NIST Resources for the Forensic DNA Community

John M. Butler
and Human Identity Project Team
National Institute of Standards and Technology

NIJ Conference
Crystal City, VA
July 22, 2008

Presentation Outline

- NIST projects overview (OLES)
- SRM update: SRM 2372, 2391b, 2392, 2395
- STR allele sequencing (Margaret Kline)
- Mixture work (Amy Decker, Angie Dolph & Michelle Burns)
- Additional STRs and 26plex (Becky Hill)
- Rapid PCR for DNA biometrics (Pete Vallone)
- Training workshops (John Butler/Pete Vallone)

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

Mark Stolorow is now Director of the NIST Office of Law Enforcement Standards (OLES)

http://www.eeel.nist.gov/oles/forensics.html

NIST Gaithersburg Campus

Located in Gaithersburg, Maryland, on approximately 234 hectares (578 acres) just off Interstate 270 about 25 miles northwest of Washington, D.C.

http://www.nist.gov

Advanced Chemical Sciences Laboratory (Building 227)

NIST Human Identity Project Team

SRMs
OLES
- Forensic DNA Work
- Microchip and microarray PCR
- Fingerprints
- Hair analysis
- Other biometrics

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http://www.eeel.nist.gov/oles/directory.html

Sue Ballou (Forensics)

http://www.eeel.nist.gov/oles/forensics.html

Physics Laboratory

OLES

Defines U.S. Time
3 Nobel Prize Winners (97, 01, 05)

Chemical Science and Technology Laboratory
Human Identity Project Team
SRMs

MSEL

NIST Center for Neutron Research

Electronic and Electrical Engineering Laboratory
Biometrics

WTC Investigation

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Forensics Research at NIST

- Computer (digital evidence) forensics
- Ballistics
- Fingerprints
- Arson investigation
- DNA

For more information, contact:
Susan Ballou
Program Manager for Forensic Sciences
susan.ballou@nist.gov
301-975-8750

NIST Human Identity Team Projects
Funded by the National Institute of Justice
http://www.cstl.nist.gov/biotech/strbase/NISTprojects.htm

33 different projects are described

Projects
- STRBase
- SRM 2461 Casing
- DNA quantitation
- Forensics
- Human DNA
- Alternative forensic DNA markers

SRM 2461

Funding and support

Pete Vallone
John Butler
Margaret Kline
Amy Decker
Becky Hill
Dave Duewer
Jan Redman

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

Why We Have Been Successful

- Well-qualified and hard-working team members
- Meet weekly and communicate daily on project specifics
- Solid financial support from NIJ
- Our own supply sergeant (Jan Redman)
- Publish and present often (maintains focus)
- Maintain a comprehensive literature collection for our field
- Share what we learn (STRBase, textbook)
- Know our customers’ needs and work hard to meet them

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
Support to the Community

- Conduct interlaboratory studies
- Perform beta-testing of new human identity testing products
- We provide input to (or have aided):
  - Scientific Working Group on DNA Analysis Methods (SWGDAM)
  - Department of Defense Quality Assurance Oversight Committee for DNA Analysis
  - American Prosecutors' Research Institute (APRI) DNA Forensics Program “Course-in-a-Box” for training lawyers
  - WTC Kinship and Data Analysis Panel (KADAP)
  - 2005 Hurricane Victim DNA Identification Expert Group (HVDIEG)

STRBase: a Community Resource...

The Revised Quality Assurance Standards (which were recently approved by the FBI Director—to be effective July 1, 2009) will be posted on STRBase tomorrow.

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Benefits of Website like STRBase

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

- Develops expertise when collecting information
- Requires me to stay up-to-date with field
- Provides transparency to our team’s work
- Training tool and resource for the world
- Respected resource for >11 years
- ~10,000 pages of information available now
- >300,000 hits cumulative

Our Team Provides Support to Other NIJ Grantees and Commercial Collaborations

Support to NIJ-Funded Projects

- Akonni Biosystems (microchip SNPs)
- Network Biosystems (microchip CE)
- Roche (mtDNA strips)
- IBIS (mass spec of STRs)
- Marshall University (NEST Project)
- Florida International University (miniSTRs)

Recent Commercial Collaborations

- Applied Biosystems – MiniFiler concordance
- Biomatrica – testing new DNA storage materials

Supplying U.S. population samples, multiplex assays, or evaluation of materials

Current Activities at NIST

Enabled by Our NIJ Partnership

- Standard Reference Materials
  - SRM 2372 (DNA quant) released Oct 2007 (>130 units in use)
  - Updates to SRM 2391b (STRs), 2395 (Y-STRs), 2392 (mtDNA)
- Technology Evaluation and Development
  - Unusual STR allele characterization
  - Y-chromosome characterization (mutation rates, deletions)
  - New STR loci and assays (26plex)
  - Rapid multiplex PCR protocol (multiplex STR amplification in <35 min)
- Training Materials
  - AAFS workshops on DNA quantitation and mixture interpretation
  - Third edition of Forensic DNA Typing textbook

Standard Reference Materials

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

Traceable standards to ensure accurate measurements in our nation’s crime laboratories

SRM 2391b – CODIS STRs
SRM 2392 – mtDNA
SRM 2395 – Y-STRs
SRM 2372 – DNA quantitation

Helps meet DAB Std. 9.5 and ISO 17025

Calibration with SRMs enables confidence in comparisons of results between laboratories

Certificate Updates – new information (loci) added and stability testing performed to enable extension of expiration dates

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
Unusual STR Allele Characterization (Free)

Send us any unusual variant or null alleles and we will sequence them...

