

Forensics: Human Identity Testing in the Applied Genetics Group

Workshop to Identify Standards Needed to Support Pathogen
Identification via Next-Generation Sequencing (SPIN)
October 20-21, 2014

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Disclaimer

NIST Disclaimer: Certain commercial equipment, instruments and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or it imply that any of the materials, instruments or equipment identified are necessarily the best available for the purpose.

Information presented does not necessarily represent the official position of the National Institute of Standards and Technology or the U.S. Department of Justice.

Our group receives or has received funding from the FBI Laboratory and the National Institute of Justice.

Applied Genetics Group

Group Leader
Peter Vallone

Advancing technology and traceability through quality genetic measurements to aid work in Forensic and Clinical Genetics

A core competency of our group is the application of *nucleic acid-based methods*
PCR – Genotyping – Sequencing – Real-time PCR – Digital PCR – DNA based SRMs

Forensic Genetics ← → Clinical Genetics

Recent focus areas: update of SRM2391c, digital PCR, & next generation sequencing

Topics

- Forensic DNA Typing
- History and SRMs supporting the DNA typing community
- Interlaboratory studies
- Education and training
- Technologies

Steps in Forensic DNA Analysis

Usually 1-2 day process (a minimum of ~8 hours)

Short Tandem Repeat (STR) Markers

Length-based polymorphism present in the human genome

TCCCAAGCTCTTCCTCTTCCTTAGATCAATACAGACAGAAGACAG
 GTG**GATAGATA**GATAGATAGATAGATAGATAGATAGATAGATA
 GATAGATA**TCATTGAAAGACAAAACAGATGGATGATAGATAC**
 ATGCTTACAGATGCACAC

= 12 GATA repeats ("12" is reported)

The number of consecutive repeat units can vary between people

The frequencies of these length-based alleles are known in the various population groups (published in the literature)

Identifiler (Applied Biosystems) 15 STR Loci Kit

Information is tied together with multiplex PCR and data analysis

The frequencies of the STR alleles are known in a population
The allele frequencies are independent and can be multiplied (product rule)

The Random Match Probability (RMP) is over 1 in 800 trillion for unrelated individuals

This test contains the 13 FBI core loci (NDIS)

Applications of Human Identity Testing

- Forensic cases: matching suspect with evidence
- Kinship determination
- Missing persons investigations
- Military DNA “dog tag”
- Convicted felon DNA databases
- Mass disasters: putting pieces back together
- Historical investigations
- Genetic genealogy

Human Identity Testing with DNA

- Always testing human DNA (one species)
- The majority of the identification tests are performed with a core set of short tandem repeat markers (STRs) 13 → 20
- Mitochondrial DNA (high copy number, maternally inherited)
- Selected SNPs (identity, biogeographical ancestry, phenotype)
- Currently the workflow is very similar in all DNA testing labs
- Extraction, qPCR quantification, multiplex PCR kit, separation and detection (capillary electrophoresis)
- Performed with very similar commercial reagents and instrumentation (no in house or home brew assays are used – validation is important)

FBI DNA Advisory Board Quality Assurance Standards for Forensic DNA Testing Laboratories

Oct 1, 1998

A lab must follow the QAS to attain accreditation

- Scope
- Definitions
- Quality Assurance Program
- Organization and Management
- Personnel
- Facilities
- Validation
- Analytical Procedures
- Equipment and Calibration Maintenance
- Reports
- Review
- Proficiency Testing
- Corrective Action
- Audits
- Safety
- Outsourcing

http://www.fbi.gov/about-us/lab/biometric-analysis/codis/qas_testlabs

Standard Reference Material 2391c : PCR-Based DNA Profiling Standard

- Components A through D are DNA extracts in liquid form
- Components E and F are cells spotted on 903 paper or FTA paper
- Certified values are for STR alleles based on length polymorphisms observed using capillary electrophoresis

