Interlaboratory Mixture Studies

AAFS 2008 Workshop #16
Washington, DC
February 19, 2008
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Outline

• Purpose of Interlaboratory Studies

• Overview of Mixture Studies and Lessons Learned

• NIST MIX05 Study Results

Interlaboratory Studies

• Purpose…
  – Not a proficiency test
  – Most labs see them as opportunity to anonymously directly compare themselves to others

• STRBase section on interlab studies

A High Degree of Variability Currently Exists with Mixture Interpretation

- “If you show 10 colleagues a mixture, you will probably end up with 10 different answers”
  - Peter Gill, Human Identification E-Symposium, April 14, 2005

- Interlaboratory studies help to better understand why variability may exist between laboratories

- Most analysts are only concerned about their own lab protocols and do not get an opportunity to see the big picture from the entire community that can be provided by a well-run interlaboratory study

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**Interlaboratory Summary**

**QuantiBlot**

Your Values

The National Institute of Standards and Technology

GratefullyAcknowledges the Participation of the Laboratory XYZ

In the 2001 Interlaboratory Challenge Exercise “Mixed Stain Study #3”

<table>
<thead>
<tr>
<th>Sample Quantitation</th>
<th>Sample Typing</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA Concentration, ng/μL</td>
<td>Donor Alleles (Major + Minor) Identified, %</td>
</tr>
<tr>
<td>Others</td>
<td>99.5</td>
</tr>
<tr>
<td>You</td>
<td>3</td>
</tr>
</tbody>
</table>

This feedback can be helpful to a laboratory to know where they stand relative to other labs to illustrate opportunities for improvement.


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**Individual Performance in an Interlaboratory Study**

**DNA Quantitation**

<table>
<thead>
<tr>
<th>DNA Concentration, ng/μL</th>
<th>Yield gel</th>
<th>QuantiBlot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Others</td>
<td>2.5%</td>
<td>25%</td>
</tr>
<tr>
<td>You</td>
<td>0.3</td>
<td>0.5</td>
</tr>
</tbody>
</table>

2 different quant methods gave different results; this lab followed the QuantiBlot results


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### Process for Interlaboratory Study

1. **Study Design**
   - Stability Testing of Materials
   - Solicitation of Participants

2. **Manufacturing and Shipping**
   - Put samples in tubes
   - Put tubes in boxes
   - Generate labels and shipping orders

3. **Receipt of Data and Analysis**
   - Enter data into common format
   - Reports back to laboratories on their performance relative to the entire study

4. **Laboratories Conduct Studies**
   - Test DNA samples over time
   - Test DNA samples with multiple methods

5. **Reports and Publications**
   - Consider lessons learned from previous studies
   - Decide on number of experiments, quantity of tests, and types of samples

### NIST Initiated Interlaboratory Studies

<table>
<thead>
<tr>
<th>Studies involving STRs</th>
<th># Labs</th>
<th>Publications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixture Interpretation Study (Jan–Aug 2005)</td>
<td>69</td>
<td>Several presentations made. Poster at 2005 Promega meeting (Sept 2005); available on STRBase.</td>
</tr>
</tbody>
</table>
Overall Lessons Learned from NIST MSS 1,2,&3

- Laboratories have instruments with different sensitivities
- Different levels of experience and training plays a part in effective mixture interpretation
- Amount of input DNA makes a difference in the ability to detect the minor component (labs that put in "too much" DNA actually detected minor components more frequently)

NIST MIX05 Summary

Purpose of MIX05 Study

- Goal is to understand the “lay of the land” regarding mixture analysis across the DNA typing community
- One of the primary benefits we hope to gain from this study is recommendations for a more uniform approach to mixture interpretation and training tools to help educate the community
Interlab Variability – J.M. Butler

MIX05 Study Design and Purpose

- Permit a large number of forensic practitioners to evaluate the same mixture data
- Provide multiple cases representing a range of mixture scenarios
- Generate data from multiple STR kits on the same mixture samples to compare performance for detecting minor components
- The primary variable should be the laboratory's interpretation guidelines rather than the DNA extraction, PCR amplification, and STR typing instrument sensitivity
- Are there best practices in the field that can be advocated to others?

