NIST Update

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

National CODIS Conference
Crystal City, VA
November 10, 2008

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

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

Sue Ballou
(Forensics)

Robert Thompson
(recent hire for forensics)

Mark Stolorow
(formerly of Orchid Cellmark)

Forensics Research at NIST
Computer (digital) forensics
Fingerprints
Ballistics
Arsenal investigation
DNA

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

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

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

33 different projects are described

[Human DNA Quantitation] [Mitochondrial DNA] [Y Chromosome] [Compressed DNA Library] [Transplantation and安东尼·杰斐逊] [General Tools and Information] [Non-Human DNA] [Alternative Forensic DNA Markers]

Methodology/Literature Project
ERS 3105 performance with various STR typing systems (April 2008-Jan 2008)
ERS 3105 protocol evaluation (Sept 2007-May 2008)

AutoPhage software to enable rapid multilocus PCR design (2005-2007) [see also software link]

Automated SNP loci (July 2010-present)

Automated STR loci beyond the CODIS mapping (Dec 2004-present) [see also nist/str.html]

Resources for storing, handling, and looking at DNA mobile study (June 2004-present)

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

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

Our Research Efforts are Similar to the Olympic Motto of “Swifter, Higher, Stronger”
What is “Rapid PCR”?

Current STR kits were optimized by manufacturers for slower PCR (~3 hours)
- Use of new commercial DNA polymerases
  - Replace the current standard polymerase (AmpliTaq Gold) and buffer but keep commercial STR kit primer mixes
    - rapid hot start (save ~10min)
    - ‘faster’ nucleotide incorporation (processivity >100 bases/sec)
- Use with common thermal cycler (GeneAmp 9700)
  - Utilize maximum ramp rate of 4 °C/sec with 9700
    - Shorten cycling hold times (to 1-5 sec vs 1 min)
    - Eliminate 60 °C adenylation soak (to save ~30-60 min)
- Explore possibilities with faster thermal cyclers (e.g., 10 °C/sec ramp) and possibly new primer mixes

Goal: to obtain full STR profiles in as little time as possible (<30 min?)

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
  - Permit analysis of individuals at a point of interest such as an embassy, an airport, or a country border

NYC Forensic DNA “X-Prize”

January 17, 2008 Press Release
From Mayor Bloomberg’s STATE OF THE CITY ADDRESS

“The City will establish a six-figure prize for anyone who can invent a device tailored to the NYPD which analyzes the DNA of potential suspects right at the crime scene - so that officers can release innocent suspects before they are arrested, and track down promising leads more quickly”


http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
Comparison of Thermal Cycling Times

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Trad</th>
<th>Rapid</th>
<th>Difference (min)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot Start</td>
<td>Min</td>
<td>10</td>
<td>1</td>
<td>9.0</td>
<td>6.3</td>
</tr>
<tr>
<td>Hold</td>
<td>Sec</td>
<td>60</td>
<td>5/10</td>
<td>72.3</td>
<td>56.6</td>
</tr>
<tr>
<td>Soak</td>
<td>Min</td>
<td>60</td>
<td>1</td>
<td>59.0</td>
<td>41.2</td>
</tr>
<tr>
<td>Ramp rate</td>
<td>(deg/sec)</td>
<td>1</td>
<td>4</td>
<td>22.4</td>
<td>15.7</td>
</tr>
<tr>
<td>Cycles</td>
<td></td>
<td>28</td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td>2:58:41</td>
<td>0:35:38</td>
<td>2:23:03</td>
<td></td>
</tr>
</tbody>
</table>

Overall time reduction on GeneAmp 9700 from 3 hours to 35 minutes

Rapid DNA Polymerases used: PyroStart (Fermentas, Glen Burnie, MD) and SpeedSTAR (Takara Bio USA, Madison, WI)

PowerPlex 16 Rapid Cycling

Rapid DNA Polymerases used: PyroStart (Fermentas, Glen Burnie, MD) and SpeedSTAR (Takara Bio USA, Madison, WI)

Rapid Multiplex PCR Protocols
Under Development

Utilizing AB 9700 cycler and ‘fast’ commercial enzymes
Manuscript in press with FSI Genetics

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Complete concordance of STR allele calls (for 60 samples) between the rapid and standard thermal cycling protocols were observed although there was incomplete adenylation at several of the loci examined and some PCR artifacts were detected. Using less than 750 pg of template DNA and 28 cycles, STR peaks for all loci were above a 150 relative fluorescent unit (RFU) detection threshold with fully adequate inter-locus balance and heterozygote peak height ratios of greater than 0.84.