Current price: \$626 USD

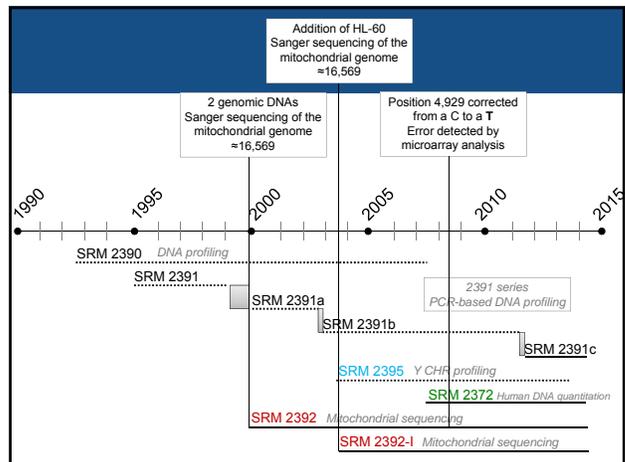
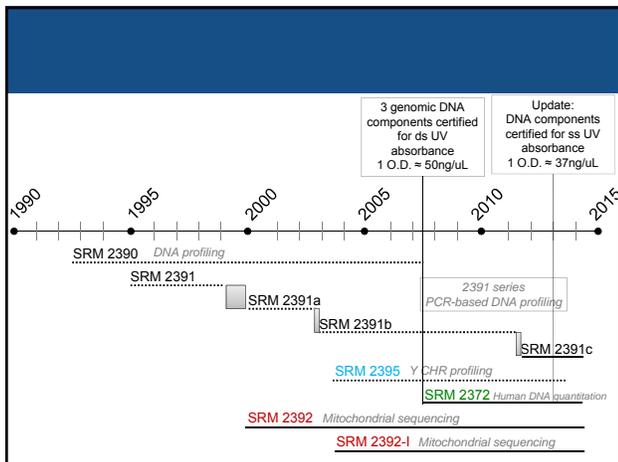
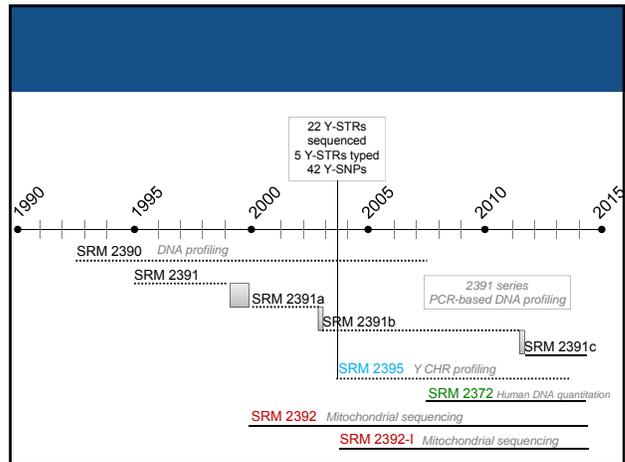
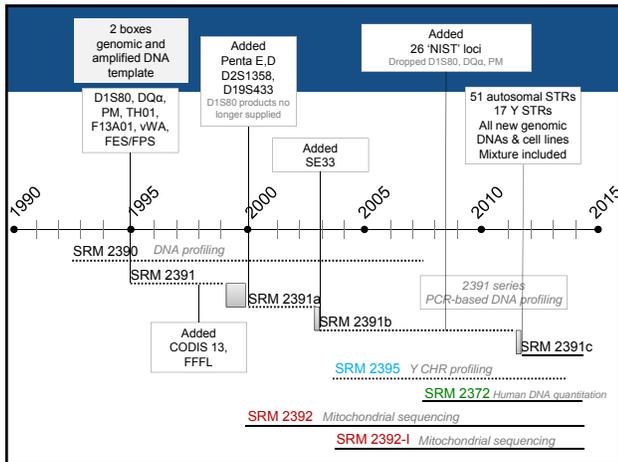
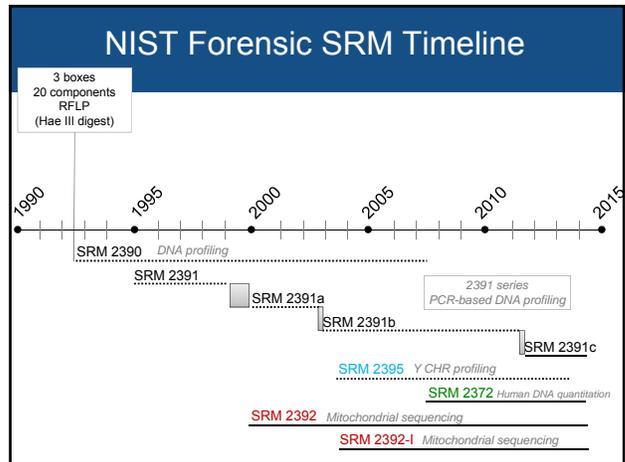
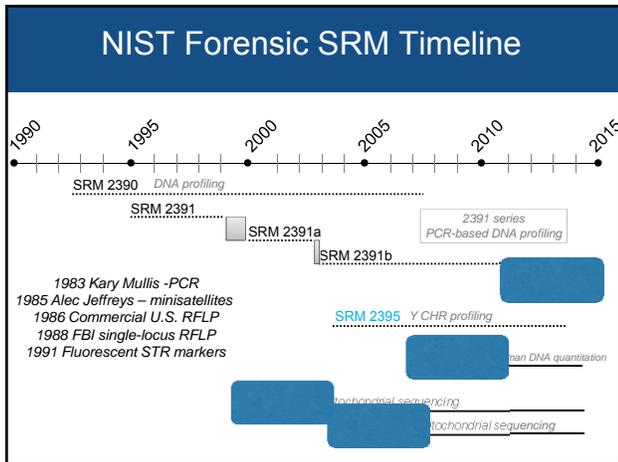
Genomic DNAs characterized for the expanded CODIS core loci and Y-STRs

