshops on DNA evidence interpretation

Handouts and reference list available at
http://www.cstl.nist.gov/strbase/training/ISFG2013workshops.htm

The Workshop Instructors

Mike Coble (NIST)  Peter Gill (U. Oslo)  Jo Bright (ESR)  John Buckleton (ESR)  Duncan Taylor (FSSA)  John Butler (NIST)
The Future of Forensic DNA is Similar to the Olympic Motto of “Swifter, Higher, Stronger”
Acknowledgments

• A great team of scientists at NIST and many wonderful collaborators

• Some slides from Pete Vallone (NIST) and Manuel Fondevila (NIST, USC)

• Funding from National Institute of Justice and FBI Biometrics Center of Excellence for work performed within the NIST Applied Genetics Group
Thank you for your attention

Acknowledgments: A great team of scientists within our NIST Applied Genetics Group and funding from the National Institute of Justice and the FBI

Contact Information

John Butler
NIST Fellow
john.butler@nist.gov
+1-301-975-4049

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

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