Mixture Interpretation Interlab Study (MIX05)

- Only involves interpretation of data – to remove instrument detection variability and quantitation accuracy issues
- 94 labs enrolled for participation
- 69 labs have returned results (17 from outside U.S.)
- Four mock cases supplied with “victim” and “evidence” electropherograms (GeneScan .fsa files – that can be converted for Mac or GeneMapper; gel files made available to FMBIO labs)
- Data available with Profiler Plus, COflle, SGM Plus, PowerPlex 16, Identifiler, PowerPlex 16 BIO (FMBIO) kits
- Summary of results will involve training materials to illustrate various approaches to solving mixtures

Perpetrator Profile(s) ???
Along with reasons for making calls and any stats that would be reported

Requests for Participants in MIX05

Mixtures representing four different case scenarios have been generated at NIST with multiple STR kits and provided to laboratories as electropherograms.

We would like to receive the following information:

1) Report the results as though they were from a real case including whether a statistical value would be attached to the results. Please summarize the perpetrator(s) alleles in each “case” as they might be presented in court—along with an appropriate statistic (if warranted by your laboratory standard operating procedure) and the source of the allele frequencies used to make the calculation. Please indicate which kit(s) were used to solve each case.

2) Estimate the ratio for samples present in the evidence mixture and how this estimate was determined.

3) Provide a copy of your laboratory mixture interpretation guidelines and a brief explanation as to why conclusions were reached in each scenario.
A MIX05 Participant Noted…

“Things we do not do:

- Calculate mixture ratios for casework
  - Calculation used for this study: Find loci with 4 alleles (2 sets of sister alleles). Make sure sister alleles fall within 70%, then take the ratio of one allele from one sister set to one allele of the second sister set, figure ratios for all combinations and average. Use peak heights to calculate ratios.
- Provide allele calls in reports
- Provide perpetrator(s) alleles or statistics in court without a reference sample to compare to the DNA profile obtained from the evidence. We will try to determine the perpetrator(s) profile for entry into CODIS.

We recognize that some of the information requested in this interlab study may not be part of a lab’s standard operating procedure.

MIX05 Case Scenarios

Genomic DNA samples with specific allele combinations (‘evidence’) were mixed in the following ratios:

- Case #1 – victim is major contributor (3F:1M)
- Case #2 – perpetrator is major contributor (1F:3M)
- Case #3 – balanced mixture (1F:1M)
  - Male lacked amelogenin X
- Case #4 – more extreme mixture (7F:1M)
  - Male contained tri-allelic pattern at TPOX

Female victim DNA profile was supplied for each case

Labs asked to deduce the perpetrator DNA profile – suspect(s) not provided

Amelogenin X allele is missing in male perpetrator DNA sample for MIX05 Case #3

## MIX05 Results on Multiple Kits

[Link to MIX05 Results](http://www.cstl.nist.gov/biotech/strbase/interlab/MIX05.htm)

### ABI 3100 Generated Data
- **Profiler Plus**
- **COflffer**
- **Identifiler**
- **PowerPlex 16**
- **SGM Plus**

ABI 3100 generated data was supplied on CD-ROM to labs as either .fsa files (for Genotyper NT or GeneMapper ID) or Mac-converted files for Genotyper Mac.

FMBIO data was also made available upon request.

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## Summary of MIX05 Responses

- **94 labs enrolled** for participation
- **69 labs returned results** (17 from outside U.S.)
- **50 labs made allele calls**
- **39 labs estimated ratios**
- **29 labs provided stats**

**STR kit results used**
- 24 Profiler Plus/COfiler
- 10 PowerPlex 16
- 7 PP16 BIO
- 5 Identifiler
- 2 SGM Plus
- 1 All ABI kit data
- 9 Various combinations

All participants were supplied with all data and could choose what kits to examine based on their experience and lab protocols.

Generally Identifiler data was of poorer quality in the electropherograms we provided...which caused some labs to not return results (they indicated a desire for higher quality data through sample re-injection to reduce pull-up prior to data interpretation).

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## What MIX05 Participants Have Received Back from NIST...

- Certificate of participation in the interlab study
- Copy of the poster presented at the Promega Sept 2005 meeting displaying "correct" results for the perpetrator in each case scenario as well as an explanation of study design and preliminary results

[Link to MIX05 Poster](http://www.cstl.nist.gov/biotech/strbase/interlab/MIX05/MIX05poster.pdf)
When is a Sample a Potential Mixture?

