Forensic DNA 201: Mixture Interpretation and Other Advanced DNA Topics

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National Institute of Standards and Technology

Indigent Criminal Defense Seminar (Richmond, VA)
April 25, 2008

Interfaces Between Disciplines Are Crucial

Law Enforcement  Judicial

Laboratory

What Every Law Enforcement Officer Should Know About DNA Evidence
http://www.dna.gov/

DNA Training for Officers of the Court

Guilt

Innocence

Colin Pitchfork
Kirk Bloodsworth Josiah Sutton
Roger Coleman

http://www.innocenceproject.org/

DNA Training for Officers of the Court

CD-ROM available from the U.S. National Institute of Justice (http://www.ncjrs.gov)

On-line training available at http://www.DNA.gov

DNA Training for Officers of the Court

http://www.dna.gov/training/otc/

Lessons from the First Case Involving DNA Testing

Describes the first use of DNA (in 1986) to solve a double rape-homicide case in England; about 5,000 men asked to give blood or saliva to compare to crime stains

• Connection of two crimes (1983 and 1986)
• Use of DNA database to screen for perpetrator (DNA only done on 10% with same blood type as perpetrator)
• Exoneration of an innocent suspect
• DNA was an investigative tool – did not solve the case by itself (confession of accomplice)

A local baker, Colin Pitchfork, was arrested and his DNA profile matched with the semen from both murders. In 1988 he was sentenced to life for the two murders.

Impact of Forensic DNA Testing

Guilt

Innocence

Colin Pitchfork
Kirk Bloodsworth Josiah Sutton
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http://www.innocenceproject.org/

DNA Training for Officers of the Court

Advancing Justice Through DNA Technology

• CD-ROM available from the U.S. National Institute of Justice (http://www.ncjrs.gov)

On-line training available at http://www.DNA.gov

DNA Training for Officers of the Court

http://www.dna.gov/training/otc/
Principles of Forensic DNA for Officers of the Court

1. Introduction
2. Biology of DNA
3. Practical Issues Specific to DNA Evidence
4. Forensic DNA Laboratory
5. Assurance Quality in DNA Testing
6. Understanding a Forensic DNA Lab Report
7. Statistics and Population Genetics
8. Mitochondrial DNA & Y-STR Analysis
9. Forensic DNA Databases
10. Collection of DNA Evidence Issues
11. Pretrial DNA Evidence Issues
12. Victim Issues
13. Trial Presentation
14. Postconviction DNA Cases
15. Emerging Trends

Common Defense Attacks
Compiled from Forensic Bioinformatics website
- Contamination
- Statistical Weight of a Match
- Degradation/PCR Inhibition of “True” Perp
- Artifacts (N+4 stutter, etc.)
- Thresholds Set Too High (missing peaks)
- Examiner Bias
- Improper Mixture Interpretation
- Meaning of a Database Hit

Presentation Outline
- How DNA Results are Obtained
  - Where do these “1 in a zillion” numbers come from?
- Mixture Interpretation
  - How to detect if a mixture is present in a DNA result?
  - Why are mixtures challenging to interpret?
- Other Topics
  - Why are partial profiles not as informative?
  - What measures exist for quality control in labs?
  - Why are protocols used in forensic labs?

How Are DNA Results Obtained?

Steps in DNA Analysis
Usually a 1-2 day process (a minimum of ~5 hours)

Steps Involved
- Collection
- Specimen Storage
- Extraction
- STR Typing
- Interpretation of Results
- Database Storage & Searching
- Calculation of Match Probability

Steps in DNA Separation and Storing
DNA separation and storing
- STR Typing
- Multiplex PCR Amplification
- DNA Extraction
- Quantitation

DNA Database Search
- Male: 13, 14, 15, 16, 12, 13, 15, 16
- Female: 13, 14, 15, 16, 12, 13, 15, 16
Short Tandem Repeat (STR) Markers