1 ng template DNA, 28 cycles

Identifier data (full profile)

Samples selected for study that are heterozygous at every locus

Peak Height Ratios (PHRs) all >0.80

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
J.M. Butler – NIST Update at National CODIS Conference
November 10, 2008

100 pg template DNA with 31 cycles of PCR
Identifier data (full profile)

50 pg template DNA with 31 cycles of PCR
Identifier data (full profile)

10 pg template DNA with 31 cycles of PCR
Identifier data (full profile)

Peak Height Ratio Measurements

<table>
<thead>
<tr>
<th>Identifier STR Kit – only FGA shown</th>
<th>Peak Heights (RFUs)</th>
<th>Average PHR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FGA-22</td>
<td>FGA-25</td>
</tr>
<tr>
<td>100 pg</td>
<td>(1) 1692</td>
<td>1517 0.90</td>
</tr>
<tr>
<td></td>
<td>(a) 1915</td>
<td>864 0.45</td>
</tr>
<tr>
<td></td>
<td>(b) 1239</td>
<td>909 0.73</td>
</tr>
<tr>
<td>50 pg</td>
<td>(1) 902</td>
<td>260 0.26</td>
</tr>
<tr>
<td></td>
<td>(a) 1422</td>
<td>419 0.29</td>
</tr>
<tr>
<td></td>
<td>(b) 896</td>
<td>806 0.90</td>
</tr>
<tr>
<td>10 pg</td>
<td>(1) 66</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(2) 54</td>
<td>107 0.50</td>
</tr>
<tr>
<td></td>
<td>(3) 130</td>
<td>219 0.59</td>
</tr>
</tbody>
</table>

All levels performed in triplicate...

Peak Height Ratio Comparisons

Samples at <1 ng tested in triplicate (std dev shown)

10 pg template DNA with 31 cycles of PCR - triplicates
Identifier data (green loci)

Consensus Profile (2 out of 3)
D3S1358 (14,19) correct
TH01 (7,8,3) correct
D13S317 (12,13) correct
D16S539 (11,13) correct
D2S1338 (24,2) partial

Allele PHR imbalance
Allele dropout

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
Mixture Analysis Efforts at NIST

- Interlaboratory Studies: MSS1,2,3 and MIX05
  - Future ones planned when software tools and guidelines are available
- Software testing (see posters from AAFS 2008 and Promega 2008)
  - DNA_DataAnalysis (USACIL) – user’s manual written
  - FSS-i3 (Promega)
  - Web-LSD (UTenn)
  - GeneMapper (D-X v.1.1 (ABI))
  - GenoProof Mixture 1.0 (Qualitype)
  - Some conversations with Mark Perlin regarding TrueAllele 3 software
  - Some work coordinated with NEST Project (Marshall University)
- Work with SWGDAM Mixture Committee
  - Case summaries
- Training workshops and discussion groups

Creating Known Mixtures for Testing Software Tools

NIST 3-person mixture (Identifiler data, 1ng DNA, 5:2:1)
NIST 2-person mixture (Identifiler data, 1ng DNA, 1:5)

Mixtures were created for research purposes and are synthetic mixtures of extracted DNA created in a controlled environment without PCR inhibitors or an unknown amount of degraded DNA as may be found in forensic casework.

Variant Alleles Cataloged in STRBase

Off-Ladder Alleles
http://www.cstl.nist.gov/biotech/strbase/var_tab.htm

Tri-Allelic Patterns

Sample Submissions

- For those that desire more assurances of confidentiality we can have MOUs signed.
- We generally re-type the samples at NIST prior to starting sequencing.
- We may run a monoplex assay (single locus).
- We return results as PowerPoint slides.
- We thank all of those agencies that have used this free service (thanks to NIJ)!
- Contact Margaret Kline: margaret.kline@nist.gov

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
In FY2008, 21 variant allele samples were submitted for NIST sequence analysis.

Summary of Variant Alleles Sequenced (only 15 shown)

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele</th>
<th>Repeat Motif</th>
<th>Summary of Variant Alleles Sequenced</th>
</tr>
</thead>
<tbody>
<tr>
<td>D7S820</td>
<td>“8.3”,10</td>
<td>9 repeat units with a deletion 22 bases downstream from the repeat region</td>
<td></td>
</tr>
<tr>
<td>D18S51</td>
<td>15.2,18</td>
<td>[AGAA], AA [AGAA]</td>
<td></td>
</tr>
<tr>
<td>D16S539</td>
<td>Variant Causing Problems during a Recent Proficiency Test</td>
<td>Control allele</td>
<td>Sample has repeat region with [GATA]11 but a base change of TÆC 83 nucleotides upstream from the repeat (sequence shown above). Second allele is a nominal 13 allele, [GATA]13</td>
</tr>
</tbody>
</table>
New STR Loci and Assays

Usefulness of new STR loci:

- **Databases**: More loci to help resolve relatives in growing national DNA databases (UK went from 6 to 10 STRs in 1999; future Pan-European database will include >10 loci)
- **Casework**: Obtaining additional information with degraded DNA samples (miniSTRs), rapid screening of multiple crime scene samples
- **Identity/Relationship Testing**: Kinship analysis, parentage testing, complex criminal paternity, **missing persons/mass disasters**, immigration testing (25 STRs are recommended)


