Topics and Techniques for Forensic DNA Analysis

miniSTRs and Degraded DNA

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National Institute of Standards and Technology
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Outline for This Section

- NIST projects funded by NIJ
- Background on miniSTRs
- MiniFiler kit and concordance studies performed
- New non-CODIS (NC) miniSTR loci

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, expert system review
- Training Materials
  - Review articles and workshops on STRs, CE, validation
  - PowerPoint and pdf files available for download

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

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

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

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

Calibration with SRMs enables confidence in comparisons of results between laboratories

Information Resource

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

Provides up-to-date information and has been used in court cases to support application of DNA technology

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

Recent STRBase Updates...

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

Includes information on:
- Core STR loci
- Validation
- STR reference list
- NIST publications
- miniSTRs
- Forensic SNPs
- Variant STR alleles
- Population data resources
- Addresses of scientists

Calibration with SRMs enables confidence in comparisons of results between laboratories

http://www.cstl.nist.gov/biotech/strbase/strbase/training.htm
**Technology: Research Programs**

- **miniSTRs**
- Y-chromosome STRs
- mtDNA
- SNPs
- qPCR for DNA quantitation
- DNA stability studies
- Variant allele characterization and sequencing
- Software tools
- Expert System review
- Assay development with collaborators

**Timeline for miniSTRs**

- 1994 – FSS finds that smaller STR loci work best with burned bone and tissue from Branch Davidian fire
- 1997 – New primers developed for time-of-flight mass spectrometry to make small STR amplicons
- 2001 – Work at NIST and OhioU with CODIS STRs; BodePlexes used in WTC investigation starting 2002
- 2004 – Work at NIST with non-CODIS (NC) miniSTRs
- 2007 – Applied Biosystems releases 9plex MiniFiler

**Recent Publications on miniSTRs**


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http://www.cstl.nist.gov/biotech/strbase/miniSTR.htm
http://www.cstl.nist.gov/biotech/strbase/miniSTR/timeline.htm

http://www.cstl.nist.gov/biotech/strbase/training.htm
## DNA Degraded With DNase I

### Isolates

<table>
<thead>
<tr>
<th>Dnase concentration: 0.01 U/μL</th>
</tr>
</thead>
</table>

- 2645 bp
- 1605 bp
- 1198 bp
- 676 bp
- 517 bp
- 460 bp
- 396 bp
- 350 bp
- 222 bp
- 179 bp
- 126 bp

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## Miniplexes improve detection of degraded DNA

### “Big Mini”

<table>
<thead>
<tr>
<th>Primer Set</th>
<th>80bp</th>
</tr>
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<tbody>
<tr>
<td>TH01</td>
<td></td>
</tr>
<tr>
<td>D8</td>
<td></td>
</tr>
<tr>
<td>D21</td>
<td></td>
</tr>
<tr>
<td>D7</td>
<td></td>
</tr>
<tr>
<td>CSF</td>
<td></td>
</tr>
</tbody>
</table>

### PowerPlex 16

<table>
<thead>
<tr>
<th>Primer Set</th>
<th>160bp</th>
</tr>
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<tbody>
<tr>
<td>TH01</td>
<td></td>
</tr>
<tr>
<td>D13</td>
<td></td>
</tr>
<tr>
<td>D16</td>
<td></td>
</tr>
<tr>
<td>D2</td>
<td></td>
</tr>
</tbody>
</table>

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## Comparison of PCR Amplification Success Rates with Commercial Kit vs. miniSTR Assays

### Single amp for 12 STR loci

<table>
<thead>
<tr>
<th>Primer Set</th>
<th>12 STR loci</th>
</tr>
</thead>
<tbody>
<tr>
<td>PowerPlex 16</td>
<td></td>
</tr>
<tr>
<td>TH01</td>
<td>12 STR loci</td>
</tr>
<tr>
<td>D8</td>
<td>12 STR loci</td>
</tr>
<tr>
<td>D21</td>
<td>12 STR loci</td>
</tr>
<tr>
<td>D7</td>
<td>12 STR loci</td>
</tr>
<tr>
<td>CSF</td>
<td>12 STR loci</td>
</tr>
</tbody>
</table>

### Three amp for 12 STR loci

<table>
<thead>
<tr>
<th>Primer Set</th>
<th>12 STR loci</th>
</tr>
</thead>
<tbody>
<tr>
<td>PowerPlex 16</td>
<td></td>
</tr>
<tr>
<td>TH01</td>
<td>12 STR loci</td>
</tr>
<tr>
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<td>12 STR loci</td>
</tr>
<tr>
<td>TH01</td>
<td>12 STR loci</td>
</tr>
</tbody>
</table>

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## Summary of Samples Typed with ABI MiniFiler kit at NIST and ABI

- 1,308 samples
  - Allele concordance = 10,437/10,464 = 99.7%

### 1,308 samples

- 656 NIST U.S. population samples
  - 260 Caucasian, 253 African American, 140 Hispanic, 3 Asian
  - Previously examined with Identifiler; also with PowerPlex 16
  - Also tested with Butler et al. (2003) published miniSTR primers

- 481 father-son pairs
  - 184 Caucasian, 196 African American, 101 Asian samples (provided by paternity testing company DDC)
  - Previously examined with Identifiler

- 171 samples from Applied Biosystems

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Concordance Conducted at NIST