An accordion-like DNA sequence that occurs between genes

TTCACTCTCTTCCTCTTTGATATACACAGACAGA
GATATGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGAT

= 12 GATA repeats (“12” is all that is reported)

The number of consecutive repeat units can vary between people

The FBI has selected 13 core STR loci that must be run in all DNA tests in order to provide a common currency with DNA profiles

**What is a DNA Profile?**

Human Genome

23 Pairs of Chromosomes (~3 billion bp)

Usually 13-15 STR targets are examined

The number of consecutive GATA repeats can vary between people

The regions are consistent of a few hundred base pairs

The copied fragments are labeled with fluorescent dyes for detection purposes

**DNA Testing Requires a Reference Sample**

A DNA profile by itself is fairly useless because it has no context...

DNA analysis for identity only works by comparison – you need a reference sample

Crime Scene Evidence compared to Suspect(s) (Forensic Case)

Child compared to Alleged Father (Paternity Case)

Victim’s Remains compared to Biological Relative (Mass Disaster ID)

Soldier’s Remains compared to Direct Reference Sample (Armed Forces ID)

**STR Allele Separation Can Be Performed by Gel or Capillary Electrophoresis with Detection of Fluorescent Dyes Labeling Each PCR Product**

**Autosomal Paternity Example**

<table>
<thead>
<tr>
<th>Locus 1</th>
<th>Locus 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 repeats</td>
<td>9 repeats</td>
</tr>
<tr>
<td>10 repeats</td>
<td></td>
</tr>
</tbody>
</table>

What would be entered into a DNA database for searching:

14, 17, 6-7, 10-13, 12, 13, 17, 25 ...

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
STR Typing with Gel Electrophoresis

Virginia State Lab

Detects the DNA Fragments by Fluorescent Dye Label

Gel Image of Multiple Samples with PCR Products from 16 Different Loci Amplicons with Green, Yellow, and Red Dye Labelled Primers

PowerPlex 16 BIO

A Locus-Specific Allelic Ladder Composed of Common Alleles is Used to Calibrate Size Measurements

Size of peaks are measured relative to an internal size standard (not shown) included in every sample

The allelic ladder defines bins for sizing STR alleles

Artificial ladder: 258.75 +/- 0.5

On-Ladder Sample

Any STR peak falling in the range of 258.25 to 258.35 bp is considered a "22" allele

Off-Ladder Sample

Almost 1 bp less than the ladder allele

"Variant Allele"

DNA analysts interpret data to sort out which peaks are STR alleles versus artifacts

Thresholds for Measuring DNA Data

Peak is called (deemed "reliable")

• Detection (analytical) threshold
  - Dependent on instrument sensitivity
  - ~50 RFU (relative fluorescence units)
  - Impacted by instrument baseline noise

• Dropout (stochastic) threshold
  - Dependent on biological sensitivity
  - ~150-200 RFU
  - Important in mixture interpretation

A Locus-Specific Allelic Ladder

Composed of Common Alleles

Sizes of peaks are measured relative to an internal size standard (not shown) included in every sample

The allelic ladder defines bins for sizing STR alleles

Allele 21.3

257.84 bp

(-0.91 bp from ladder allele)

Allele 22

258.69 bp

(-0.06 bp from ladder allele)

Any STR peak falling in range of 258.25 to 259.25 bp is considered a "22" allele

The allelic ladder defines bins for sizing STR alleles

Almost 1 bp less than the ladder allele

"Variant Allele"

STR alleles stutter products

Biological (PCR) artifacts

DNA analysts interpret data to sort out which peaks are STR alleles versus artifacts

Thresholds for Measuring DNA Data

Peak is called (deemed "reliable")

• Detection (analytical) threshold
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  - ~150-200 RFU
  - Important in mixture interpretation

STR Data Interpretation Involves Determining What is a True Allele (Peak)

