An Investigation of Software Programs Using “Drop-out” and “Continuous” Methods for Complex Mixture Interpretation

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NIST and NIJ Disclaimer

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Challenging Mixtures - Uncertainty

• If allele dropout is a possibility (e.g., in a partial profile), then there is uncertainty in whether or not an allele is present in the sample…and therefore what genotype combinations are possible

• If different allele combinations are possible in a mixture, then there is uncertainty in the genotype combinations that are possible…
Challenging Mixtures

12 Allele
56 RFU

13 Allele
60 RFU

“Q” Allele
??
How to handle low level data

• Continue to use RMNE (CPI, CPE)
CPI = 1 in 119

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How to handle low level data

• Continue to use RMNE (CPI, CPE) (not optimal)
• Use the Binary LR with 2p
The Binary LR approach

\[ LR = \frac{1}{2pq} \]

\[ LR = \frac{0}{2pq} \]

\[ LR = \frac{?}{2pq} \]

“2p”
How to handle low level data

- Continue to use RMNE (CPI, CPE) (not optimal)
- Use the Binary LR with 2p (not optimal)
- Semi-continuous methods with a LR (Drop models)

\[ \Pr(D_{out}) = 0.89 \]
Some Drop Model Examples

- LR mix (Haned and Gill)
- LikeLTD (Balding and Buckleton)
- Lab Retriever (Lohmueller, Rudin and Inman)
- FST (NYOCME, Mitchell et al.)
- Kelly et al. (University of Auckland, ESR)
- Puch-Solis et al. (LikeLiRa and LikeLiRaHT)

The drop models only use the alleles present in the mixture
How to handle low level data

• Continue to use RMNE (CPI, CPE) (not optimal)
• Use the Binary LR with 2p (not optimal)
• Semi-continuous methods with a LR (Drop models).
• Fully continuous methods with LR.
Continuous Models

- Mathematical modeling of “molecular biology” of the profile (mix ratio, PHR (Hb), stutter, etc…) to find optimal genotypes, giving **WEIGHT** to the results.

<table>
<thead>
<tr>
<th>Probable Genotypes</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>AC</td>
<td>40%</td>
</tr>
<tr>
<td>BC</td>
<td>25%</td>
</tr>
<tr>
<td>CC</td>
<td>20%</td>
</tr>
<tr>
<td>CQ</td>
<td>15%</td>
</tr>
</tbody>
</table>
Some Continuous Model Examples

- TrueAllele (Cybergenetics)
- STRmix (ESR and Australian collaboration)
- Cowell et al. (FSI-G (2011) 5:202-209)

Weights are determined by performing simulations of the data (Markov Chain Monte Carlo - MCMC)
Software Examined

• LR Mix (Gill and Haned) – open source R program with GUI.

• Lab Retriever (Rudin, Lohmueller, Inman) – free software, based on the Balding/Buckleton approach.

• TrueAllele (Cybergenetics) – Continuous approach, publications and presentations by Perlin et al.

• STRmix (Australia/NZ) – Continuous approach, publications by Taylor, Bright and Buckleton.
Some Ground Rules

• For LRmix and Lab Retriever, the same values for $\text{Pr}(D_{\text{out}})$ and $\text{Pr}(D_{\text{in}})$ were used.

• The NIST (2003) allele frequencies for Western Europeans were used for all systems.

• TrueAllele analyses were performed at both 10 RFU (default) and 50 (2p mixtures) or 30 (3p mixture) RFUs.
Example 1 – Low-level 2p with $D_{out}$

$ST = 150$ RFU

$AT = 50$ RFU
Loci with Drop-out

POI = 13, 14

POI = 21, 24

2 loci with allele drop-out
logLR

N = 8
Time of Analysis

2p - 2DO

LRmix < 1 sec.
Lab Ret. < 1 sec.
TrueAllele 8-12 hours
STRmix 25.2 sec.
Example 2 – Low-level 2p more $D_{out}$

$ST = 150 \text{ RFU}$

$AT = 50 \text{ RFU}$
Loci with Drop-out

POI = 7, 9

POI = 11, 12

5 loci with allele drop-out; 1 locus drop-out (CSF)
## Time of Analysis

<table>
<thead>
<tr>
<th></th>
<th>2p - 2DO</th>
<th>2p - 6DO</th>
</tr>
</thead>
<tbody>
<tr>
<td>LRmix</td>
<td>&lt; 1 sec.</td>
<td>&lt; 1 sec.</td>
</tr>
<tr>
<td>Lab Ret.</td>
<td>&lt; 1 sec.</td>
<td>1 sec.</td>
</tr>
<tr>
<td>TrueAllele</td>
<td>8-12 hours</td>
<td>8-12 hours</td>
</tr>
<tr>
<td>STRmix</td>
<td>25.2 sec.</td>
<td>14.8 sec.</td>
</tr>
</tbody>
</table>
Example 3 – Low-level 3 person mixture

