NIST Standards for Genetic Testing

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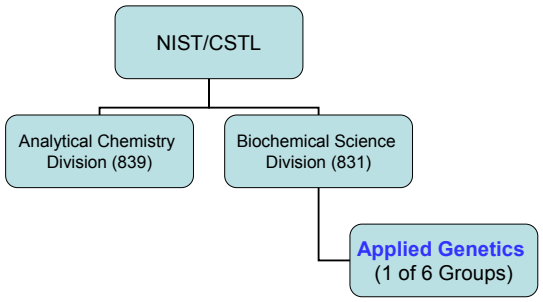
NIST Visit to IRMM
September 4-5, 2008

An Abbreviated Organizational Chart



Responsibilities for Forensic DNA Testing and Clinical and Agricultural Diagnostics (GMOs)

NIST Forensic DNA Project Team

Publications and presentations available on STRBase: http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

FY 2007 Achievements:
14 publications
44 presentations
9 workshops

Since 2000:
98 publications
254 presentations
29 workshops

National Institute of Justice

The Research, Development, and Evaluation Agency of the U.S. Department of Justice

Current Areas of NIST Effort with Forensic DNA

- **Standards** http://www.cstl.nist.gov/biotech/strbase/
  - Standard Reference Materials
  - Standard Information Resources (STRBase website)
  - Interlaboratory Studies
- **Technology**
  - Research programs in SNPs, miniSTRs, Y-STRs, mtDNA, qPCR
  - Assay and software development
- **Training Materials**
  - Review articles and workshops on STRs, CE, validation
  - PowerPoint and pdf files available for download

NIST DNA Reference Materials

Forensic Applications
- **STR PCR DNA Profiling** (SRM 2391b)
- Mitochondrial DNA Sequencing (SRM 2392-I, 2392)
- **Human Y-Chromosome DNA Profiling** (SRM 2395)
- RFLP DNA Profiling (SRM 2390) – now obsolete

Clinical Applications
- Fragile X Human DNA Triplet Repeat (SRM 2399)
- Huntington's Disease CAG Repeats (SRM 2393)

Platform Testing
- **Human DNA Quantitation** (SRM 2372)
- Heteroplasmic Mitochondrial DNA Mutation Detection (SRM 2394)
- DNA Sequence Library for External RNA Controls (SRM 2374)

A few others are in various stages of development

Congress Passed **the DNA Identification Act of 1994** (Public Law 103 322)

Formalized the FBI's authority to establish a national DNA index for law enforcement purposes.

FBI's DNA Advisory Board

Quality Assurance Standards for Forensic DNA Testing Laboratories

(October 1, 1998)

STANDARD 9.5

The laboratory shall check its DNA procedures annually or whenever substantial changes are made to the protocol(s) against an appropriate and available NIST standard reference material or standard traceable to a NIST standard.

The Tools of DNA Typing and SRM Needs

- RFLP Testing (Late 1980's) ~~SRM 2390~~
  - Radioactive Based
  - Chemiluminescent Based

  Technology no longer used
- PCR-Based Testing (Mid 1990's)
  - Dot-Blot
  - VNTR
  - STR (Fluorescent markers used today)

  SRM 2391..a..b

  Growth area
- DNA Sequencing (Late 1990's) SRM 2392, 2392-I
  - Mitochondrial DNA
- Y-Chromosome Testing (early 2000's) SRM 2395

  Growth area

Steps in Forensic DNA Analysis

Usually 1-2 day process (a minimum of ~5 hours)

Steps Involved: Collection; Specimen Storage; Extraction; Quantitation; Multiplex PCR; STR Typing; Interpretation of Results; Database Storage & Searching; Calculation of Match Probability

Blood Stain, Buccal swab — Sample Collection & Storage

DNA Extraction — DNA Quantitation (Slot Blot: 1 ng, 0.3 ng, No DNA, 0.5 ng, 0.5 ng, 0.7 ng, 1 ng, 1 ng)

Biology

Multiplex PCR Amplification

Genetics: **If a match occurs**, comparison of DNA profile to population allele frequencies to generate a case report with probability of a random match to an unrelated individual