All of these issues impact mixture interpretation

Peak is NOT called (deemed "unreliable")

Baseline noise

Deciphering Artifacts from the True Alleles

Biological (PCR) artifacts

STR alleles

Dye blob

Stutter product

Pull-up (bleed-through)

Incompletely adenylated

DNA analysts interpret data to sort out which peaks are STR alleles versus artifacts

Thresholds for Measuring DNA Data

Peak is called (deemed "reliable")

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STR Data Interpretation Involves Determining What is a True Allele (Peak)

All of these issues impact mixture interpretation

Peak detection threshold

Peak height ratio (PHR)

Stutter percentage

Signal >3x sd of noise (or S/N >3)

PHRs consistent with single source are typically above 60%

Stutter is usually one repeat position less and <15% than true allele

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
A Report is Generated Based on the STR Allele Calls

How Are Such Large Numbers Generated with Random Match Probabilities?

- Each allele is sampled multiple times to produce a statistically stable allele frequency in the populations of interest (e.g., African American, Caucasian, and Hispanic); each population is calculated assuming no population substructure (using only $p^2$ and $2\cdot pq$).

- Using a theoretical model from genetics called Hardy-Weinberg equilibrium, the predicted frequency of a genotype at a particular locus is calculated ($p^2$ for homozygotes and $2\cdot pq$ for heterozygotes).

- Since the forensic STR loci are on separate chromosomes and thus inherited independently, the result from each locus can be multiplied together with the other tested loci to produce an estimate of the rarity of a particular multi-locus DNA profile, often referred to as the product rule.

The Same 13 Locus STR Profile in Different Populations

1 in 837 trillion ($10^{15}$) in U.S. Caucasian population
1 in 0.84 quadrillion ($10^{16}$) in U.S. Caucasian population
1 in 1.86 quadrillion ($10^{15}$) in Canadian Caucasian population
1 in 16.6 quadrillion ($10^{16}$) in African American population
1 in 17.6 quadrillion ($10^{16}$) in African American population
1 in 18.0 quadrillion ($10^{16}$) in U.S. Hispanic population

These values are for unrelated individuals assuming no population substructure (using only $p^2$ and $2\cdot pq$).

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

http://www.cstl.nist.gov/biotech/strbase/NISTpop.htm
DNA Mixtures: Detection and Interpretation

Single Source vs. Mixture Samples

<table>
<thead>
<tr>
<th>Locus 1</th>
<th>Locus 2</th>
<th>Locus 3</th>
<th>Locus 4</th>
<th>Locus 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>16,16</td>
<td>9,0,3</td>
<td>8,2,12</td>
<td>9,9</td>
<td>17,19</td>
</tr>
</tbody>
</table>

One or two peaks observed at each locus (tested DNA region)

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<tr>
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More than two peaks observed at more than two loci (tested DNA regions)

Different possible combinations could have given rise to the particular mixture observed

With Some Mixtures, Multiple Genotype Combinations Are Possible

- AC
- BD
- AB
- CD
- BC
- AD

Peak Height Ratios (PHR)
Minimum Peak Height (mPH)
Proportion (p) or mixture proportion (Mx)

Depends on PHR and proportion of mixture components from the various contributors

From Report to the Virginia Scientific Advisory Committee by the DNA Subcommittee – Addendum January 8, 2008
(authored by Dr. Norah Rudin and Dr. Artie Eisenberg)

- "Among the many reasons that Forensic DNA analysis has become the gold standard for forensic science is the relatively discrete nature of the data. For strong, single source samples, a profile can readily be determined, and is subject to little or no analyst judgment. However, ambiguity may arise when interpreting more complex samples, such as those containing multiple contributors, of poor quality (e.g. degraded or inhibited DNA), of low quantity (e.g. contact samples), or various combinations of these challenging situations..."

http://www.dfs.virginia.gov/about/minutes/saCommittee/20080108.pdf

From Report to the Virginia Scientific Advisory Committee by the DNA Subcommittee – Addendum January 8, 2008
(authored by Dr. Norah Rudin and Dr. Artie Eisenberg)

- "...These kinds of samples are encountered with increasing frequency, as the sensitivity of the technology has increased, and as law enforcement has become more sophisticated about the kinds of samples they submit for analysis. Difficult samples are also frequently encountered when reanalyzing historical cases, in which samples were not collected and preserved using the precautions necessary for DNA analysis..."