Calibration with SRMs enables confidence in comparisons of results between laboratories

NIST Nucleic acid-based standards

SRM	NIST DNA-based SRMs
2366	Cytomegalovirus (CMV) for DNA Measurements ²
2393	CAG Repeat Length Mutation in Huntington's Disease ¹
2374	DNA Sequence Library for External RNA Controls ³
2372	Human DNA Quantitation Standard ¹
2391c	PCR Based DNA Profiling Standard ^{1,5}
2392, 2392-I	Mitochondrial DNA Sequencing ¹
2394	Heteroplasmic Mitochondrial DNA Mutation Detection Std ⁴
	Candidates currently under characterization
	BK Virus ³
	HER2 Copy Number Measurement ¹
	Pathlength Standard for Nanoliter Spectrophotometers ⁶
	Genome in a Bottle (NA 12878) ¹

¹extracted genomic DNA (human); ²extracted genomic DNA (viral in BAC); ³extracted DNA (plasmid) ⁴PCR products; ⁵cell lines on paper substrate; ⁶Uracil and Tryptophan solutions

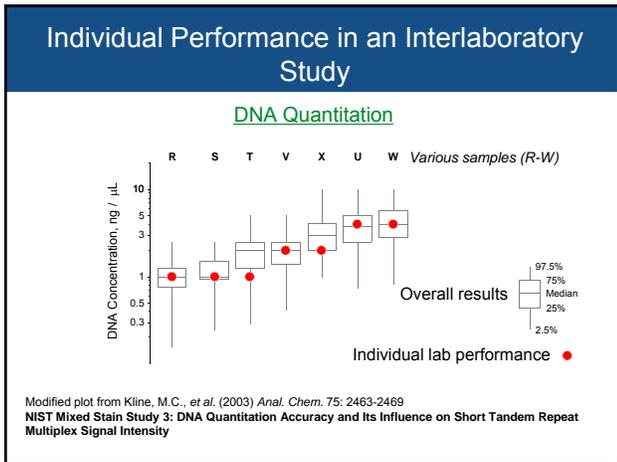


Purpose of an Interlaboratory Study

Interlaboratory studies (ILS) are a way for multiple laboratories to compare results and demonstrate that the **methods or instrument platforms** used in one's own laboratory are reproducible in another laboratory