Charactertization of 20 MiniSTR Loci for Improved Analysis of Degraded DNA Samples

NIST 26plex

**Gender identification + 25 autosomal STR loci in a single amplification**


Other Topics

- **SRM updates**
  - SRM 2372
  - SRM 2391b, 2395, 2392
- **DNA stability testing** (on-going)
- **Training workshops**
- **SNPs for ethnicity estimation**
- **NIST group re-organization**
  - Human Identity Project Team now part of Applied Genetics Group that also covers clinical genetics and agricultural biotechnology areas

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)

- **SRM 2395** (Y-STR and Y-SNP Loci)
  - Yfiler loci sequenced (DYTS635 now included)
  - Additional Loci: 20 new Y-STR loci
  - Demonstrating extended stability (new quantitation data and no significant degradation to existing components)

Revised Certificates available since September 5, 2008

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
Room Temperature Storage of Dried Bloodstains

11 year time point

June 2008

Identifier profiles after DNA IQ Extraction

<table>
<thead>
<tr>
<th>DNA IQ</th>
<th>Mixture Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>FTA paper</td>
<td>500 pg amplified</td>
</tr>
<tr>
<td>S&amp;S 903 paper (untreated)</td>
<td>500 pg amplified</td>
</tr>
</tbody>
</table>

Training Workshops in the Past Year

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

• AAFS Meeting (February 2008, Washington, DC)
  – DNA Quantitation by qPCR (158 page handout)
  – Mixture Interpretation (196 page handout)

• Florida Statewide Training Meeting (May 2008, Indian Rocks Beach, FL)
  – STRs and CE
  – Mixture Interpretation

• Int. Symposium on Human Identification (Promega) Meeting (October 2008, Hollywood, CA)
  – Troubleshooting CE and PCR Systems

qPCR for DNA Quantitation Workshop

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

AAFS (February 18th, 2008)

Human DNA Quantification Using Real-Time PCR Assays

– Peter Vallone (NIST)
– Margaret Kline (NIST)
– Eric Buei (Vermont)
– Jan Nicklas (Vermont)
– Marie Allen (Uppsala)
– Mark Timken (CA DOJ)
– David Foran (Michigan State)
– Melanie Richard (CFS – Toronto)
– Toni Diegoli (AFDIL)

Mixture Interpretation Workshop


AAFS (February 19, 2008)

DNA Mixture Interpretation: Principles and Practice in Component Deconvolution and Statistical Analysis

– John Butler (NIST)
– Ann Gross (MN)
– George Carmody (Carleton U.)
– Gary Shutler (WA)
– Joanne Sgueglia (MA)
– Angela Dolph (Marshall U./NIST)
– Tim Kalafut (USACIL)

Lawyer Training…

• Virginia Defense Attorneys in Richmond, VA
  – April 25, 2008
  – Addressed general topics with DNA and mixtures

• New Jersey County Prosecutors in Jersey City, NJ
  – September 27, 2008
  – Spoke on past, present, and future issues with DNA

DNA Biometrics and Other Applications of Human Identity Testing

• Interactions with intelligence community and homeland security personnel (e.g., DoD, DHS) seeking to learn more about forensic DNA challenges and opportunities

• Aid to international humanitarian efforts (e.g., International Committee of the Red Cross) seeking to use DNA to aid remains identification in natural or man-made disasters

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

We welcome new collaborations...

Current Activities at NIST, Enabled by Our NIJ Partnership

- Standard Reference Materials
  - SRM 2372 (DNA quant) released Oct 2007 (>150 units in use)
  - Updates to SRM 2391b (STRs), 2395 (Y-STRs), 2392 (mtDNA)
- Technology Evaluation and Development
  - Rapid multiplex PCR protocols (multiplex STR amplification in <35 min)
  - Low-level DNA studies underway
  - Mixture interpretation – research and training materials
  - Unusual STR allele characterization
  - New STR loci and assays (STR 26plex, SNP testing)
  - Y-chromosome characterization (mutation rates, deletions, nomenclature)
- Training Materials
  - AAFS workshops on DNA quantitation and mixture interpretation
  - Third edition of Forensic DNA Typing textbook

Forensic DNA Typing Textbook

<table>
<thead>
<tr>
<th>Edition</th>
<th>Chapters</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>1-18</td>
<td>335</td>
</tr>
<tr>
<td>2nd</td>
<td>1-25</td>
<td>688</td>
</tr>
<tr>
<td>3rd</td>
<td>1-25</td>
<td>~1000</td>
</tr>
</tbody>
</table>

Now available in Chinese (Yiping Hou) and Japanese in preparation (Yoshiya Fukuma)

Thank you for your attention...

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

http://www.cstl.nist.gov/biotech/strbase
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301-975-4049

Questions?

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