Rapid Forensic DNA Typing: Protocols and Instrumentation

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Forensic STR Typing

Collection
Specimen Storage

Extraction

Quantitation

Multiplex PCR

STR Typing

Interpretation of Results

Database
Storage & Searching

Calculation of Match Probability

Usually 1-2 day process (a minimum of ~8 hours)

Blood Stain
Sample Collection & Storage

Buccal swab

DNA Extraction

DNA Quantitation

DNA separation and sizing

Statistics Calculated
DNA Database search
Paternity test
Reference sample

Applied Use of Information

1.5 hours

Multiplex PCR Amplification

1.5 hours

1.5 hours

3.5 hours

STR Typing

Interpretation of Results

Calculation of Match Probability
What is Rapid Forensic DNA Typing or Rapid DNA (R-DNA)?

• Generating a STR profile in **minutes vs hours**
  – 90 minutes versus 6-8 hours
  – Single-source reference samples (not casework)

• Non-integrated
  – Laboratory based (existing equipment)
  – Specially trained analysts
  – Robotics, fast PCR, direct PCR, quick extraction, etc

• Integrated approach
  – Fully integrated microfluidic platform
  – ‘Swab in – answer out’
  – Non-expert user
Benefits and Applications

• Faster sample-to-answer turnaround times
• Increased throughput for databasing labs

• Impact of Integrated R-DNA platforms
  – Booking stations, investigative leads
  – Rapid intelligence, field testing
  – Mass fatality, disaster victim investigation
  – Kinship determination, immigration, border security
  – Interest in R-DNA by FBI, DHS, DoD
Important Questions

• Can a quality result be obtained with rapid techniques?
  – Uphold DNA as the gold standard for human identification
  – Reference/database or casework samples?
  – How do we validate rapid techniques and instruments?

• Robustness
• Reliability
• Reproducibility
• Concordance ‘the correct answer’
• Sensitivity
• Contamination, mixtures
• Stutter, peak height balance, artifacts
Ongoing Projects that Support R-DNA

• Non-integrated
  – Developing rapid PCR protocols for STR kits
  – Faster thermal cyclers and DNA polymerases
  – Direct PCR kit evaluation
  – **Rapid typing workflows (Sampling through Profile)**

• Integrated approach
  – Performance assessment of prototype R-DNA instruments
  – Inter-laboratory study
Rapid PCR Protocols

• Reducing the time required for PCR
  – 3 hours down to sub-30 minute
• Accomplish this by optimizing conditions for:
  – Faster DNA polymerases
  – Faster thermal cyclers
**PCR Thermal Cyclers**

<table>
<thead>
<tr>
<th>Cycler</th>
<th>Cycling Time (min)</th>
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<tbody>
<tr>
<td>GeneAmp 9700</td>
<td>36</td>
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<tr>
<td>Mastercycler Pro S</td>
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<tr>
<td>Rotor-Gene Q</td>
<td>36</td>
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<tr>
<td>SmartCycler</td>
<td>22</td>
</tr>
<tr>
<td>Philisa</td>
<td>17</td>
</tr>
<tr>
<td>Piko</td>
<td>30</td>
</tr>
<tr>
<td>SpeedCycler2</td>
<td>22</td>
</tr>
<tr>
<td>Palm PCR</td>
<td>17</td>
</tr>
</tbody>
</table>

- **95°C for 1 min**
- **28x (95°C for 5s, 58°C for 10s, 72°C for 10s)**
- **72°C 1 min**

Peter Vallone: Green Mountain DNA Conference (Burlington, VT), August 3, 2012, "Development of Protocols for Rapid Amplification of STR Typing Kits: The Use of 'Non-Standard' Thermal Cyclers"
DNA Polymerases

- **AmpliTaq Gold®** is typically used
  - Heat activated (avoid non-specific PCR products)

- **SpeedSTAR™ HS DNA Polymerase**
  - Extension times of 100 bp/s are possible (compared to 20 bp/s for other polymerases)
  - Hot-start formulation is antibody mediated

- **Qiagen**
  - QIAGEN Fast Cycling PCR Kit

- **New England Biolabs/Finnzymes**
  - Phusion and Phire DNA Polymerases

- **KAPA Biosystems**
  - KAPA2G Fast PCR Kits

- **Biotium**
  - Cheetah™ Taq

- **Fermentas**
  - PyroStart Master Mix

- **EMD Millipore**
  - KOD DNA Polymerase
GeneAmp 9700 31 min PCR

1 ng of DNA template
Philisa 17 min PCR

1 ng of DNA template
Benefits of Direct PCR

- Sample set-up convenience: ‘punch and go’
- Amplify unpurified DNA - skip extraction and quantitation
- Amenable to automation
- Applications: offender DNA database samples, paternity samples, casework reference samples
PowerPlex 18D: 1.2 mm Blood punch off FTA paper