"Cold cases" or Innocence Project samples...

http://www.dfs.virginia.gov/about/minutes/saCommittee/20080108.pdf

From Report to the Virginia Scientific Advisory Committee by the DNA Subcommittee – Addendum January 8, 2008
(authored by Dr. Norah Rudin and Dr. Artie Eisenberg)

- "It is for these types of challenging samples, where the evidence profile may not exactly "match" a reference profile, that confirmation bias becomes a concern. The interpretation of an evidentiary DNA profile should not be influenced by information about a subject’s DNA profile. Each item of evidence must be interpreted independently of other items of evidence or reference samples. Yet forensic analysts are commonly aware of submitted reference profiles when interpreting DNA test results, creating the opportunity for confirmatory bias, despite the best intentions of the analyst..."

http://www.dfs.virginia.gov/about/minutes/saCommittee/20080108.pdf
Mixture Basics

- Mixtures arise when two or more individuals contribute to the sample being tested.
- Mixtures can be challenging to detect and interpret without extensive experience and careful training.
- Differential extraction can help distinguish male and female components of many sexual assault mixtures.

Sources of DNA Mixtures

- **Two (or more) individuals** contribute to the biological evidence examined in a forensic case (e.g., sexual assault with victim and perpetrator or victim, consensual sexual partner, and perp)
- **Contamination** of a single source sample from:
  - evidence collection staff
  - laboratory staff handling the sample
  - Low-level DNA in reagents or PCR tubes or pipet tips

Differential extraction can help here in some cases to isolate male DNA...

Even more challenging with poor quality data when degraded DNA is present...

Steps in the Interpretation of Mixtures
(Clayton et al. 1998)

1. Identify the Presence of a Mixture
2. Designate Allele Peaks
3. Identify the Number of Potential Contributors
4. Estimate the Relative Ratio of the Individuals Contributing to the Mixture
5. Consider All Possible Genotype Combinations
6. Compare Reference Samples

Statistical Approaches with Mixtures

- **Inferring Genotypes of Contributors** - Separate major and minor components into individual profiles and compute the random match probability estimate as if a component was from a single source
- **Calculation of Exclusion Probabilities** - CPE/CPI (RMNE) – The probability that a random person (unrelated individual) would be excluded as a contributor to the observed DNA mixture
- **Calculation of Likelihood Ratio Estimates** – Comparing the probability of observing the mixture data under two (or more) alternative hypotheses; in its simplest form LR = 1/RMP

Detecting the Presence of a Mixture

- The probability that a mixture will be detected improves with the use of more loci and genetic markers that have a high incidence of heterozygotes.
- The detectability of multiple DNA sources in a single sample relates to the ratio of DNA present from each source, the specific combinations of genotypes, and the total amount of DNA amplified.
- Some mixtures will not be as easily detectable as other mixtures.

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
The DNA Advisory Board (DAB) Recommendations on Statistics
February 23, 2000
Forensic Sci. Comm. 2(3); available on-line at

“The DAB finds either one or both PE or LR calculations acceptable and strongly recommends that one or both calculations be carried out whenever feasible and a mixture is indicated”

- Probability of exclusion (PE)
- Likelihood ratios (LR)

Probability of exclusion (PE)


Likelihood ratios (LR)


Mixture Example
Comparing Alleles Only

Locus 1 Locus 2 Locus 3

Mixed stain
15 16 17 18 12 13 14 10 11 12

Reference
15 16 12 14 11

Mixture Example
Showing Importance of Using Peak Height Information

Locus 1 Locus 2 Locus 3

Mixed stain
15 16 17 18 12 13 14 10 11 12

Reference
15 16 12 14 11

Yes, the reference alleles are present in the evidence mixed stain
BUT the peak height patterns do not fit...

