10-28-2004

Forensic DNA Evidence: Collection, Mixtures, and Degradation

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Forensic DNA evidence: collection, mixtures and degradation

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Three generations of DNA testing

RFLP  AUTORAD  Allele = BAND

DQ-alpha  TEST STRIP  Allele = BLUE DOT

Automated STR  ELECTROPHEROGRAM  Allele = PEAK
DNA content of biological samples:

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Amount of DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>30,000 ng/mL</td>
</tr>
<tr>
<td>stain 1 cm² in area</td>
<td>200 ng</td>
</tr>
<tr>
<td>stain 1 mm² in area</td>
<td>2 ng</td>
</tr>
<tr>
<td>Semen</td>
<td>250,000 ng/mL</td>
</tr>
<tr>
<td>Postcoital vaginal swab</td>
<td>0 - 3,000 ng</td>
</tr>
<tr>
<td>Hair</td>
<td></td>
</tr>
<tr>
<td>plucked</td>
<td>1 - 750 ng/hair</td>
</tr>
<tr>
<td>shed</td>
<td>1 - 12 ng/hair</td>
</tr>
<tr>
<td>Saliva</td>
<td>5,000 ng/mL</td>
</tr>
<tr>
<td>Urine</td>
<td>1 - 20 ng/mL</td>
</tr>
</tbody>
</table>
Basic terminology: Genetics

- **DNA Polymorphism** ("many forms")
  - Regions of DNA which differ from person to person
- **Locus** (plural = loci)
  - Site or location on a chromosome
- **Allele**
  - Different variants which can exist at a locus
- **DNA Profile**
  - The combination of alleles for an individual
Basic terminology: Technology

- Amplification or PCR (Polymerase Chain Reaction)
  - A technique for ‘replicating’ DNA in the laboratory (‘molecular Xeroxing’)
  - Region to be amplified defined by PRIMERS
  - Can be ‘color coded’
- Electrophoresis
  - A technique for separating molecules according to their size
STR

• **Short tandem repeat**
• Describes a type of DNA polymorphism in which:
  – a DNA sequence repeats
  – over and over again
  – and has a short (usually 4 base pair) repeat unit
• A length polymorphism -- alleles differ in their length

3 repeats: AATG AATG AATG
4 repeats: AATG AATG AATG AATG
5 repeats: AATG AATG AATG AATG AATG
6 repeats: AATG AATG AATG AATG AATG AATG
Reading an electropherogram

Peaks correspond to alleles

Amelogenin
XX = female
XY = male

Red = ROX size standard
Automated STR Test
Crime Scene Samples & Reference Samples

- Extract and purify DNA

Differential extraction in sex assault cases separates out DNA from sperm cells
Extract and Purify DNA

- Add primers and other reagents
PCR Amplification

- DNA regions flanked by primers are amplified

Groups of amplified STR products are labeled with different colored dyes (blue, green, yellow)
The ABI 310 Genetic Analyzer: SIZE, COLOR & AMOUNT
ABI 310 Genetic Analyzer: Capillary Electrophoresis

- Amplified STR DNA injected onto column
- Electric current applied
- DNA pulled towards the positive electrode
- DNA separated out by size:
  - Large STRs travel slower
  - Small STRs travel faster
- Color of STR detected and recorded as it passes the detector
Profiler Plus: Raw data
Statistical estimates: the product rule

\[ 0.222 \times 0.222 \times 2 = 0.1 \]
Statistical estimates: the product rule

Locus D3S1358
Race Caucasian
(N = 203)

<table>
<thead>
<tr>
<th>Allele</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>0.012</td>
</tr>
<tr>
<td>13</td>
<td>0.012</td>
</tr>
<tr>
<td>14</td>
<td>0.140</td>
</tr>
<tr>
<td>15</td>
<td>0.246</td>
</tr>
<tr>
<td>16</td>
<td>0.222</td>
</tr>
<tr>
<td>17</td>
<td>0.222</td>
</tr>
<tr>
<td>18</td>
<td>0.163</td>
</tr>
<tr>
<td>19</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Locus vWA
Race Caucasian
(N = 196)

<table>
<thead>
<tr>
<th>Allele</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>0.012</td>
</tr>
<tr>
<td>12</td>
<td>0.012</td>
</tr>
<tr>
<td>13</td>
<td>0.012</td>
</tr>
<tr>
<td>14</td>
<td>0.102</td>
</tr>
<tr>
<td>15</td>
<td>0.082</td>
</tr>
</tbody>
</table>

1 in 79,531,528,960,000,000,000
1 in 80 quadrillion
What more is there to say after you have said: “The chance of a coincidental match is one in 80 quadrillion?”
What more is there to say after you have said: “The chance of a coincidental match is one in 80 quadrillion?”