Developmental validation of the PowerPlex® 18D System, a rapid STR multiplex for analysis of reference samples

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90 minute PCR cycling time
Example Rapid Typing Workflow
Non-Integrated (Lab) Setting
single source reference samples

- DNA extraction
- Rapid PCR
- CE separation and detection

- Extraction: Prep-N-Go Buffer
- PCR: Rapid Identifier (Philisa cycler)
- Separations: 8 capillary 3500 Genetic Analyzer

- Direct PCR
- CE separation and detection

- PP18D (9700 cycler)
- Separations: 8 capillary 3500 Genetic Analyzer

8 unique samples were typed in parallel
Extraction → rPCR → Separation/Detection

Total time from swab to answer: 57 min 42 sec
Includes set up times
Total time from swab to answer: **2 hours 16 min 45 sec**

Includes set up times

**Erica Butts poster at the 23rd International Symposium on Human Identification (ISHI) meeting (Nashville, TN), October 16-17, 2012, "Rapid DNA testing approaches for reference samples"** Lecture Room B
Integrated Approach to Rapid DNA

Fully automated (hands free) process of developing a CODIS Core STR profile from a reference sample buccal swab

R-DNA Instrument

DNA extraction
PCR
Separation and Detection
Allele calling

- D8S1179 {15,16}
- D21S11 {29,29}
- D7S820 {9,11}
- CSF1PO {10,11}
- D3S1358 {16,17}
- TH01 {6,7}
- D13S317 {8,12}
- D16S539 {10,11}
- VWA {15,17}
- TPOX {8,12}
- D18S51 {11,15}
- D5S818 {9,11}
- FGA {19,22}
- Amel {X,Y}

Buccal swab
Developers of R-DNA Instrumentation

- IntegenX
- NetBio
- ZyGem/Lockheed Martin
- Univ of Az
Performance Testing Goals

• Testing of R-DNA platforms for baseline performance of Robustness, Reliability, and Reproducibility

• Type similar sample sets on multiple instruments and from multiple vendors

• Results will help guide platform improvements and additional testing

Carry this out through an inter-laboratory study
NIST Inter-laboratory Test Samples

• 50 samples (buccal swabs) will be provided to each participant
  – Five replicates of 10 anonymous individuals
  – NIST IRB approval
  – Each individual typed at NIST (PowerPlex 16 HS)
What will this data provide?

High level

- Is the correct profile obtained?
- Typing success
  - Per lane, chip, overall
- Incorrect profiles
- Partial profiles
- Allele drop out
- Contamination
- General operational issues
  - Instrument/chip failures
  - Hardware and software

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<table>
<thead>
<tr>
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<tbody>
<tr>
<td><strong>Total Runs</strong></td>
<td>44</td>
</tr>
<tr>
<td><strong>Total Lanes</strong></td>
<td>220</td>
</tr>
<tr>
<td>Lanes with correct CODIS 13</td>
<td>90</td>
</tr>
<tr>
<td>% CODIS 13 loci</td>
<td>41%</td>
</tr>
<tr>
<td>Lanes with correct PP16</td>
<td>82</td>
</tr>
<tr>
<td>% PP16 loci</td>
<td>37%</td>
</tr>
<tr>
<td>Failed lanes (CODIS 13)</td>
<td>130</td>
</tr>
<tr>
<td>Failed chip eq</td>
<td>26</td>
</tr>
</tbody>
</table>
What will this data provide?
Detailed-expert user; developer

- Electropherogram characteristics
  - Signal intensity
  - Peak balance (inter- and intra locus)
  - Stutter, PCR artifacts, adenylation
  - Sizing precision of peaks

- Manual versus automated allele calls
  - Confirm optimal software allele calling parameters

33.6% stutter

Peak balance
Inter-laboratory Testing Results

• Provide participants and sponsor with data and feedback
  ✓ Each participant and will receive their specific performance feedback
  ✓ The sponsor (FBI) will get a cumulative report for dissemination

Data and results will assist ongoing developmental validation studies and other decisions in the adoption of R-DNA
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Erica Butts

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Initial rapid PCR work

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Thank you for your attention!