According to several MIX05 participant interpretation guidelines:

- **Number of Observed Peaks**
  - Greater than two peaks at a locus
  - More than two alleles are present at two or more loci, although three banded patterns can occur
  - Presence of 3 alleles at a single locus within a profile
  - 4 peaked patterns (if observed at any locus), 3 peaked patterns (if observed at two or more loci), significant imbalances (peak height ratios <60%) of alleles for a heterozygous genotype at two or more loci with the exception of low template amplifications, which should be interpreted with caution

- **Imbalance of heterozygote alleles**
  - thresholds range from 50-70%

- **Stutter above expected levels**
  - generally 15-20%

These protocol differences can lead to variation in reported alleles and therefore the deduced profile and resulting statistics.

Summary of Some MIX05 Reported Results

**Case #2 has perpetrator as major component and thus is the easiest to solve…**

Some Mixture Ratios Reported in MIX05

<table>
<thead>
<tr>
<th>LabID</th>
<th>Case 1 (F/M)</th>
<th>Case 2 (F/M)</th>
<th>Case 3 (M/F)</th>
<th>Case 4 (F/M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>2</td>
<td>5</td>
<td>&lt;2</td>
<td>10</td>
</tr>
<tr>
<td>70</td>
<td>1.8-3.6</td>
<td>3.8-4.7</td>
<td>1.6-1.8</td>
<td>6.2-7.6</td>
</tr>
<tr>
<td>59</td>
<td>68%/32%</td>
<td>85%/15%</td>
<td>64%/36%</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>2.1</td>
<td>6.1</td>
<td>2.1</td>
<td>not determined</td>
</tr>
<tr>
<td>54</td>
<td>2.1</td>
<td>6.1</td>
<td>2.1</td>
<td>6.1</td>
</tr>
<tr>
<td>90</td>
<td>male3/39%</td>
<td>not determined</td>
<td>male64/71%</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>3 or 4</td>
<td>4 or 5</td>
<td>1.4</td>
<td>10/1</td>
</tr>
<tr>
<td>4</td>
<td>10/1</td>
<td>6.1</td>
<td>1.1</td>
<td>not determined</td>
</tr>
<tr>
<td>33</td>
<td>male80/20%</td>
<td>male80/20%</td>
<td>male87/13%</td>
<td>victim8%</td>
</tr>
<tr>
<td>12</td>
<td>male52%</td>
<td>male65%</td>
<td>male68/32%</td>
<td>unknown</td>
</tr>
<tr>
<td>67</td>
<td>1.2.3</td>
<td>6.4.1</td>
<td>2.1</td>
<td>1.6.9</td>
</tr>
<tr>
<td>66</td>
<td>2.1</td>
<td>6.5.1</td>
<td>1.6-2.1</td>
<td>4.4.5.4</td>
</tr>
<tr>
<td>79</td>
<td>~3.1 to ~2.1</td>
<td>~6.1 to ~1.1</td>
<td>~2.1</td>
<td>a lot of victim</td>
</tr>
<tr>
<td>77</td>
<td>2.1</td>
<td>6.1</td>
<td>2.1</td>
<td>10.1</td>
</tr>
<tr>
<td>68</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Some Reported Stats for MIX05 Case #1

Many of the 29 labs providing statistics used PopStats 5.7

<table>
<thead>
<tr>
<th>LabID</th>
<th>Kits Used</th>
<th>Caucasians</th>
<th>African Americans</th>
<th>Hispanics</th>
</tr>
</thead>
<tbody>
<tr>
<td>77</td>
<td>Identifier</td>
<td>PE calculated</td>
<td>PE calculated</td>
<td>PE calculated</td>
</tr>
<tr>
<td>73 ProPlus/Cipher</td>
<td>none provided</td>
<td>none provided</td>
<td>none provided</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>ProPlus/Cipher</td>
<td>none provided</td>
<td>none provided</td>
<td>none provided</td>
</tr>
<tr>
<td>12</td>
<td>ProPlus/Cipher</td>
<td>none provided</td>
<td>none provided</td>
<td>none provided</td>
</tr>
<tr>
<td>20</td>
<td>Identifier</td>
<td>none provided</td>
<td>none provided</td>
<td>none provided</td>
</tr>
<tr>
<td>80 ProPlus/Cipher</td>
<td>1.10E+15</td>
<td>2.10E+14</td>
<td>3.00E+15</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>ProPlus/Cipher</td>
<td>2.40E+11</td>
<td>7.00E+09</td>
<td>9.00E+10</td>
</tr>
<tr>
<td>48</td>
<td>ProPlus/Cipher</td>
<td>2.94E+08</td>
<td>3.00E+07</td>
<td>5.00E+08</td>
</tr>
<tr>
<td>33</td>
<td>ProPlus/Cipher</td>
<td>2.10E+08</td>
<td>1.12E+07</td>
<td>1.74E+08</td>
</tr>
<tr>
<td>6</td>
<td>ProPlus/Cipher</td>
<td>4.00E+00</td>
<td>3.50E+00</td>
<td>2.00E+00</td>
</tr>
<tr>
<td>9</td>
<td>ProPlus/Cipher</td>
<td>1.14E+02</td>
<td>1.97E+02</td>
<td>1.54E+03</td>
</tr>
<tr>
<td>61</td>
<td>Identifier</td>
<td>1.40E+06</td>
<td>2.50E+06</td>
<td>2.40E+07</td>
</tr>
<tr>
<td>79 ProPlus/Cipher</td>
<td>5.00E+00</td>
<td>4.70E+00</td>
<td>1.350E+00</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>ProPlus/Cipher</td>
<td>4.54E+02</td>
<td>3.17E+02</td>
<td>3.59E+03</td>
</tr>
</tbody>
</table>