NIST Initiated Interlaboratory Studies

ILS	Participating Labs	Publications/Dissemination
Evaluation of CSF1PO, TPOX, and TH01	34	Kline MC, Duerwer DL, Newell P, Redman JW, Reeder DJ, Richard M. (1997) Interlaboratory evaluation of STR triplex CTT. <i>J. Forensic Sci.</i> 42: 897-906
Mixed Stain Studies #1 and #2 (1997 and 1999)	45	Duerwer DL, Kline MC, Redman JW, Newell PJ, Reeder DJ. (2001) NIST Mixed Stain Studies #1 and #2: interlaboratory comparison of DNA quantification practice and short tandem repeat multiplex performance with multiple, genuine samples. <i>J. Forensic Sci.</i> 46: 1100-1110
Mixed Stain (2000)		The ILS lead to publications, reference materials, and dissemination of results to the community
DNA Quantification (2005)		Independent measure of how the DNA typing community is performing
Mixture Interpretation Study (2005)	69	http://www.cstl.nist.gov/strbase/interlab/mixub/mixubposter.pdf
Mixture Interpretation Study (2013)	108	Manuscript in preparation
Rapid DNA Testing (Fall 2013)	3	http://www.cstl.nist.gov/strbase/pub_pres/Vallone_BCC_Talk_Sept2013.pdf



- ### Results...
- Quantitation Methods and Frequency of Use
 - Interpretation of Semi-Quantitative Data
 - Method Sensitivities
 - Combining Within-Analyst Replicates and Within-laboratory Duplicates
 - Distributions of the Among-laboratory Results
 - Measurement Variability
 - Measurement Performance Characteristics
 - Consensus Values and Variability as Functions of [DNA]
 - Blot-Based Vs. Q-PCR Methods
 - Single-Source Vs. Multiple-Source Materials
 - Polypropylene Vs. Teflon Sample Containers

Education and Training

Since 10/1997 Information related to:

- Basics of STR typing
- Variant allele reports
- STR typing kit
- STR loci 'fact sheets'
- Population data
- Software tools

Team outputs:
 Since 1998 ≈ 200 papers
 Since 2000 ≈ 865 posters/presentations/workshops

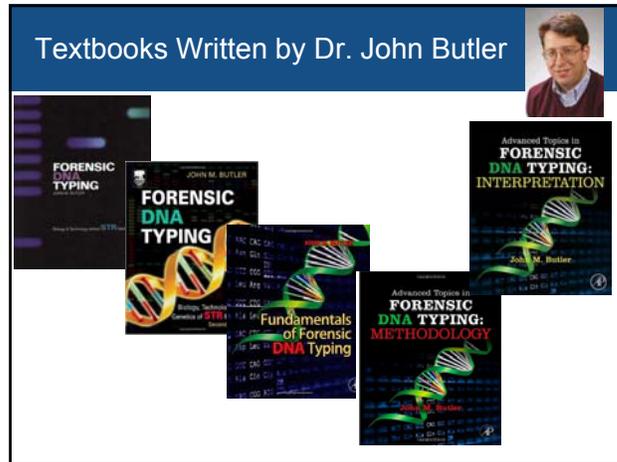
Created by John M. Butler and Dennis J. Reeder, NIST's National Science Director, with invaluable help from Jan Redman, Christian Esberg and Michael Tang. The current version was available using instructions.

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

General Information

- Report of STRbase 2008, 2001 Paper, Describing STRbase Overview Presentation
- Publication and Documentation from NIST, Human Identification Project Team
- NIST-Forensic Database
- Forensic Materials
- Links to other web sites
- Glossary of commonly used terms

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



Impact Examples

miniSTRs

*J Forensic Sci. Jan. 2005, Vol. 50, No. 1
Pages 82-93
Available online at: www.asmf.org*

Michael D. Coble, Ph.D. and John M. Butler, Ph.D.