DNA Database Search

Technology: DNA separation and sizing — STR Typing

Male: 13,14-15,16-12,13-10,13-15,16

Interpretation of Results

Short Tandem Repeat (STR) Markers

PCR primers anneal to unique sequences bracketing the variable STR repeat region

The overall PCR product size is measured

PCR product size generated

Fluorescent dye; Forward PCR primer; DNA template containing STR marker; Reverse PCR primer

GATA GATA GATA GATA

STR repeat region

PCR Product Size (bp)

Allelic Ladder; Sample #1; Sample #2

TCCCAAGCTCTTCCTCTTCCCTAGATCAATACAGACAGA
AGACAGGTG**GATAGATAGATAGATAGATAGATAGATA**
GATAGATAGATAGATATCATTGAAAGACAAAACAGAGA
TGGATGATAGATACATGCTTACAGATGCACAC

= 11 GATA repeats ("11" is all that is reported)

Position of Forensic STR Markers on Human Chromosomes

13 Core U.S. STR Loci

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

1997

1 2 3 4 5 6 7 8 9 10 11 12

8 STR loci overlap between U.S. and Europe

AMEL

Sex-typing

D13S317, D16S539, D18S51, D21S11, AMEL

13 14 15 16 17 18 19 20 21 22 X Y

Short Tandem Repeat (STR) Typing

Fluorescent dye-labeled primer

STR Repeat Region

(Maternal) 1 2 3 4 5 6

(Paternal) 1 2 3 4 5 6 7 8

GATA

forward primer hybridization region

reverse primer hybridization region

(size in bp)

75….80….100….120….140….160….180….200….220….240….260…..

RFUs 1000 500

6 139bp

8 147bp

DNA Separation and Detection

PATERNITY TESTING

Family Inheritance of STR Alleles (D13S317)

PCR product size (bp)

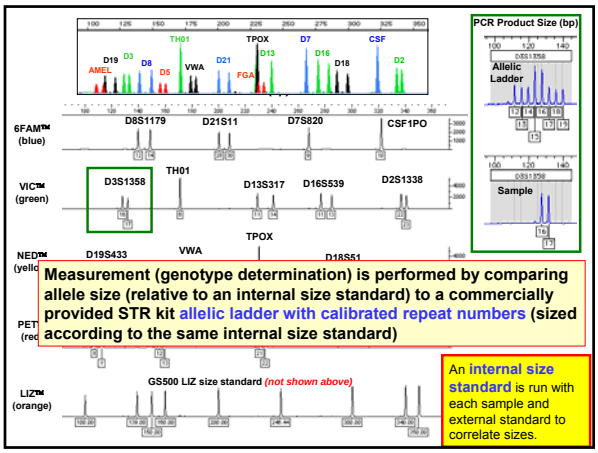
Father: 11, 14

Child #1: 12, 14

Child #2: 8, 14

Child #3: 11, 12

Mother: 8, 12



Different Genetic Tests Can Give Different Results Based on PCR Primer Positions

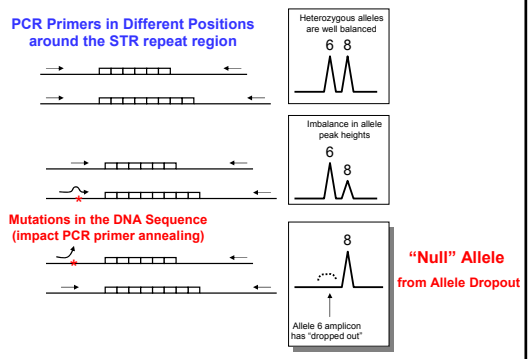
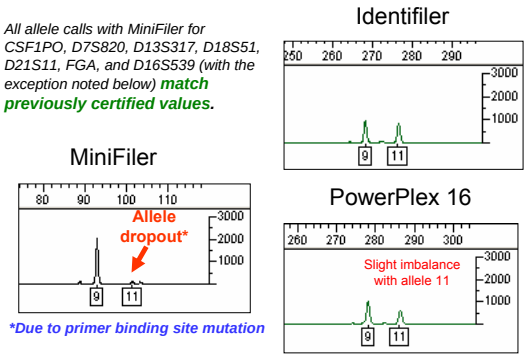