Send 10-20 ng of DNA (or 2-3 FTA bloodstain punches)
Contact margaret.kline@nist.gov or john.butler@nist.gov
Information will be posted on STRBase .../STRseq.htm
Sequence details provided back to sender

D18S51 deletion results in 5.3 Allele

Genotypes as 8
Forward Primer
Genotypes as 5.3
Reverse Primer
9 base deletion
5 repeats
5.3 based on size

STR Locus Sequence Variability

• Collaboration with Tom Hall (IBIS): has analyzed some of our NIST U.S. population samples by their mass spec methods

• In many samples the mass spec detected SNPs (base pair changes) within specific STR loci

• Margaret Kline has gone back and sequenced some of these samples to verify the mass spec results and determine where the SNPs are located

SNPs within the D8S1179 repeat
Repeat is TCTA
Three NIST samples have genotypes 13,13.
Analysis by Mass Spec indicates the presence of SNPs (Tom Hall, IBIS)
Confirmation of the Mass Spec by sequencing at NIST indicates:
There are 4 different “13” alleles in these three samples.

Base Pair difference between Repeats

D8S1179

<table>
<thead>
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<th>Allele</th>
<th>AVG</th>
<th>SD</th>
<th>N</th>
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<tbody>
<tr>
<td>8</td>
<td>123.82</td>
<td>0.02</td>
<td>10</td>
</tr>
<tr>
<td>9</td>
<td>127.90</td>
<td>0.02</td>
<td>7</td>
</tr>
<tr>
<td>10</td>
<td>132.03</td>
<td>0.06</td>
<td>42</td>
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<td>11</td>
<td>136.17</td>
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<td>12</td>
<td>140.42</td>
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<td>13</td>
<td>144.93</td>
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<td>17</td>
<td>162.92</td>
<td>0.04</td>
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</table>

D16S539

Mass Spec detected fewer SNPs in this locus.
Less average bp variability seen between repeat sizes.

<table>
<thead>
<tr>
<th>Allele</th>
<th>AVG</th>
<th>SD</th>
<th>N</th>
</tr>
</thead>
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<td>5</td>
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<td>0.01</td>
<td>2</td>
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<tr>
<td>6</td>
<td>264.09</td>
<td>0.11</td>
<td>17</td>
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<tr>
<td>7</td>
<td>268.14</td>
<td>0.11</td>
<td>93</td>
</tr>
<tr>
<td>8</td>
<td>272.20</td>
<td>0.12</td>
<td>56</td>
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<tr>
<td>9</td>
<td>276.18</td>
<td>0.14</td>
<td>162</td>
</tr>
<tr>
<td>10</td>
<td>280.25</td>
<td>0.14</td>
<td>158</td>
</tr>
<tr>
<td>11</td>
<td>284.30</td>
<td>0.12</td>
<td>72</td>
</tr>
<tr>
<td>12</td>
<td>288.35</td>
<td>0.07</td>
<td>8</td>
</tr>
</tbody>
</table>

Three Banded Patterns: FGA 20, 25, 26 Alleles

This particular tri-allelic pattern has not been reported in STRBase
Is this an FGA - Tri-allelic pattern identified using Identifier?

PK HT Ratio
12/10 - 0.48

D5S818

PK HT Ratio
19/24 - 0.56
25/24 - 0.89

Or is this a D13S317 - Tri-allelic pattern identified using Powerplex 16?

PK HT Ratio
12/10 - 0.48

D5S818

PK HT Ratio
13/11 - 0.83
14.3/11 - 0.42

It’s really a D5S818 Tri-allelic pattern identified using multiple STR Kits

PK HT Ratio
12/10 - 0.48
12+29/10 - 0.87

D5S818 monoplex results

144.97 bp

153.69 bp

221.76 bp

The 68 bp size difference between the 12 allele and the variant allele sizing as an “apparent 29” allele.

D5S818 Apparent 29 Allele Sequencing Results

There is a 4 bp deletion, the last 4 bases of the PP16 reverse primer binding site, followed by an insertion of 5 repeats. The 10 and 12 alleles of this sample have been sequenced and have the expected sequences.

Are there other large D5S818 alleles?