Which loci are included in each calculation?

Some Differences in Reporting Statistics

<table>
<thead>
<tr>
<th>LabID</th>
<th>Kits Used</th>
<th>Caucasians</th>
<th>African Americans</th>
<th>Hispanics</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>ProPlus/Cipher</td>
<td>1.10E+15</td>
<td>2.10E+14</td>
<td>3.00E+15</td>
</tr>
<tr>
<td>34</td>
<td>ProPlus/Cipher</td>
<td>2.40E+11</td>
<td>7.00E+09</td>
<td>9.00E+10</td>
</tr>
<tr>
<td>33</td>
<td>ProPlus/Cipher</td>
<td>2.94E+08</td>
<td>3.00E+07</td>
<td>5.00E+08</td>
</tr>
<tr>
<td>6</td>
<td>ProPlus/Cipher</td>
<td>4.00E+00</td>
<td>3.50E+00</td>
<td>2.00E+00</td>
</tr>
<tr>
<td>9</td>
<td>ProPlus/Cipher</td>
<td>1.14E+02</td>
<td>1.97E+02</td>
<td>1.54E+03</td>
</tr>
<tr>
<td>79</td>
<td>ProPlus/Cipher</td>
<td>5.00E+00</td>
<td>4.70E+00</td>
<td>1.350E+00</td>
</tr>
<tr>
<td>18</td>
<td>ProPlus/Cipher</td>
<td>4.54E+02</td>
<td>3.17E+02</td>
<td>3.59E+03</td>
</tr>
</tbody>
</table>

~10 orders of magnitude difference (10^7 to 10^15) based on which alleles were deduced and reported

Remember that these labs are interpreting the same MIX05 electropherograms

Further Examination of These 7 Labs

<table>
<thead>
<tr>
<th>LabID</th>
<th>Kits Used</th>
<th>Caucasians</th>
<th>ASCLD-LAB accredited?</th>
<th>Solved loci listed?</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>ProPlus/Cipher</td>
<td>1.10E+15</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>34</td>
<td>ProPlus/Cipher</td>
<td>2.40E+11</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>33</td>
<td>ProPlus/Cipher</td>
<td>2.94E+08</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>ProPlus/Cipher</td>
<td>4.00E+00</td>
<td>Yes</td>
<td>No (CPE)</td>
</tr>
<tr>
<td>9</td>
<td>ProPlus/Cipher</td>
<td>4.14E+07</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>79</td>
<td>ProPlus/Cipher</td>
<td>9.00E+00</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>18</td>
<td>ProPlus/Cipher</td>
<td>4.34E+00</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Possible Reasons for Variability in Reported Statistics:

- Different types of calculations (CPE vs RMP)
- Different loci included in calculations (due to different thresholds used)
- Different allele frequency population databases (most use PopStats)
- Use of victim (e.g., major component in Case 1) profile stats

Different Stats Used

- Lab 9 \((4.14 \times 10^7)\) used \(1/CPI\)
- Lab 6 \((4.0 \times 10^7)\) used selected loci and summed all possible genotypes for loci not completely deduced
- Lab 90 \((1.18 \times 10^{15})\) used theta value of 0.03 and deduced alleles at all 13 loci (correctly deduced all perpetrator alleles)

