M.C.Kline – VIFSM Conf
NIST Research Update

March 29, 2006

NIST Research Update
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Human Identity Project Team, U.S. National Institute of Standards and Technology

2nd Annual Present & Future Technological Advances in Human Identification (Roanoke, VA) March 27-29, 2006

Disclaimers and Collaborations

Funding: Interagency Agreement 2003-IJ-R-029 between the National Institute of Justice and NIST Office of Law Enforcement Standards

Points of view are those of the authors and do not necessarily represent the official position or policies of the US Department of Justice. 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 endorsement by the National Institute of Standards and Technology nor does it imply that any of the materials, instruments or equipment identified are necessarily the best available for the purpose.

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

Past and Present Collaborators (also funded by NIJ):
Mike Hammer and Alan Redd (U. AZ) for Y-chromosome studies
Tom Parsons, Rebecca Just, Jodi Irwin (AFDIL) for mtDNA coding SNP work
Sandy Calloway (Roche) for mtDNA LINEAR ARRAYS
Bruce McCord and students (FL Int. U.) for miniSTR work
Marilyn Raymond and Victor David (NCI-Frederick) for cat STR work
Artie Eisenberg and John Planz (U. North Texas) for miniSTR testing on bones

Team Impact on Forensic Community

• 28 publications (published or submitted) since Nov 2004
• 51 presentations to the community since Nov 2004
• All NIST publications and presentations available on STRBase: http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

• Training materials from 2 workshops
– Albany DNA Academy (June 13-14, 2005) with Bruce McCord
– NFSTC Validation Workshop (August 24-26, 2005) with Robyn Ragsdale

• AAFS 2006 Workshop #6 (John Butler and Bruce McCord) Advanced Topics in Capillary Electrophoresis and DNA Typing

Current Areas of NIST Effort with Forensic DNA

• Standards
  – 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

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

Formulated 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, 1996)

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.

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
Standard Reference Materials

- Relevant Forensic DNA SRMs
  - SRM 2391b (DNA profiling – STRs, D1S80, DQA1/PM)
  - SRM 2392-I (mtDNA)
  - SRM 2395 (Y-chromosome)
  - SRM 2372 (Human DNA quantitation); in development
- Provides national/international traceability and compatibility (aids in ISO 17025 compliance)

SRM 2372
Human DNA Quantitation Standard

Component A: Male (blood)
Component B: Female (blood)
Component C: Mixture (placenta)

- Genomic DNA isolated by Salt out procedure
- Treated with RNase and re-precipitated
- UV spectroscopy 340-220 nm on a NIST calibrated spectrophotometer
- Assume $A_{260} = OD_{260} = 1$ for a 50 µg/mL solution

Not Available at This Time: Fall 2006?
Planned Amounts: Each component 50 µL of Human Genomic DNA with a concentration targeted @ 50 ng/µL. The [DNA] for each component will be listed in the materials Certificate of Analysis.

Interlaboratory Comparisons
Laboratory Performances with Real-Time PCR Methods

Comparing results from 60 data sets
60 data sets
- Methods 1 through 5
- Dilutions 1 - 7

Samples run at each concentration were plotted as a function of Method/Calibrant...
**Comparison of Methods**

Using C1 as the Calibrant

<table>
<thead>
<tr>
<th>Method</th>
<th>1-Qfiler</th>
<th>2-QfilerY</th>
<th>3-Alu</th>
<th>4-CFS</th>
<th>5-CADOJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 ng/µL</td>
<td><img src="Graph1.png" alt="Graph" /></td>
<td><img src="Graph2.png" alt="Graph" /></td>
<td><img src="Graph3.png" alt="Graph" /></td>
<td><img src="Graph4.png" alt="Graph" /></td>
<td><img src="Graph5.png" alt="Graph" /></td>
</tr>
</tbody>
</table>

C1 and C2 are cell line DNAs; C3-C6 are single/multiple source DNAs.