Characterization of New MiniSTR Loci to Aid Analysis of Degraded DNA*

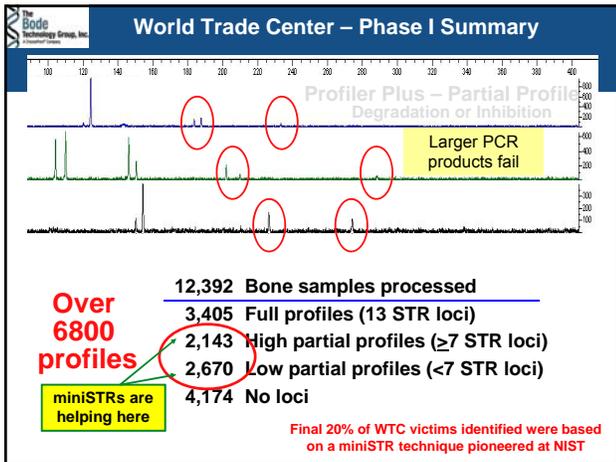
**Smaller PCR product size (<125 bp)
Utility: typing degraded samples**

ABSTRACT: A number of studies have demonstrated that successful analysis of degraded DNA specimens from mass fatality investigations with smaller sized polymerase chain reaction (PCR) products. We have examined the literature for new STR loci markers, which can generate amplicons less than 125 bp in size and would therefore be helpful in typing degraded DNA. A panel of 26 loci was designed and tested for the STR loci D10S1248, D22S1045, D10S1248, D10S1248, D10S1248, and D10S1248. All loci show a moderate degree of polymorphism among 474 U.S. population samples tested and were suitable for DNA template under controlled laboratory conditions and forensic DNA samples. The utility of these new loci for the analysis of the miniSTR assays for typing degraded bone samples while partial profiles were obtained with the most commercial STR kits.

KEYWORDS: forensic science, DNA typing, degraded DNA, STR, miniSTR, D10S1248, D22S1045, D10S1248

Initial work developing miniSTRs was started for WTC identifications
John Butler (NIST), Bruce McCord (FIU), Bode Technology Group (Lorton, VA)

Technology adopted by U.S. commercial STR kit vendors
(Life Tech: MiniFiler, Promega: S5)



Rapid PCR

Forensic Science International: Genetics

Demonstration of rapid multiplex PCR amplification involving 16 genetic loci*

Peter M. Vallone*, Carolyn M. Hill, John M. Butler

Up until 2008 PCR amplification times required approximately 3 hours

Utilizing new (faster) DNA polymerases and rapid PCR thermal cyclers we demonstrated results in 36 minutes

Enabling faster commercial STR typing kits (37 min) and fully integrated 'Rapid DNA' typing instruments (swab to profile in 90 minutes)

New STR Loci

Characterization of 26 MiniSTR Loci for Improved Analysis of Degraded DNA Samples

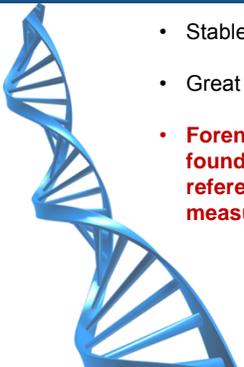
- The U.S. DNA database is expanding their core STR loci from 13 to 20
- 3 of the new candidates come from research performed at NIST (D2S441, D10S1248, D22S1045)
- NIST is providing the allele frequencies for U.S. populations
- Hill, C.R., Duewer, D.L., Kline, M.C., Coble, M.D., Butler, J.M. (2013) U.S. population data for 29 autosomal STR loci. *Forensic Sci. Int. Genet.* 7: e82-e83.

Emerging: Next-generation sequencing

Characterization of forensic SRMs with NGS technologies

Samples	Markers	Platforms
SRM 2391c	STR	MiSeq
SRM 2392/2392i (mtDNA)	mtDNA	PGM
NIST Population Samples	SNP	

Acknowledgements



- Stable funding (NIJ, NIST, FBI)
- Great team of people (past and present)
- **Forensic DNA typing built on a foundation of science, QAS, and reference materials to ensure quality measurements**

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- Stable funding
- Great team of people
- Focused goals
- Forensic DNA typing built on a foundation of science and QAS