Mixture Example
Solving Components Prior to Comparison to Suspect Reference

Locus 1 Locus 2 Locus 3

Component 1: 15 17 12 13 11 12

Component 2: 16 18 14,14 10,10

Reference (suspect) does not match either component of the mixed stain and therefore could not have contributed to the evidence sample.

Another Mixture Example

Conclusions from the evidence:

1. Major contributor = 13,15 (victim) – to be expected with an intimate sample like a fingernail or vaginal swab
2. Alleles 12 and 14 are likely stutter products of the major contributor’s 13 and 15 alleles but could also be masking minor contributor alleles
3. A number of minor contributor combinations are possible (e.g., 10,11 or 10,12 or 10,13 or 11,13, etc.)
4. Could have more than two contributors present in this mixture

“Suspect cannot be excluded” BUT statement needs to be qualified by statistics because a large percentage of the population might also not be able to be excluded.

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
Probability of Exclusion Calculation for a Single STR Locus

Evidence (mixture)

The fact that in this case a suspect is included is not very informative because ~9 out of 10 people examined from any population could potentially be included in the evidence mixture...

From VA DFS STR Allele Frequencies

<table>
<thead>
<tr>
<th>STR Locus</th>
<th>H</th>
<th>C</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1045/134</td>
<td>4.9</td>
<td>12.3</td>
<td>16.9</td>
</tr>
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</table>

PE (%) = 1 - PI

SUM = PI

0.1114 0.1231 0.1692

0.8886 0.8769 0.8308

DNA Degradation

Intact sample

Target region for PCR

300 base pair PCR product can be produced

Degraded sample

Target region for PCR is fragmented

300 base pair PCR product can not be produced or only in limited quantities

DNA Degradation Means Less Loci Work

Impact of Degraded DNA Samples

- Comparison to a phone number (string of 13 numbers)

001-301-975-4049

- If you only had “4049”…this information would be of limited value since it is not as specific (and could match other phone numbers from different area codes)

- DNA profiles are essentially a string of numbers – if the DNA is damaged, then the string of numbers is shorter and less informative...

---------4049 or ----301-9------

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
The Statistic (Determining the Weight of the Evidence) Should Be Calculated from the Evidence

<table>
<thead>
<tr>
<th>Evidence (partial profile):</th>
<th>Reference (full profile):</th>
</tr>
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<tbody>
<tr>
<td>Type</td>
<td>Statistic</td>
</tr>
<tr>
<td>Locus 1</td>
<td>16,17</td>
</tr>
<tr>
<td>Locus 2</td>
<td>17,18</td>
</tr>
<tr>
<td>Locus 3</td>
<td>21,22</td>
</tr>
<tr>
<td>Locus 4</td>
<td>12,14</td>
</tr>
<tr>
<td>Locus 5</td>
<td>28,30</td>
</tr>
</tbody>
</table>

Product = 1 in 171,000

The reference sample is still a “match” – just not as much information is available from the evidence for comparison

The Statistic (Determining the Weight of the Evidence) Should Be Calculated from the Evidence

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</tr>
<tr>
<td>Locus 5</td>
<td>28,30</td>
</tr>
<tr>
<td>Locus 6</td>
<td>14,16</td>
</tr>
<tr>
<td>Locus 7</td>
<td>12,13</td>
</tr>
<tr>
<td>Locus 8</td>
<td>11,14</td>
</tr>
<tr>
<td>Locus 9</td>
<td>9,9</td>
</tr>
<tr>
<td>Locus 10</td>
<td>9,11</td>
</tr>
<tr>
<td>Locus 11</td>
<td>6,6</td>
</tr>
<tr>
<td>Locus 12</td>
<td>8,8</td>
</tr>
<tr>
<td>Locus 13</td>
<td>10,10</td>
</tr>
</tbody>
</table>