Different Thresholds of Detection Influence Allele Calls

- Lab 90 has specific, detailed mixture interpretation guidelines with worked examples and a fabulous flowchart
- Lab 16 has vague guidelines that begin with “mixture interpretation is not always straightforward. Analysts must depend on their knowledge and experience…”

Different Detection Thresholds Used

<table>
<thead>
<tr>
<th>Lab</th>
<th>Kits Used</th>
<th>Case 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>ProPlus/Collier</td>
<td>118E+16</td>
</tr>
<tr>
<td>34</td>
<td>ProPlus/Collier</td>
<td>2.4E+11</td>
</tr>
<tr>
<td>33</td>
<td>ProPlus/Collier</td>
<td>2.9E+06</td>
</tr>
<tr>
<td>6</td>
<td>ProPlus/Collier</td>
<td>40,000,000</td>
</tr>
<tr>
<td>9</td>
<td>ProPlus/Collier</td>
<td>4.1E+07</td>
</tr>
<tr>
<td>79</td>
<td>ProPlus/Collier</td>
<td>930,000</td>
</tr>
<tr>
<td>16</td>
<td>ProPlus/Collier</td>
<td>434,000</td>
</tr>
</tbody>
</table>

Case 1:
- 75 RFUs: all 13 STRs; all results correct
- Not stated: 8 STRs, 2 partial, 3 INC
- Not provided: 3 STRs, 6 partial, 4 INC
- 150 RFUs: 2 STR, 5 partial, 6 INC

LOQ (77 RFU)
LOD (23 RFU)
Manually Solving Mixture Component Profiles

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele</th>
<th>Peak height</th>
<th>Possible Component profile giving rise to observed mixture</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Lab 90 – correctly deduced all perpetrator alleles in Case #1 (highest of the 7 listed stats for ProPlus/COfiler at 1.18 x 10^15)
Also prepared a CODIS Search/Upload Request with the deduced profile

A Model Report of Analysis...

• “The Profile Plus and COfiler sample files were evaluated by four different analysts, using both NT and MAC analysis platforms. The analysts checked for concordance, and a single conclusion for each mock case has been issued.”

• They detailed all assumptions made outside the course of routine casework:
  – Assumed intimate samples
  – That a comparison of deduced “foreign” alleles had been made with the perpetrator’s known standard in order to calculate the significance of the inclusion with the evidentiary profile

• For Case #4: “A Combined Probability of Inclusion was calculated and reported for only those loci where all the alleles were above threshold [75 RFUs]. However, a minor profile(s) could not be deduced from this sample. Please note that our laboratory may employ strategies to gain more information from the sample, such as a 10 second injection of the CE and Y-STR analysis.”

Quotes from One Lab’s MIX05 Report

• Case 1: STR typing results from the Evidence sample indicate a DNA mixture profile. The victim cannot be excluded as a possible donor of the genetic material in the Evidence sample. No statistics will be generated at this time.

• The Evidence samples would have to be rerun in order to verify any alleles called in the final profiles. This is true for any mixed sample profiles as per our laboratory guidelines.

• Our laboratory does not “pull out” any profile from a mixture for interpretation or statistical purposes. The exception to this is for CODIS profiles where the alleles that can be unambiguously attributed to the victim are removed.

• We currently do not calculate and report statistics on mixture samples.
Examples of MIX05 Report Formats

All examples with Case #1
(~3:1 mixture with female victim as the major component – and victim profile is provided)

Manual Solving of MIX05 Peak Ratios and Possible Mixture Combinations

Another MIX05 Participant Manually Solving a Mixture
Semi-Automated Locus-by-Locus Interpretation Performed by One MIX05 Participant

Excel spreadsheet used to examine possible component combinations

Different Reporting Formats for MIX05 Data

No attempt to deduce perpetrator alleles (foreign profile)
The community would benefit from more uniform reporting formats and mixture solving strategies...
Some Protocols Have Flow Charts to Help Make Decisions in Mixture Resolution

Value of the MIX05 Study

- Data sets exist with multiple mixture scenarios and a variety of STR kits that can be used for training purposes
- A wide variety of approaches to mixture interpretation have been applied on the same data sets evaluated as part of a single study
- Interpretation guidelines from many laboratories are being compared to one another for the first time in an effort to determine challenges facing future efforts to develop "expert systems" for automated mixture interpretation
- We are exploring the challenges of supplying a common data set to a number of forensic laboratories (e.g., if a standard reference data set was ever desired for evaluating expert systems)