**Comparison of Methods**

Using C3 as the Calibrant

<table>
<thead>
<tr>
<th>Method</th>
<th>1-Qfiler</th>
<th>2-QfilerY</th>
<th>3-Alu</th>
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**STRBase:**

A Standard Information Resource

Primary updates performed monthly

- Summary of variant alleles and tri-allelic patterns
- List of STR references (Reference Manager database)
- NIST publications and presentations

- New content is being added regularly to aid training and to support forensic DNA laboratories

http://www.cstl.nist.gov/biotech/strbase/
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
http://www.cstl.nist.gov/biotech/strbase/var_tab.htm

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**Validation Information**

- Survey initiated at June 2004 NIJ meeting and conducted last summer resulted in 53 responses
- Talk at Promega meeting Oct 2004
- Validation summary sheets
- Validation website on STRBase
- Workshop conducted August 2005 at NFSTC (DVD to be released as part of President’s DNA Initiative training)

- We invite submission of your internal validation studies for inclusion in the NIST validation website
  http://www.cstl.nist.gov/biotech/strbase/validation.htm

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**Validation Workshop**

(Aug 24-26, 2005 at NFSTC)

http://www.cstl.nist.gov/biotech/strbase/validation/validationworkshop.htm

**COURSE CONTENTS**

**Day #1**

- Validation Overview (John)
- Introduction to DAB Standards (Robyn & John)
- Developmental Validation (John)

**Day #2**

- Inconsistency in Validation between Labs (John)
- Internal Validation (Robyn)
- Method Modifications and Performance Checks (Robyn)

**Day #3**

- Practical Exercises (Robyn)

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http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
### Laboratory Internal Validation Summaries

**Soliciting Information on Studies Performed by the Community**

- **DNA Quantitation Study (QS04)**
  - 8 DNA samples supplied
  - 84 laboratories signed up (80 labs returned results)
  - 267 data sets using 19 different methods
  - 60 data sets with real-time qPCR (37 Quantifiler data sets)

- **Mixture Interpretation Study (MIX05)**
  - 94 labs signed up (69 labs returned data)
  - Interpretation requested of provided e-grams for 4 mock sexual assault cases
  - Data analysis is still on-going...
  

### NIST Initiated Interlaboratory Studies

**Studies Involving STRs**

- **Evaluation of CSF1PO, TPOX, and TH01**
  - 34 labs

- **Mixed Stain Studies #1 and #2 (Apr-Nov 1997 and Jan-May 1999)**
  - 45 labs

- **Mixed Stain Study #3 (Oct 2000-May 2001)**
  - 74 labs

- **DNA Quantitation Study (Jan-Mar 2004)**
  - 80 labs

- **Mixture Interpretation Study (Jan-Mar 2005)**
  - 69 labs

**Publications**

- **Mixed Stain Studies #1 and #2**

- **Mixed Stain Study #3**

### Genetic Markers Under Examination

- **New miniSTRs**
- **mtDNA**
- **CORE STR Loci**
- **Variant allele sequencing**
- **Y-chromosome STRs**

### STR Allele Sequencing and Characterization

- **Variant characterization**
  - TPOX 10.3 (Maryland State Police)
  - D18S51 null alleles (FFS and Kuwait govt)
  - D18S51 allele 40 (Nebraska State Crime Lab)
  - D18S51 allele “5.3” (DNA Solutions)
  - VWA allele “15.1” (Peter de Knijff)
  - DYS19 allele 9 (Italy)
  - DYS392 allele “10.2” (AFDIL)
  - DYS439 allele 7 (Italy)

[http://www.cstl.nist.gov/biotech/strbase/STRseq.htm](http://www.cstl.nist.gov/biotech/strbase/STRseq.htm)
STR Allele Sequencing and Characterization

- Locus duplication or deletion
  - DYS390 deletion (CFS Toronto)
  - DYS19 duplication (Portugal)
  - DYS392 deletion (MN BCA) - H4 duplication (Portugal)
  - DYS385 triplication (Italy)
  - DYS389II duplication (Italy)
  - DYS439 duplication (Italy)

- Forensic labs are sending us unusual STR alleles for sequence characterization
  
  http://www.cstl.nist.gov/biotech/strbase/STRseq.htm

Genetic Markers Under Examination

New miniSTRs

CORE STR Loci

Variant allele sequencing

A miniSTR is a reduced size STR amplicon that enables higher recovery of information from degraded DNA samples

- Original miniSTR paper with CODIS loci, D2, D19, Penta D, Penta E
- Many CODIS loci are too big and make poor miniSTRs
- New miniSTRs and assays: NC01, NC02
- New miniSGM miniplex: AMEL, TH01, FGA, D18, D16, D2
- EDNAP/ENFSI degraded DNA study coordinated by Peter Gill
- Creation of miniSTR information on STRBase
  
  http://www.cstl.nist.gov/biotech/strbase/miniSTR.htm

miniSTRs for Degraded DNA

- PCR product size (bp)
  - TH01 191 bp
  - TPOX 117 bp
  - CSF1PO 146 bp
  - FGA 191 bp
  - D21S11 148 bp
  - D7S820 105 bp
  - D2 117 bp

http://www.cstl.nist.gov/biotech/strbase/miniSTR.htm
Describes new primer sequences for all CODIS loci and initial assays developed in 2003 issue of J. Forensic Sci.

