State-of-the-Art Forensic DNA

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Gaithersburg, Maryland
United States of America

8 May 2013 (São Paulo, Brazil)
O.J. Simpson: Helped Bring DNA Testing to Knowledge of the General Public
Progress Since 1995...

Almost 8 weeks needed to get results

O.J. Simpson DNA testing was performed with RFLP

Now <8 hours to get results
Steps in Forensic DNA Testing

- **Collection/Storage/Characterization**
- **Extraction/Quantitation**
- **Amplification/Marker Sets**
- **Separation/Detection**
- **Interpretation**
- **Report**

**Sample Collection & Storage**
- Blood Stain
- Buccal swab

**DNA Extraction & Quantitation**

**Multiplex PCR Amplification of STR Markers**

**CE with LIF Detection**

**Data Interpretation, Review & Reporting**

**Male:** 13, 14, 15, 16-...

**Mixture interpretation**

**GeneAmp 9700 Thermal Cycler**

**ABI 3500 Genetic Analyzer**

**GeneMapper ID-X software**

**capillary electrophoresis**
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
- ~ 2600 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
National Academies Report on Forensic Science

Harry T. Edwards
U.S. Court of Appeals (DC)
Co-Chair, Forensic Science Committee

- Released February 18, 2009
- Entitled “Strengthening Forensic Science in the United States: A Path Forward”
- 13 recommendations provided to Congress
- Recommends establishing a National Institute of Forensic Science (NIFS)
- NIST and the U.S. Department of Justice announced plans on February 15, 2013 to establish a National Commission on Forensic Sciences
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

DNA quantity measurement calibration

Autosomal and Y-chromosome short tandem repeat (STR) measurement calibration
Forensic DNA Typing Textbook
3rd Edition is Three Volumes

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

For beginning students, general public, & lawyers

Currently being written

Sept 2009
~500 pages

August 2011
~700 pages

Fall 2014
~500 pages
Historical Perspective on DNA Typing

2013: DNA is an important part of the criminal justice system

1985
PCR developed
RFLP

1990
First STRs developed
FSS
Quadruplex

1992
Capillary electrophoresis of STRs first described

1994
1996
CODIS loci defined

1998
NDIS launched (October 13, 1998)

1999
Gill et al. (1985) Forensic application of DNA 'fingerprints'. Nature 318:577-9

2000
Identifiler 5-dye kit and ABI 3100

2002
PowerPlex 16 (16 loci in single amp)

2004
www.dna.gov
President’s DNA Initiative
Debbie Smith Act Backlog Reduction (>1B from 2004-2010)

2006
2013
DNA is an important part of the criminal justice system

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# Stages of Forensic DNA Progression

<table>
<thead>
<tr>
<th>Stages</th>
<th>Time Frame</th>
<th>Description</th>
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<tbody>
<tr>
<td><strong>Exploration</strong></td>
<td>1985-1995</td>
<td>Beginnings, different methods tried (RFLP and early PCR)</td>
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<tr>
<td><strong>Stabilization</strong></td>
<td>1995-2005</td>
<td>Standardization to STRs, selection of core loci, implementation of Quality Assurance Standards</td>
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<tr>
<td><strong>Growth</strong></td>
<td>2005-2013</td>
<td>Rapid growth of DNA databases, extended applications pursued</td>
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<tr>
<td><strong>Sophistication</strong></td>
<td>The Future</td>
<td>Expanding tools available, <em>confronting privacy concerns</em></td>
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</table>
306 exonerated as of May 7, 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>
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<tbody>
<tr>
<td>Liquid Blood</td>
<td>Never</td>
<td>Best</td>
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<td>Urine</td>
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<td>Swabs with Biological Material</td>
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<td>Best (dried)</td>
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<td>Vaginal Smears</td>
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<td>Buccal Swabs</td>
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<td>DNA Extracts</td>
<td>Best (liquid)</td>
<td>Acceptable (liquid)</td>
<td>Acceptable (dried)</td>
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</tbody>
</table>

Released April 2013
• Report published in Nov 2000

• Asked to estimate where DNA testing would be 2, 5, and 10 years into the future

Conclusions

STR typing is here to stay for a few years because of DNA databases that have grown to contain millions of profiles
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

As of March 2013:
- **10,252,900** offender profiles
- **1,429,000** arrestee profiles

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

As of March 2013: 205,700 hits aiding >197,400 investigations

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 28 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

8 STR loci overlap between U.S. and Europe

Sex-typing
Expanding the U.S. CODIS Core Loci


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

13 CODIS loci

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

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

7 ESS loci

Currently there are 24 autosomal STR markers present in commercial kits

ESS = European Standard Set

3 miniSTR loci developed at NIST

D6S1043

5 loci adopted in 2009 to expand to 12 ESS loci

Locus used in China

Core locus for Germany

D12S391
D1S1656
D2S441
D10S1248
D22S1045
SE33

3 miniSTR loci developed at NIST

Locus used in China

Core locus for Germany
## STR Loci Covered in Currently Available Commercial Kits

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STR Marker Layouts for New U.S. Kits

**PowerPlex Fusion**
- **2012**
  - AM, D3S1358, D1S1656, D2S441, D10S1248, D13S317, D16S539, D18S51, D2S1338, CSF1PO, TH01
  - D8S1179, D12S391, D19S433, D21S11, D7S820, FGA
  - D12S391, D18S51, D21S11, D10S1248, D1S1656

**24plex (5-dye)**
- Penta E
- Penta D

**GlobalFiler**
- **2012**
  - Y, AM, D8S1179, D21S11, CSF1PO, TPOX, D1S1656
  - D2S441, D19S433, TH01, FGA
  - D2S441, D13S317, D22S1045, D5S818, D2S1338