Conclusions from the MIX05 Study (Opportunities for Improvement)

- It is worth taking a closer look at protocol differences between labs to see the impact on recovering information from mixture data
- Training should help bring greater consistency
- Expert systems (when they become available and are used) should help aid consistency in evaluating mixtures and help produce more uniform reporting formats
NIST Software Programs to Aid Mixture Work

Excel-based programs developed by David Duewer (NIST)

- **mixSTR** (developed at request of Palm Beach Sheriff’s Office)
  - Does not interpret data (relies on user inputted alleles following STR data review)
  - Aids in the organization of STR mixture information
  - Considers only the presence/absence of alleles (no peak heights used)

- **Virtual MixtureMaker** (developed to aid MIX05 sample selection)
  - Creates mixture combinations through pairwise comparisons of input STR profiles
  - Returns information on the number of loci possessing 0, 1, 2, 3, 4, 5, or 6 alleles in each 2-person mixture (also reports number of loci in each sample with 0, 1, 2, or 3 alleles)
  - Useful for selection of samples in mixture or validation studies with various degrees of overlapping alleles in combined STR profiles
  - Useful in checking for potentially related individuals in a population database

Programs can be downloaded from NIST STRBase web site:
http://www.cstl.nist.gov/div831/strbase/software.htm

mixSTR Program

Comparisons are made between

- suspect and evidence (S/E) alleles,
- suspect and suspect (S/S) alleles (to look for potential close relatives),
- evidence and other evidence (E/E) sample(s) alleles (to see how various evidentiary samples compare to one another), and
- controls to evidence (C/E) and controls to suspect (C/S) alleles (as a quality control contamination check).

mixSTR S/E output

Example of suspect to evidence (S/E) comparisons made in this case. Note that the suspect is 21,23 at FGA while the evidence contains 23,24* (* indicates that allele 24 is a minor component). Thus this suspect has allele 23 in common and is missing allele 24 in the evidence.
When the STR profiles for these two individuals are combined to create a 2-person mixture, the mixture profile will contain 1 locus with a single allele, 7 loci with two alleles, 4 loci with three alleles, and 3 loci with four alleles (and no loci with 5 or 6 alleles, which is only possible if one or both samples possess tri-allelic patterns at the same STR locus).

16 loci examined with 31 distinguishable alleles
One tri-allelic locus
13 heterozygous loci
2 homozygous loci

Virtual MixtureMaker Output

Some Final Thoughts…

• It is of the highest importance in the art of detection to be able to recognize out of a number of facts, which are incidental and which vital. Otherwise your energy and attention must be dissipated instead of being concentrated (Sherlock Holmes, The Reigate Puzzle).

• "Don't do mixture interpretation unless you have to" (Peter Gill, Forensic Science Service, 1998).

• Mixture interpretation consumes a large part of DNA analysts' time – software tools that improve consistency in analysis will speed casework reporting and hopefully cases solved.
Conclusion

"Mixture interpretation theory is well established and used in forensic laboratories. Most mixtures detected in casework are satisfactorily solved. But from this revision we can conclude that the behaviour of each mixed sample can be different and multifactorial and occasionally its interpretation turns out to be complicated—sometimes paralleling the importance of the evidence in the resolution of the case. In some casework mixtures our experience has proved that theoretical assumptions from studies with laboratory samples, albeit very useful, can turn out to be impracticable. We consider that more sharing of day to day forensic laboratory problems is needed to refine our technical procedures in the resolution of specially difficult evidence."

Acknowledgments

Funding from interagency agreement 2003-JJ-R-029 between NIJ and the NIST Office of Law Enforcement Standards

NIST Human Identity Project Team — Leading the Way in Forensic DNA...

Role in MIX05

- Margaret Kline (running study, sample prep, data review)
- John Butler (study design and data review)
- Becky Hill (GeneMapper® data review)
- Jan Redman (Access database entry, shipping)
- Dave Duewer (Virtual MixtureMaker to aid sample selection; mixSTR program)
- Chris Tomsey & Frank Krist (FMBIO Mac data)
- Kermit Channel & Mary Robnett (FMBIO NT data)
- Mandy Sozer for early discussions on study design

The many forensic scientists and their supervisors who took time out of their busy schedules to examine the MIX05 data provided as part of this interlaboratory study