**Initial Testing Results with Potential miniSTR Loci**

- TH01 Allelic Ladder
  - Size relative to ABI kits
- TPOX Allelic Ladder
- CSF1PO Allelic Ladder
- D21S11 Allelic Ladder
- D7S820 Allelic Ladder
- FGA Allelic Ladder
- D2S1338 Allelic Ladder
- D16S539 Allelic Ladder
- D18S51 Allelic Ladder

**NC01 Loci**

- D10S1248
- D14S1434
- D22S1045

**Plans to characterize ~27 new miniSTRs**

**Forthcoming Article Advocating miniSTRs**

- They recommend that miniSTRs “be adopted as the way forward to increase both the robustness and sensitivity of analysis.”

- They recommend that European laboratories adopt three new mini-STR loci, namely: D10S1248, D14S1434, and D22S1045.

**New Autosomal STR Loci**

- NC01 loci: D10S1248, D14S1434, D22S1045
- Peter Gill and the EDNAP/ENFSI group have recommended the NC01 loci as an extension of current European core loci following their demonstrated success in the recent EDNAP degraded DNA interlab study
- Being adopted/explored in multiple U.S. paternity testing labs (BRT Labs, Orchid Cellmark East Lansing, DNA Diagnostics Center)
- Population data, locus characterization, and allelic ladders for 27 new autosomal STRs under development as new miniSTRs
- All new STR loci are physically unlinked to CODIS and ENFSI core loci

www.elsevier.com/life...www.elsevier.com/life...www.elsevier.com/life...

http://www.cstl.nist.gov/biotech/strbase/miniSTRs.htm
Genetic Markers Under Examination

New miniSTRs

CORE STR Loci

Variant allele sequencing

Y-chromosome STRs

Work with Y-STRs

- Beta-testing of all commercial Y-STR kits
- U.S. population data supplied to Yfiler haplotype database
- 49 Y-STR loci evaluated with ~650 U.S. samples
- New Y-chromosome information on STRBase linking to all available haplotype databases
- Human Y-Chromosome DNA Profiling Standard Reference Material (SRM 2395) – updates with DYS635 for Yfiler
- Separation of two brothers with 47 Y-STRs

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

New Y-Chromosome Information

Resources on STRBase

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

NIST Work with New Y-STR Loci

14,535 types generated across 27 new loci

YHRD has 9,634 haplotypes (from 61 populations) with SWGDAM recommended loci

Subdividing Common Types with More Loci

657 males from 3 U.S. populations

U.S. Haplotype

Identical: DYS444,446,485,509,505,508,534,540,556
Subdivide into two groups (2/1): DYS...449,465,520,532,533,557,594,643
Subdivide into three groups (1/1/1): DYS522, DYS576

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http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
Genetic Markers Under Examination

- New miniSTRs
- SNPs
- Y-chromosome STRs

CORE STR Loci

Variant allele sequencing

Work with SNP Loci

- U.S. population frequencies with 70 autosomal SNPs
- U.S. population information with 50 Y-SNPs
- Construction of 12plex autosomal SNP assay
  - Vallone et al. (2005) Progress in Forensic Genetics 11
- Creation of Forensic SNP Information website on STRBase

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

Work with mtDNA

- Collaboration with Armed Forces DNA Identification Laboratory to develop multiplex mtDNA SNP assays for coding region polymorphisms
- Beta-testing and automation of Roche LINEAR ARRAY HV1/HVII probes
- Population study performed with LINEAR ARRAY HV1/HVII probes
- Exploration of effective strategies for forensic analysis in the mitochondrial DNA coding region

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

Summary of Our Population Typing with Roche mtDNA LINEAR ARRAYS

LINEAR ARRAY summary
- 282 different types
- 185 were unique (occurred only once)
- 51 samples had "Most Common Type"

HV1/HV2 sequencing summary
- 518 different types
- 454 were unique (occurred only once)
- 17 samples had "Most Common Type"