Product = 1 in 665 trillion

Quality Control Measures Used in Forensic Laboratories

Checks and Controls on DNA Results

<table>
<thead>
<tr>
<th>Community</th>
<th>FBI DNA Advisory Board’s Quality Assurance Standards (also interlaboratory studies)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory</td>
<td>ASCCL/LAB Audits and Accreditation</td>
</tr>
<tr>
<td>Analyst</td>
<td>Proficiency Tests &amp; Continuing Education</td>
</tr>
<tr>
<td>Method/instrument</td>
<td>Validation of Performance (along with traceable standard samples)</td>
</tr>
<tr>
<td>Protocol</td>
<td>Standard Operating Procedure is followed</td>
</tr>
<tr>
<td>Data Sets</td>
<td>Allelic ladders, positive and negative amplification controls, and reagent blanks are used</td>
</tr>
<tr>
<td>Individual Sample</td>
<td>Internal size standard present in every sample</td>
</tr>
<tr>
<td>Interpretation of Result</td>
<td>Second review by qualified analyst/supervisor</td>
</tr>
<tr>
<td>Court Presentation of Evidence</td>
<td>Defense attorneys and experts with power of discovery requests</td>
</tr>
</tbody>
</table>

Standard Operating Procedures (SOPs)

- Based on validation studies performed in a laboratory
- Validation studies help define a range over which reliable results can be expected (e.g., a detection threshold of 150 RFU with DNA profile peaks)
- An SOP helps to ensure consistency from case-to-case and analyst-to-analyst within a laboratory and should keep analysts within the scope of reliable results defined by the validation studies
- SOPs may differ between labs (e.g., Virginia vs. FBI)

Virginia’s State Forensic Laboratory Makes Their Standard Operating Procedures Available

http://www.dfs.virginia.gov/manuals/manuals.cfm?id=5

Summary

- “DNA” + “Match” ➔ “Guilty” in the minds of many jurors
- Consider the assumptions with the weight of the evidence particularly for mixtures
- The technology is advancing rapidly with new capabilities becoming available...
- Training for both the scientific and legal communities is vital to make the most effective use of the wonderful power of DNA technology
If You Want to Know More Regarding Recent Advances...
See Review Article on "Forensic Science" in Analytical Chemistry

Describes 181 forensic DNA articles published in 2005 and 2006
(560 references covering DNA, trace evidence, drugs and poisons)

<table>
<thead>
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<th></th>
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<tbody>
<tr>
<td>T.A. Brettell</td>
<td>Forensic science, Anal. Chem. 79: 4365-4384</td>
</tr>
<tr>
<td>J. M. Butler</td>
<td>Bioclinical Science Division, National Institute of Standards and Technology, Gaithersburg, Maryland</td>
</tr>
<tr>
<td>J. R. Almirall</td>
<td>Department of Chemistry and Biochemistry, and International Forensic Research Institute, Florida International University, University Park, Miami, Florida 33199</td>
</tr>
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Available at http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

Thank you for your attention...
Our team publications and presentations are available at:
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

Questions?
See also http://www.dna.gov/research/nist
http://www.cstl.nist.gov/biotech/strbase
john.butler@nist.gov

Status of Genetic Marker Systems Used in Forensic DNA Testing

- STRs – widely used in casework and national databases world-wide
- miniSTRs – smaller versions of STR loci that can work well on degraded DNA
- Y-STRs – permits examination of male-only DNA
- mtDNA – used in specialty labs for highly degraded specimens or hair that contains limited amounts of DNA
- SNPs – potential for identifying ethnicity of evidence sample; still in research and likely to be limited in use