- Two samples really do have the same source
- Samples match coincidentally
- An error has occurred
Sources of ambiguity in DNA testing results

- Mixtures: deconvolution and relatives
- Degradation, inhibition
- Background noise
- Stutter (n+4)
- Pull-up
- Spikes and blobs
Opportunities for subjective interpretation?

Electropherograms showing a DNA profile for the D3, vWA and FGA loci for two samples. Top sample is:

<table>
<thead>
<tr>
<th>Locus</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3S1358</td>
<td>12, 47</td>
</tr>
<tr>
<td>vWA</td>
<td>17, 107</td>
</tr>
<tr>
<td>FGA</td>
<td>15, 17, 71, 100, QL Allele 36</td>
</tr>
</tbody>
</table>

Saliva Sample:

<table>
<thead>
<tr>
<th>Locus</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3S1358</td>
<td>12, 47</td>
</tr>
<tr>
<td>vWA</td>
<td>17, 107</td>
</tr>
<tr>
<td>FGA</td>
<td>15, 17, 71, 100, QL Allele 36</td>
</tr>
</tbody>
</table>

Defendant:

<table>
<thead>
<tr>
<th>Locus</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3S1358</td>
<td>17, 1646</td>
</tr>
<tr>
<td>vWA</td>
<td>15, 17, 1151, 1160</td>
</tr>
<tr>
<td>FGA</td>
<td>25, 1708</td>
</tr>
</tbody>
</table>

QL Allele 95

-100 -50 100
-1500 -1000 -500 1000 1500
Opportunities for subjective interpretation?

Electropherograms showing a DNA profile for the D3, vWA and FGA loci for two samples. Top sample is

D3: 12, 17  
vWA: 15, 17  
FGA: 22, 26
Sources of ambiguity in DNA testing results

- Mixtures: deconvolution and relatives
- Degradation, inhibition
- Background noise
- Stutter (n+4)
- Pull-up
- Spikes and blobs
Mixed DNA samples
<table>
<thead>
<tr>
<th>Maximum # of alleles observed in a 3 person mixture</th>
<th># of occurrences</th>
<th>Percent of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>3</td>
<td>8,151</td>
<td>0.02</td>
</tr>
<tr>
<td>4</td>
<td>310</td>
<td>0.00</td>
</tr>
<tr>
<td>11,526,219</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2,498,139</td>
<td>5.53</td>
</tr>
<tr>
<td>11,526,219</td>
<td>5.53</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>32,078,976</td>
<td>25.53</td>
</tr>
<tr>
<td>29,938,777</td>
<td>25.53</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1,526,550</td>
<td>71.07</td>
</tr>
<tr>
<td>6</td>
<td>12,702,670</td>
<td>66.32</td>
</tr>
<tr>
<td>6</td>
<td>3.38</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>28.14</td>
<td></td>
</tr>
</tbody>
</table>

There are 45,139,896 possible different 3-way mixtures of the 648 individuals in the MN BCI database.
Sources of ambiguity in DNA testing results

- Mixtures: deconvolution and relatives
- Degradation, inhibition
- Background noise
- Stutter (n+4)
- Pull-up
- Spikes and blobs
Accounting for relatives

The graph shows the distribution of shared alleles between relatives, with different curves representing synthetic cousins, cousins, and siblings. The x-axis represents the number of shared alleles, while the y-axis represents the percent of total shared alleles. The graph illustrates how the distribution of shared alleles varies among different types of kinship:

- Synthetic cousins show a peak around 11 shared alleles, indicating a higher similarity compared to siblings and cousins.
- Cousins have a peak around 9 shared alleles, suggesting a moderate level of similarity.
- Siblings display a peak around 15 shared alleles, reflecting the highest level of similarity.

This visual representation helps in understanding the genetic relationships and the extent of shared ancestry among relatives.
Likelihood ratios for allele sharing:

- Synthetic
- Cousins
- Siblings

Number of Shared Alleles

Likelihood
Sources of ambiguity in DNA testing results

- Mixtures: deconvolution and relatives
- Degradation, inhibition
- Background noise
- Stutter (n+4)
- Pull-up
- Spikes and blobs
Degradation

- When biological samples are exposed to adverse environmental conditions, they can become degraded
  - *Warm, moist, sunlight, time*
- Degradation breaks the DNA at random
- Larger amplified regions are affected first
- Classic ‘ski-slope’ electropherogram
- Degradation is unusual.
Degradation
The Leskie Inquest

- Undegraded samples can have “ski-slopes” too.
- How negative does a slope have to be to an indication of degradation?
- Experience, training and expertise.
- Positive controls should not be degraded.
Degradation
The Leskie Inquest