- STRBase Tri-allelic reports for FGA for 19,*,* patterns with AB amplification kits.
  - 5 reports:
    - 19,20,21; 19,20,23; 19,20,24; 19,22,23; 19,24,25
  - But there we have sequenced true tri-allelic FGA samples

- STRBase Tri-allelic reports for D13S317 for *,OL patterns with PP16 amplification kits.
  - NO tri-allelic patterns with Off-Ladder alleles reported
**Y-STR Mutation Rates Measured at NIST**


- **389 father/son sample pairs**
  - U.S. Caucasians, African Americans, Hispanics and Asians
- **17 Y-STR loci** in the Yfiler kit
- **24 differences** between father and son
  - 13 mutations resulted in the gain of a repeat in the son
  - 11 resulted in a loss of a repeat
- All single step repeat mutations
  - except a two repeat loss at Y-GATA-H4
- **2 sample pairs were found to have two mutations**
  - African American pair: mutations at DYS458 and DYS635
  - Asian pair: mutations at DYS439 and Y-GATA-H4
- Also observed 4 duplications, 1 triplication, and 4 deletions that were seen in both father and son

**Mixture Work**

- Testing software tools
  - FSS-i3
  - DNA_DataAnalysis (US Army Crime Lab)
- Examining reproducibility of mixture replicates to see how well mixture ratios hold across loci
- Peak height ratio studies with multiple data sets to understand mixture ratio ranges

**New STR Loci Characterized**


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

- Primer sequences, GeneMapper bins and panels, genotypes on common samples, and allele frequency information available on STRBase

**Promega Meeting Proceedings Paper**

NIST 26plex

- **32 Cycles, 50 pg**

Heterozygote peak imbalance due to stochastic effects with low DNA template

- 42 page article available on STRBase and Promega site
- Describes 26 miniSTR loci
- Covers 23plex STR assay
- Includes world-wide Yfiler data review

The 26plex assay (including primer sequences) has been submitted for publication in the Journal of Forensic Sciences

**http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm**
STRs vs SNPs Article

- Describes challenges with SNPs in terms of mixture detection and interpretation
- Most likely use of SNPs is as ancestry-informative markers (AIMs)


Typical STR DNA Analysis Workflow

- Sample Extraction ~2 h
- Quantitation ~1.5 h
- PCR ~3 h
- CE Run ~1.5 h

How can we reduce the time needed for cycling?
What happens when we alter cycling parameters?
How well existing commercial kits work?
How will different polymerases perform?
Can we develop novel assays and further the understanding/limits of rapid multiplex PCR?

Rapid PCR Project at NIST

Parameter | Unit | Trad | Rapid | Difference (min) | %
---|---|---|---|---|---
Hot Start | Min | 10 | 1 | 9.0 | 6.3
Hold | Sec | 60 | 5/10 | 72.3 | 50.6
Soak | Min | 60 | 1 | 59.6 | 41.2
Ramp rate | (deg/sec) | 1 | 4 | 22.4 | 15.7
Cycles | | 28 | 28 | |%
Time | | 2:58:41 | 0:35:38 | 2:23:03 |%

Parameter | Purpose
---|---
Hot Start | Primer Dimer, non-specific amplification
Hold | Denature, annealing, elongation, Inter and intra locus balance
Soak | Full adenylation of PCR products

Evaluate robustness and reproducibility
Using different DNA polymerases besides TaqGold

PowerPlex 16 Rapid Cycling

Identifer Rapid Cycling

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
Seminars and Training Workshops to Individual Forensic DNA Laboratories

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<td>Aug 7, 2006</td>
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<tr>
<td>Int. Symposium on Human Identification</td>
<td>June 6, 2006</td>
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<tr>
<td>NEAFS Meeting</td>
<td>Dec 5-6, 2006</td>
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<td>AAFS Meeting</td>
<td>Apr 3-4, 2007</td>
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<td>ISFG Meeting</td>
<td>Nov 15, 2006</td>
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<td>Int. Symposium on Human Identification</td>
<td>June 8, 2005</td>
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<td>Apr 27-28, 2006</td>
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Training Workshops in the Past Year

- ISFG Meeting (August 2007, Copenhagen, Denmark)
  - CE Fundaments and Troubleshooting
  - Validation
- Int. Symposium on Human Identification (Promega) Meeting (October 2007, Hollywood, CA)
  - Validation
- NEAFS Meeting (November 2007, Bolton Landing, NY)
  - Mixture Interpretation
  - Low-copy Number DNA Issues
  - ministrs
- AAFS Meeting (February 2008, Washington, DC)
  - DNA Quantitation by qPCR (158 page handout)
  - Mixture Interpretation (196 page handout)

Planned Promega 2008 Meeting

Troubleshooting Workshop

- Title: “Principles of Interpretation and Troubleshooting of Forensic DNA Typing Systems”
- Instructors: John Butler (NIST) and Bruce McCord (FIU)
- Date: October 16, 2008 with Promega Int. Symp. Human ID

The workshop will consist of three parts:
1. A through examination of theoretical issues with capillary electrophoresis PCR amplification of short tandem repeat markers
2. A discussion of how to properly set instrument parameters to interpret data (including mixtures), and
3. A review of specific problems seen by labs submitting problematic data and commentary on possible troubleshooting solutions.

Seeking input of problems observed with CE systems

Forensic Science Review Article


Funding from the National Institute of Justice (NIJ) through NIST Office of Law Enforcement Standards

Questions?

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