

IV Congresso Brasileiro de Genética Forense

07 a 10 de maio de 2013 Memorial da América Latina • São Paulo • SP • Brasil

State-of-the-Art Forensic DNA

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NST

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



Steps in Forensic DNA Testing



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.



\$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
- 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



Advisers to the Nation on Science, Engineering, and Medicine



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
- Third edition of *Forensic DNA Typing* textbook (2010, 2012, & 2014)

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)



John Butler

For beginning students, general public, & lawyers



Sept 2009

~500 pages



August 2011 ~700 pages



Fall 2014 ~500 pages



Stages of Forensic DNA Progression

Stages	Time Frame	Description
Exploration	1985-1995	Beginnings, different methods tried (RFLP and early PCR)
Stabilization	1995-2005	Standardization to STRs, selection of core loci, implementation of Quality Assurance Standards
Growth	2005-2013	Rapid growth of DNA databases, extended applications pursued
Sophistication	The Future	Expanding tools available, confronting privacy concerns



http://www.innocenceproject.org

KNOW THE CASES : UNDERSTAND THE CAUSES : FIX THE SYSTEM

ABOUT : DONATE : NEWS & I

Rickey Johnson Served 25 years in Louisiana for a crime he didn't commit.



New Handbook on Biological Evidence Preservation

Available (as free pdf): http://nvlpubs.nist.gov/nistpubs/ir/2013/NIST.IR.7928.pdf



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



National Commission on the Future of DNA Evidence



U.S. Department of Justice Office of Justice Programs Natural Justice of Justice



The Future of Forensic DNA Testing

redictions of the Research and Development Working Group

A Report From

http://www.ojp.usdoj.gov/nij/pubs-sum/183697.htm

•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



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 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



Expanding the U.S. CODIS Core Loci

D.R. Hares (2012) Expanding the CODIS Core Loci in the United States. *Forensic Sci. Int. Genet.* 6(1): e52-e54 Addendum to expanding the CODIS core loci in the United States, Forensic Sci. Int. Genet. (2012) 6(5): e135



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 D.R. Hares (2012) Forensic Sci. Int. Genet. 6(1):e52-e54

- 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



STR Loci Covered in Currently Available Commercial Kits

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Butler, J.M., & Hill, C.R. (2013) *Topics on Forensic DNA Analysis: Current Practices and Emerging Technologies* (CRC Press). Chapter 9. Biology and Genetics of New Autosomal STR Loci Useful for Forensic DNA Analysis (in press)

STR Marker Layouts for New U.S. Kits



DNA Mixture Detected with PowerPlex Fusion (24plex STR kit)



Size standard not shown

GlobalFiler Allelic Ladder



STR Marker Layouts for Y-STR Kits



Expanding the Forensic Core Competency



Level of Certainty

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

Arrest Made in L.A. 'Grim Sleeper' Killings

Published July 07, 2010 | Associated Press

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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.



Lonnie David Franklin Jr.

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 **NetBlo**



http://ishinews.com/wp-content/uploads/2012/10/Rapid-DNA-Miles-1.58MB.pc

RapidHIT 200 developed by IntegenX





Pete Vallone Erica Butts

- 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"

Fastest results swab-to-profile (Identifiler): 57 minutes

Rapid PCR Thermal Cycling Profile Identifiler STR kit 28 cycles of PCR



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

Slide from Peter Vallone (NIST)

Full Identifiler 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



STRs vs SNPs Article

Butler et al. (2007) STRs vs SNPs: thoughts on the future of forensic DNA testing. Forensic Science, Medicine and Pathology 3:200-205.

Forensic Sci Med Pathol (2007) 3:200–205 DOI 10.1007/s12024-007-0018-1

ORIGINAL PAPER

STRs vs. SNPs: thoughts on the future of forensic DNA testing

John M. Butler · Michael D. Coble · Peter M. Vallone

- 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)

Sufficient DNA quantity (ng)

Lower amounts of DNA being tested Direct Matching (or Parentage) Challenging kinship search questions

Familial Searching Attempts (fishing for brothers or other relatives)

Solution: Additional Markers (Y-chromosome, more STRs) and Multiple Reference Samples

Touch DNA Attempts (poor quality, mixtures, low-level stochastic effects)

Solution: Replicate Testing

Be very cautious when outside the box...



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



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
- Calcinated remains

HID 52plex Auto 1: success 100%









+SNPs

STRs

P:

Geographical Origin Prediction



- Lao O, van Duijn K, et al. (2006) Proportioning whole-genome single-nucleotide-polymorphism diversity for the identification of geographic population structure and genetic ancestry. Am J Hum Genet 78: 680-90.
- Phillips, C., Salas, A., et al. (2007) Inferring ancestral origin using a single multiplex assay of ancestry-informative marker SNPs. FSI: Genetics 1: 273-280.
- Halder, I., Shriver, M., et al. (2008) A Panel of Ancestry Informative Markers for Estimating Individual Biogeographical Ancestry and Admixture From Four Continents: Utility and Applications. Hum Mut 29: 648-658.
- Pereira R., Phillips C., et al. (2012) Straightforward inference of ancestry and admixture proportions through ancestry-informative insertion deletion multiplexing. PLoS One;7(1):e29684.

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.



Slide from Manuel Fondevila (NIST, USC)

Phenotypic trait prediction



Liu F., et al. (2009). Eye color and the prediction of complex phenotypes from genotypes, Curr. Biol. 19:R192–R193

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:
 - <u>http://www.nist.gov/mml/bmd/genetics/ngs_hid_workshop.cfm</u>
- 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)

Forensic Science International: Genetics 6 (2012) 677-678

Contents lists available at SciVerse ScienceDirect



Forensic Science International: Genetics

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

Editorial

Focus issue—Analysis and biostatistical interpretation of complex and low template DNA samples

December 2012 – Forensic Science International: Genetics, volume 6, issue 6

Forensic Science International: Genetics 6 (2012) 679-688



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

P. Gill^{a,b,*}, L. Gusmão^c, H. Haned^d, W.R. Mayr^e, N. Morling^T, W. Parson^g, L. Prieto^h, M. Prinz¹, H. Schneider^J, P.M. Schneider^k, B.S. Weir¹

April 12, 2013 Webcast

http://www.nist.gov/oles/forensics/dna-analysttraining-on-mixture-interpretation.cfm

- 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"



Action

Resources Training

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

Swifter PCR Amplification



Stronger Powers of Discrimination

J Forensic Sci, September 2009, Vol. 54, No. 5 doi: 10.1111/j.1556-4029.2009.01110.x Available online at: www.blackwell-synergy.com

Carolyn R. Hill,¹ M.S.; John M. Butler,¹ Ph.D.; and Peter M. Vallone,¹ Ph.D.

Forensic Science International: Genetics Supplement Series 2 (2009) 23-24

Contents lists available at ScienceDirect

Forensic Science International: Genetics Supplement Series

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

Research article

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

John M. Butler^{a,*}, Carolyn R. Hill^a, Margaret C. Kline^a, David L. Duewer^a, Cynthia J. Sprecher^b, Robert S. McLaren^b, Dawn R. Rabbach^b, Benjamin E. Krenke^b, Douglas R. Storts^b

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- 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

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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