ST = 150 RFU
AT = 30 RFU
Example 3 – Low-level 3 person mixture

125 pg input DNA
1:2:1 ratio

A = 13,16
B = 11,13
C = 14,15

B = female
Conditioning

- $H_P = B$ (vic) and $C$ (suspect 1) and $A$ (suspect 2)

- (1) $H_D = B$ (vic) and $C$ (suspect 1) and 1 Unk

- (2) $H_D = B$ (vic) and $A$ (suspect 1) and 1 Unk

Suspect A, $\Pr(\text{DO}) = 0.02$

Suspect C, $\Pr(\text{DO}) = 0.529$
$H_D = B$ (vic) and $C$ (suspect 1) and 1 Unk (A)

![Graph](image-url)

$N = 8$
# Time of Analysis

<table>
<thead>
<tr>
<th></th>
<th>2p - 2DO</th>
<th>2p - 6DO</th>
<th>3p - A unk</th>
</tr>
</thead>
<tbody>
<tr>
<td>LRmix</td>
<td>&lt; 1 sec.</td>
<td>&lt; 1 sec.</td>
<td>3 sec.</td>
</tr>
<tr>
<td>Lab Ret.</td>
<td>&lt; 1 sec.</td>
<td>1 sec.</td>
<td>8 sec.</td>
</tr>
<tr>
<td>TrueAllele</td>
<td>8-12 hours</td>
<td>8-12 hours</td>
<td>16 hours +</td>
</tr>
<tr>
<td>STRmix</td>
<td>25.2 sec.</td>
<td>14.8 sec.</td>
<td>63.1 sec.</td>
</tr>
</tbody>
</table>
\( \mathcal{H}_D = B \) (vic) and A (suspect 1) and 1 Unk (C)
# Time of Analysis

<table>
<thead>
<tr>
<th></th>
<th>2p - 2DO</th>
<th>2p - 6DO</th>
<th>3p - A unk</th>
<th>3p - C unk</th>
</tr>
</thead>
<tbody>
<tr>
<td>LRmix</td>
<td>&lt; 1 sec.</td>
<td>&lt; 1 sec.</td>
<td>3 sec.</td>
<td>3.4 sec.</td>
</tr>
<tr>
<td>Lab Ret.</td>
<td>&lt; 1 sec.</td>
<td>1 sec.</td>
<td>8 sec.</td>
<td>7.5 sec.</td>
</tr>
<tr>
<td>TrueAllele</td>
<td>8-12 hours</td>
<td>8-12 hours</td>
<td>16 hours +</td>
<td>16 hours +</td>
</tr>
<tr>
<td>STRmix</td>
<td>25.2 sec.</td>
<td>14.8 sec.</td>
<td>63.1 sec.</td>
<td>50.5 sec.</td>
</tr>
</tbody>
</table>
Summary

• Probabilistic Methods make better use of the data than RMNE or the binary LR with 2p.

• The goal of the software programs should not be to simply “get bigger numbers” but to understand the details of these approaches and not treat the software as a “black box.”

• Semi-continuous approaches will produce a LR that could be replicated by hand if necessary.
Summary

• Each approach has its own advantages and disadvantages.

• “When analysed using a discrete model such as that of [Balding and Buckleton], it is necessary to rely on an analyst designations of low peaks as allelic, stutter, or masking and hence ambiguous.”

- Puch-Solis et al. (2013)
Future Studies

• Examination of other probabilistic programs.

• Include challenging four person mixtures.

• Determining the risk of including a suspect not in the mixture using randomly generated profiles.
NIST MIX13 Interlaboratory Study

- DNA Advisory Board Quality Assurance Standards
- Interlaboratory Studies
- NIST Mixture 2005 Interlab Study MIX05 Data
- NIST Mixture 2013 Interlab Study MIX13 Data
- Validation information
- DNA Quantitation - SRM 2372
- Technology for resolving STR alleles

Study Details

Five cases are provided each with an evidentiary sample file (a mixture of at least one suspect profile, suspect(s) profiles and other known references. We have generated .fsa files on an (BU)/IdentifilerPlus (NIST) kits. Allelic ladders, positive and negative controls are also provided.

Case #1 – represents sexual assault evidence from the sperm fraction of a vaginal swab. This is for comparison. Data available in Identifiler Plus and PP16HS.

Case #2 – represents evidence swabbed from the handle of a handgun retrieved outside the home of the victim. DNA profiles from four gang members are provided for comparison. Data generated by BU in either Identifiler or Identifiler sample – all other examples used the GS500 LIZ size standard.
Case Information
Reference Profiles for Comparison Purposes
(Excel file with different suspects for each case in individual tabs)

Identifier Plus Data
Zip file containing all samples from the 5 cases

Case 1

<table>
<thead>
<tr>
<th>Evidence</th>
<th>Ladder</th>
<th>Positive control</th>
<th>Negative control</th>
</tr>
</thead>
</table>

Case 2

<table>
<thead>
<tr>
<th>Evidence</th>
<th>Ladder</th>
<th>Positive control</th>
<th>Negative control</th>
</tr>
</thead>
</table>

PowerPlex 16 HS Data
Zip file containing all samples from the 5 cases
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