27 Discordant Calls
656 NIST U.S. population samples

<table>
<thead>
<tr>
<th>Identifier</th>
<th>MiniFiler</th>
<th>ABI</th>
<th>PowerPlex 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td></td>
<td>16</td>
<td>4 (13%)</td>
</tr>
<tr>
<td>14</td>
<td>10</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

AB miniFiler - 532
miniSTRs - 532

0.26% discordance (primarily D13, D16)

10,464 genotype comparisons
(1,308 samples x 8 loci)

27 Discordant Calls

481 father-son samples
171 ABI samples

Concordance Studies Reveal Potential Primer Binding Site Mutations with Different Primer Sets

<table>
<thead>
<tr>
<th>D16S539</th>
<th>Identifier</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ABI</td>
</tr>
</tbody>
</table>

Appears to be an allele 11 dropout/reduction due to primer binding site mutation

D13S317 Flanking Region Deletion

A 4 bp deletion outside the miniSTR primers causes the commercial kit produced allele to appear one repeat smaller...

<table>
<thead>
<tr>
<th>NIST Identifier data</th>
<th>Sequence analysis identified two regions where 4 bp deletions occur to cause this 1 repeat variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB miniSTR beta-test</td>
<td>Really “11-1” allele</td>
</tr>
<tr>
<td>Reverse primer is</td>
<td>Reverse primer is</td>
</tr>
<tr>
<td>on top of deletion</td>
<td>Reverse primer is outside deletion</td>
</tr>
</tbody>
</table>

Examination of D13S317 Concordance:
African American sample ZT79305

<table>
<thead>
<tr>
<th>NIST Identifier data</th>
<th>Ohio U miniSTR data</th>
<th>AB miniSTR beta-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>D13S317</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reverse primer is</td>
<td>Reverse primer is</td>
<td>Reverse primer is</td>
</tr>
<tr>
<td>outside deletion</td>
<td>inside deletion</td>
<td>on top of deletion</td>
</tr>
</tbody>
</table>

Full MiniFiler Profile for NIST Sample with D13S317 Allele Dropout

<table>
<thead>
<tr>
<th>“Null” allele</th>
<th>D13S317</th>
<th>D7S820</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMEL</td>
<td>D2S1338</td>
<td></td>
</tr>
<tr>
<td>D16S539</td>
<td>D18S51</td>
<td></td>
</tr>
<tr>
<td>CSF1PO</td>
<td>FGA</td>
<td></td>
</tr>
</tbody>
</table>

Note the Relative D13 Peak Heights (Suggests Allele Dropout)

<table>
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<th>“Null” allele</th>
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</tr>
</tbody>
</table>

Note the level of the D13 single “homozygous” allele relative to all other peaks that are heterozygous.

A true homozygous allele is taller than other heterozygous alleles
Why Go Beyond the CODIS Loci?

1. Large Allele Ranges (e.g. FGA)

2. “Unclean” Flanking Sequences (e.g. D7S820)

Characterization of New miniSTR Loci

**Computer Work**
- Candidate STR marker selection (e.g. Marshfield Clinic Center of Medical Genetics)
- Pull-down sequence data from the web
- Quality Control
- Screen for PCR Primers
- Test primers for Multiplex-ability

**Laboratory Work**
- Test markers on population samples
- Sequence homologues to determine allele sizes
- Build libraries for development
- Construct Allelic Ladders

Initial Testing Results with Potential miniSTR Loci

26 new miniSTRs (NC01-NC09)

Miniplex "NC01"

20 additional loci characterized across U.S. population groups

Standard U.S. Population Dataset

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

260 Caucasians, 260 African Americans, 140 Hispanics, 3 Asians = 636 males

DNA extracted from whole blood (anonymous; self-identified ethnicities) received from Interstate Blood Bank (Memphis, TN) and Millennium Biotech Inc. (Ft. Lauderdale, FL)

To date: >100,000 allele calls
- STRs 22 loci—27 amplions (17,388)
- STRs 27 new loci (14,535)
- Yfiler kit 17 loci (11,237)
- Y SNPs 50 markers on sub-set of samples (11,498)
- Orchid 70 autosomal SNPs on sub-set (13,230)
- miniSTR testing-new loci and CODIS concordance (9,228)

Genotypes with various human identity testing markers
New miniSTR
Non-CODIS (NC) Loci

Mike Coble
Becky Hill
John Butler

32 STR loci tested on NIST 665 U.S. population samples
26 STR loci with allele sizes below 140 bp and good heterozygosities (above TPOX level)
All new STR loci are physically unlinked to the 13 CODIS core loci
Submitted articles regarding primer sequences and locus characterization including population statistics
SRM 2391b components are being certified
Submitted articles
All new STR loci are
26 STR loci with allele sizes below 140 bp and good heterozygosities
32 STR loci tested on NIST

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

Cobble

J.M. Butler – Houston DNA Training Workshop April 3-4, 2007

Characterization of miniSTR D12ATA63
GenBank accession AC00971: positions 55,349–55,437

Trinucleotide
[TAA|CAA] repeat

Position of Forensic STR Markers on Human Chromosomes

European Labs Have Adopted the NIST-Developed NC miniSTRs


The evolution of DNA databases—Recommendations for new European STR loci
Peter Gill, Lynn Fryday, Niels Morling, Peter M. Schneider

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

- **Reduced size amplicons improve success rates with degraded DNA** or samples possessing PCR-inhibitors – European leaders view miniSTRs as “the way forward”

- A new kit will be available soon from ABI called MiniFiler – concordance testing done at NIST

- **New miniSTR loci are being characterized** at NIST – 26 loci in development