**24plex (6-dye)**
- D22S1045, D5S818, D13S317, D7S820, SE33

22 core and recommended loci + 2 additional loci
DNA Mixture Detected with PowerPlex Fusion (24plex STR kit)

Size standard not shown
GlobalFiler Allelic Ladder

343 alleles across these 24 loci
Expanding the Forensic Core Competency

Type of Search

Direct

1-to-1

13 core loci

Standard STR Typing

Indirect

1-to-many

Level of Certainty

High

Low

Type of Match

Direct

Indirect

Standard STR Typing for Database Search

Additional loci may be beneficial

Familial Searching/Missing Persons

Additional loci may be beneficial

High

Low

Paternity Testing

~15 loci

Kinship Analysis

Additional loci may be beneficial

13 core loci

~15 loci

Slide originally from Kristen O’Connor (NIST) presentation at 21st International Symposium on Human Identification
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.

Lonnie David Franklin Jr.
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 → 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”

RapidHIT 200 developed by IntegenX

Fastest results swab-to-profile (Identifiler): 57 minutes
Rapid PCR Thermal Cycling Profile

**Identifiler STR kit**

*28 cycles of PCR*

- **95°C**
  - 10 min
  - 1 min

- **95°C**
  - 1 min

- **59°C**
  - 1 min

- **72°C**
  - 1 min

- **60°C**
  - 60 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

**Core set of markers**
(e.g., CODIS 13 STRs)

**Past and Present**

(a)

**Future**

(b)

(c)

Some loci may be dropped to enable replacement with better loci

Extra loci would be included (due to large PCR multiplexes)

(d)

Highly unlikely to start over with new loci

Maintaining connection to legacy data is essential
• **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**
Compromised Sample Improvements

- Better DNA extraction/recovery
- Continued use of miniSTRs
  - to improve success rates for recovery of information from compromised DNA evidence
- Replicate results for reproducibility
  - to improve reliability with low-template DNA testing
Going Beyond the Core Competencies of Forensic DNA Testing

Core Competency

Standard STR Typing (DNA Profile)
- Direct Matching (or Parentage)
- Sufficient DNA quantity (ng)
  - Lower amounts of DNA being tested
  - Touch DNA Attempts (poor quality, mixtures, low-level stochastic effects)

Challenging kinship search questions

Familial Searching Attempts (fishing for brothers or other relatives)
- Solution: Additional Markers (Y-chromosome, more STRs) and Multiple Reference Samples

Solution: Replicate Testing

Be very cautious when outside the box…

Figure 18.3 in forthcoming Butler (2009) Fundamentals of Forensic DNA Typing
Highly degraded DNA

SNP genotyping in an extreme degradation case

Corpse half buried in a forest for ten years

- Uncovered by a forest fire
- Calcinated remains

Identifiler success 0%

Slide from Manuel Fondevila (NIST, USC)
Highly degraded DNA

SNP genotyping in extreme degradation case
Corpse half buried in a forest for ten years
• Uncovered by a forest fire
• Calcined remains

### HID 52plex Auto 1:
- success 100%

### HID 52plex Auto 2:
- success 100%

### MiniFiler:
- success 30%

---

STRs  +SNPs

P: - 99.993

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Slide from Manuel Fondevila (NIST, USC)
Geographical Origin Prediction


Phenotypic Trait Prediction

Traits of interest

• Traits whose variation may be classified on discrete categories.

• Regulated by a relatively low number of genes.

• Fine example: Iris and hair pigmentation.
Phenotypic trait prediction

For this particular marker, predictive values for blue iris are high, but intermediate or brown phenotypes are not predicted.


Figure 1. Contribution of 24 SNPs to the prediction accuracy of human eye (iris) color in Dutch Europeans of the Rotterdam Study.

Slide from Manuel Fondevila (NIST, USC)
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)
Specific issues with STRs

- Typically comprised of tetra nucleotide repeats
- Range 70 - 450+ bp regions
- Longer STRs can be difficult to assemble based on read length
- Illumina GAIIx (read length 150 bp)
  - Generated 1000-2500 bp amplicons (13 core loci)
  - Problems detecting D21S11 32.2 and 34.2 alleles
  - Issues detecting D18S51
  - Custom informatics tools for assembling STRs

Bornman et al., 2012 Biotechniques Rapid Dispatch: 1-6
Next Generation Sequencing

• Challenges
  – Repeating sequences (STRs) and read lengths
  – Sample amount requirements (10 ng to 5 µg)
  – **Cost** and **time** per unit of information
  – Data analysis (storage, assembly, interpretation)
  – Policy, privacy, disease related markers
  – Validation
  – Standards/reference materials
    • Nomenclature
    • Accuracy of sequence information
    • Errors, platform and bioinformatics-based bias
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 commission of the International Society of Forensic Genetics: Recommendations on the evaluation of STR typing results that may include drop-out and/or drop-in using probabilistic methods

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)
The Future of Forensic DNA is Similar to the Olympic Motto of “Swifter, Higher, Stronger”

- Portable Devices
- New Loci & Assays
- Expert Systems
- Expanding Toolbox
- Mixture Analysis

Resources    Training    Action
Recent NIST Publications Demonstrating “Swifter, Higher, Stronger” DNA Analysis

**Swifter PCR Amplification**

*Rapid amplification of commercial STR typing kits*

Peter M. Vallone, Carolyn R. Hill, Daniele Podini, John M. Butler

**Higher Levels of Multiplexing**

*Carolyn R. Hill, M.S.; John M. Butler, Ph.D.; and Peter M. Vallone, Ph.D.*

*A 26plex Autosomal STR Assay to Aid Human Identity Testing*†

**Stronger Powers of Discrimination**

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

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

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