"Most Common Type" evaluated further with mtDNA coding region SNP assay


Typing frequencies for 666 NIST population samples

<table>
<thead>
<tr>
<th># in Group</th>
<th>Freq.</th>
<th>% Types</th>
<th>% People</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>155</td>
<td>10.6</td>
<td>27.8</td>
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<tr>
<td>2</td>
<td>40</td>
<td>10.3</td>
<td>13.6</td>
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<td>3</td>
<td>18</td>
<td>3.4</td>
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<td>1.9</td>
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<tr>
<td>19</td>
<td>1</td>
<td>0.1</td>
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</tr>
</tbody>
</table>

Population data has been collected on >1,000 samples (662 U.S. and 375 world samples) from 10 different population groups.
Software Tools

- AutoDimer – multiplex PCR primer screening tool
  http://www.cstl.nist.gov/biotech/strbase/AutoDimerHomepage/AutoDimerProgramHomepage.htm

- mixSTR – mixture component resolution tool

- Multiplex_QA – quality assessment tool for monitoring instrument performance over time

- NIST U.S. population database (internal Access database)
  http://www.cstl.nist.gov/biotech/strbase/software.htm

Multiplex_QA Overview

- Research tool that provides quality metrics to review instrument performance over time (e.g., examines resolution on internal size standard peaks)

- Runs with Microsoft Excel macros. Requires STR data to be converted with NCBI’s BatchExtract program into numerical form.

Available for download from STRBase:
  http://www.cstl.nist.gov/biotech/strbase/software.htm

Training Materials and Review Articles

- Workshops on STRs and CE (ABI 310/3100)
  – Taught with Bruce McCord (Florida Int. Univ.)
  – NEAFS (Sept 29-30, 2004)
  – U. Albany DNA Academy (June 13-14, 2005)
  – AAFS Feb 2006 Workshop #6 (February 20, 2006)

- Validation Workshop
  – Taught with Robyn Ragsdale (FDLE) at NFSTC (August 24-26, 2005)

- PowerPoint slides from Forensic DNA Typing, 2nd Edition
  – >150 slides available now (~1,000 planned) for download
  http://www.cstl.nist.gov/biotech/strbase/software.htm

- Review articles

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

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

AAFS Workshop #6 (Feb 2006, Seattle)
Advanced Topics in STR DNA Analysis
Instructors: John Butler and Bruce McCord
For DNA analysts using the ABI 310 or ABI 3100 who would like to better understand the underlying issues and science involved with STR DNA typing

- STR Biology, Markers, and Methods
- Capillary Electrophoresis Instrumentation: Theory and Application
- Validation Aspects to Consider in Bringing a New STR Kit “On-line”
- CE Troubleshooting
- STR Mixture Interpretation
- DNA Quantitation with Real-Time qPCR
- Low-copy Number Issues
- Y-STRs and mtDNA

Workshop Slide Handouts
Handouts available as downloadable pdf files from
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm/NEAFSworkshop

2 day workshop with >500 slides describing STRs and CE (ABI 310 and ABI 310)

NEAFS CE-DNA Workshop (Butler and McCord)
Sept 29-30, 2004

Capillary Electrophoresis in DNA Analysis

Outline for Workshop

- Introduction
  - STR Analysis
    - Distribution, CE and ABI 310/3100
    - Data Interpretation
    - Additional Topics: STR protocols for real-world cases
    - Troubleshooting the ABI 310/3100 (Part II: Troubleshooting)
    - Additional Topics: Y-STRs, validation, and more
- Review and Test

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

Review Article on Core STR Loci

- J.M. Butler “Genetics and Genomics of Core STR Loci Used in Human Identity Testing”

  Journal of Forensic Sciences, in press (March 2006)

  Reviews STR kits, genomic locations, mutation rates, potential genetic linkage, and known variant alleles for autosomal STR and Y-STR loci

  Covers characteristics of 18 autosomal loci (13 core CODIS loci, D2, D19, Penta D, Penta E, SE33) and 11 SWGDAM-recommended Y-STR loci
Acknowledgments

Funding from interagency agreement 2003-IJ-R-029 between NIJ and the NIST Office of Law Enforcement Standards

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- Bruce McCord and students (FL Int. U.) for miniSTR work
- Marilyn Raymond and Victor David (NCI-Frederick) for cat STR work
- Artie Eisenberg and John Planz (U. North Texas) for miniSTR testing on bones
- Murray Brilliant (U. AZ) for phenotype markers
- Ken Kidd (Yale U.) for SNP typing population samples

The many forensic scientists and their supervisors who took time out of their busy schedules to examine the MIX05 data provided as part of this interlaboratory study.

Thank you for your attention...

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

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