- DNA profiles in a rape and a murder investigation match.
- Everyone agrees that the murder samples are degraded.
- If the rape sample is degraded, it could have contaminated the murder samples.
- Is the rape sample degraded?
Degradation

The Leskie Inquest
Sources of ambiguity in DNA testing results

- Mixtures: deconvolution and relatives
- Degradation, inhibition
- Background noise
- Stutter (n+4)
- Pull-up
- Spikes and blobs
**Background noise**

Averaged PH: 13.04 RFUs

Standard Deviation: 6.57

<table>
<thead>
<tr>
<th>Dye/Sample Peak</th>
<th>Minutes</th>
<th>Size</th>
<th>Peak Height</th>
<th>Peak Area</th>
<th>Data Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>B, 397</td>
<td>23.32</td>
<td>387.91</td>
<td>21</td>
<td>19</td>
<td>6336</td>
</tr>
<tr>
<td>B, 398</td>
<td>23.42</td>
<td>388.23</td>
<td>21</td>
<td>19</td>
<td>6386</td>
</tr>
<tr>
<td>B, 399</td>
<td>23.43</td>
<td>388.48</td>
<td>14</td>
<td>14</td>
<td>6388</td>
</tr>
<tr>
<td>B, 400</td>
<td>23.47</td>
<td>389.99</td>
<td>6</td>
<td>8</td>
<td>6400</td>
</tr>
<tr>
<td>B, 401</td>
<td>23.50</td>
<td>390.88</td>
<td>7</td>
<td>14</td>
<td>6407</td>
</tr>
<tr>
<td>B, 402</td>
<td>23.58</td>
<td>393.67</td>
<td>25</td>
<td>25</td>
<td>6429</td>
</tr>
<tr>
<td>B, 403</td>
<td>23.58</td>
<td>393.93</td>
<td>16</td>
<td>32</td>
<td>6431</td>
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<tr>
<td>B, 404</td>
<td>23.60</td>
<td>394.57</td>
<td>18</td>
<td>28</td>
<td>6436</td>
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<tr>
<td>B, 405</td>
<td>23.61</td>
<td>394.95</td>
<td>14</td>
<td>22</td>
<td>6439</td>
</tr>
<tr>
<td>B, 406</td>
<td>23.63</td>
<td>395.59</td>
<td>14</td>
<td>31</td>
<td>6444</td>
</tr>
<tr>
<td>B, 407</td>
<td>23.65</td>
<td>396.11</td>
<td>6</td>
<td>11</td>
<td>6448</td>
</tr>
<tr>
<td>B, 408</td>
<td>23.66</td>
<td>396.49</td>
<td>9</td>
<td>17</td>
<td>6451</td>
</tr>
<tr>
<td>B, 409</td>
<td>23.67</td>
<td>395.88</td>
<td>8</td>
<td>11</td>
<td>6454</td>
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<tr>
<td>B, 410</td>
<td>23.68</td>
<td>397.14</td>
<td>11</td>
<td>11</td>
<td>6466</td>
</tr>
<tr>
<td>B, 411</td>
<td>23.69</td>
<td>397.66</td>
<td>28</td>
<td>65</td>
<td>6460</td>
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<tr>
<td>B, 412</td>
<td>23.70</td>
<td>398.18</td>
<td>10</td>
<td>28</td>
<td>6464</td>
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<tr>
<td>B, 413</td>
<td>23.72</td>
<td>398.70</td>
<td>19</td>
<td>43</td>
<td>6468</td>
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<tr>
<td>B, 414</td>
<td>23.75</td>
<td>399.74</td>
<td>17</td>
<td>40</td>
<td>6476</td>
</tr>
</tbody>
</table>
Stutter peaks
Pull-up (software differences)

Advanced

Classic
“Spikes” and “blobs”
Spikes and blobs

Blob: \( \frac{\text{Peak Area}}{\text{Peak Height}} > 10 \)

Spike: \( \frac{\text{Peak Area}}{\text{Peak Height}} < 4.5 \)
Resources

• Books

• Internet
  – Applied Biosystems Website: http://www.appliedbiosystems.com/
    (see human identity and forensics)
  – Forensic Bioinformatics Website: http://www.bioforensics.com/
  – STR base: http://www.cstl.nist.gov/biotech/strbase/ (very useful)

• Scientists
  – Larry Mueller (UC Irvine)
  – Simon Ford (Lexigen, Inc. San Francisco, CA)
  – William C. Thompson (UC Irvine)
  – William Shields (SUNY, Syracuse, NY)
  – Marc Taylor (Technical Associates, Ventura, CA)
  – Keith Inman (Forensic Analytical, Haywood, CA)

• Testing laboratories
  – Technical Associates (Ventura, CA)
  – Indiana State Police (Indianapolis, IN)

• Other resources
  – Forensic Bioinformatics (Dayton, OH)