Figure 6.9, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

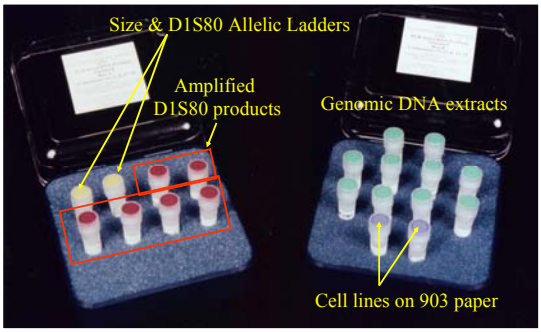
SRM 2391b Genomic 8 with D16S539

All allele calls with MiniFiler for CSF1PO, D7S820, D13S317, D18S51, D21S11, FGA, and D16S539 (with the exception noted below) ***match previously certified values.***



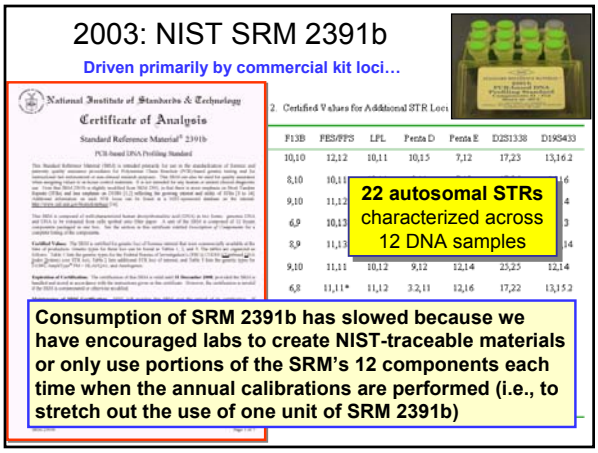
**Due to primer binding site mutation*

1995: SRM 2391 PCR-based DNA Profiling Standard



2003: NIST SRM 2391b

Driven primarily by commercial kit loci…



Consumption of SRM 2391b has slowed because we have encouraged labs to create NIST-traceable materials or only use portions of the SRM's 12 components each time when the annual calibrations are performed (i.e., to stretch out the use of one unit of SRM 2391b)

SRM 2391b and 2395 Certificate Updates

- **SRM 2391b** (Autosomal STR Loci)
  - **MiniFiler examined** (allele dropout with component 8 and D16S539)
  - **Additional Loci: 26 new miniSTR loci**
  - Demonstrating extended stability (new quantitation data and no significant degradation to existing components)

  http://www.cstl.nist.gov/biotech/strbase/srm2391b.htm
- **SRM 2395** (Y-STR and Y-SNP Loci)
  - **Yfiler loci sequenced** (DYS635 now included)
  - **Additional Loci: 20 new Y-STR loci**
  - Demonstrating extended stability (new quantitation data and no significant degradation to existing components)

  http://www.cstl.nist.gov/biotech/strbase/srm2395.htm

Awaiting Final Document Approval of Revised Certificates

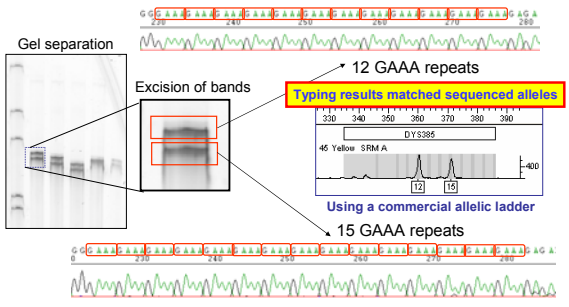
STR Typing Measurement Issues

- STR genotypes are generated using PCR amplification and electrophoretic sizing that involves an internal size standard with each sample.
- The forensic DNA community almost exclusively uses STR typing kits to obtain results (there are different kits available that examine the same common markers).
- PCR amplification is expected to generate consistent genotypes as long as primer positions are not changed between kits. Primer changes can result in allele dropout due to primer site mutations.
- Occasionally new commercial kits are created with additional loci.
- General STR repeat nomenclature rules have been established but do have some subjectivity in them permitting possible differences in how STR alleles are named.

Two Different Independent Methods Used

- Size Analysis/Genotyping
  - Electrophoretic separation and sizing of PCR product compared to an internal size standard followed by **comparison to the sizes of one or more sequenced alleles** (could be commercially available allelic ladder) run in-house with the same conditions, instrument, and internal size standard
- DNA Sequence Analysis
  - Isolation of each individual allele
  - DNA sequence analysis followed by **direct counting of the number of repeats** (and correlation to size variation observed during STR typing)

Comparing DNA Sequencing Information to STR Typing Data



SRM 2395 Component A (DYS385 a/b alleles)

Other Considerations for STR Typing SRMs

- Homogeneity
  - Single lot in a single container aliquoted to individual tubes packaged as components in each SRM unit
- Purity (absence of significant impurities)
  - Single source DNA samples used; while it is not certified to be "mixture-free", foreign, contaminating alleles should not be seen; thus, the solutions can be considered >~90% pure (mixture detection limit ~10%)
- Stability
  - Generally certified for 5-6 years but likely stable much longer under appropriate storage conditions (refrigerated or frozen, out of sunlight)
- Concentration
  - Not certified; some variability in amount of DNA present can be expected; samples generally supplied at near "ready-to-use" concentrations (~1-2 ng/μL)

Consistency of genotype matters not consistency in amount provided…

Mitochondrial DNA Heteroplasy (Mixture)

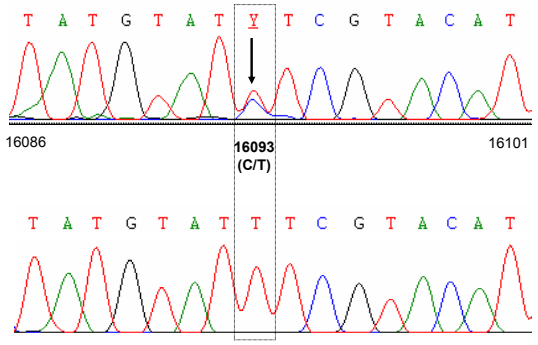


Figure 10.9, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

Summary of Issues Faced

- Initial selection of material (SRM components) was for a specific purpose usually and may not address every need in the future (a new locus may not exhibit a diverse set of alleles)
- The forensic community uses commercial STR typing kits – and only wants a confirmation of the allele calls against an allelic ladder – should we fully sequence every sample?
- Some genetic loci will not be able to have every allele sequenced
- There are lots of loci that could be "certified" – how do we decide which ones to include in future certificate updates?

SRM 2372
Human DNA Quantitation Standard

Components

A: Male/single donor/RNased/NIST
B: Female/multiple donors/NIST
C: Mixture/male & female/commercial

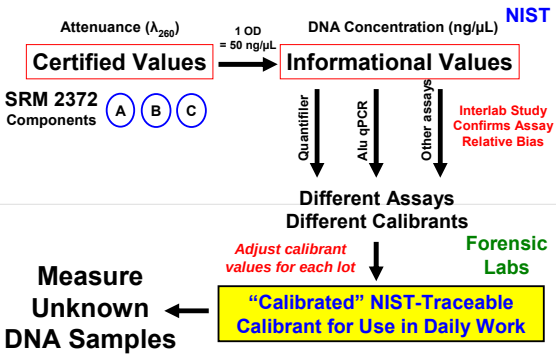
Quantities supplied:
110 µL of Human Genomic DNA ≈ 50ng/µL

Certification

Decadic Attenuance (Absorbance) by a US National Reference Spectrophotometer
Homogeneity by a Cary 100 Bio Spectrophotometer
Validation of conventional [DNA] by Interlaboratory Study and NIST qPCR studies

Overview of SRM 2372 Values and Use

See http://www.cstl.nist.gov/biotech/strbase/training/AAFS2008_qPCRworkshop.htm



Thank you for your attention…

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

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

Questions?

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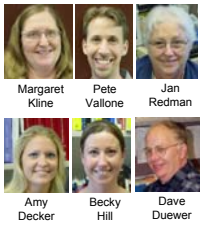
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