An Assessment of the GeneMapper® ID-X Mixture Analysis Software Tool for the Interpretation of Mixtures with Two Contributors

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INTRODUCTION

The development of expert systems to aid scientists in the analysis of forensic DNA profiles is of great interest to the forensic community. Of particular appeal are computer software programs that can analyze DNA results with the same proficiency as manual interpretation conducted by an experienced analyst. GeneMapper® ID-X (GMID-X, v1.3) is a software package developed as an expert system for single source samples, and includes a mixture interpretation tool to minimize the amount of time analysts spend on data interpretation. This latter element of the software was used to interpret two-person mixtures in a controlled study.

EXPERIMENTAL DESIGN

Eight different DNA samples, provided by NIST, were selected with a minimum number of shared alleles and a maximum number of heterozygote loci. From these samples, five sets of mixtures were prepared in ratios of 1:1, 1:2, 1:4, 1:6, 1:8, 1:10, 2:1, 4:1, 6:1, 8:1, and 10:1, and amplified using the Identifiler® and Identifiler® Plus kits following the recommended protocols. The GMID-X mixture analysis and deconvolution feature was compared to software-driven manual interpretation.

RESULTS AND CONCLUSIONS

TABLE 1: A total of 110 mixtures, accounting for 5918 alleles were analyzed. Alleles were classified as “NOT Called” when they were not listed in either the selected or unselected options for major and minor components of the GMID-X software.

<table>
<thead>
<tr>
<th>Category</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total # of Alleles Called by GMID-X</td>
<td>5667</td>
</tr>
<tr>
<td>% of Total Alleles Called</td>
<td>93%</td>
</tr>
<tr>
<td># of Alleles NOT Called at 3 Allele Loci</td>
<td>31</td>
</tr>
<tr>
<td>% of Alleles NOT Called at 3 Allele Loci</td>
<td>2.6%</td>
</tr>
<tr>
<td># of Alleles NOT Called at 4 Allele Loci</td>
<td>236</td>
</tr>
<tr>
<td>% of Alleles NOT Called at 4 Allele Loci</td>
<td>5.5%</td>
</tr>
</tbody>
</table>

Overall, the mixture analysis tool within GMID-X was easy to use and has the potential to make the mixture interpretation process faster and easier for forensic DNA analysts; remembering that GMID-X (v1.3) is not currently considered an expert system for mixture analysis.

The mixture analysis module of GMID-X was superior to manual analysis for the 1:2, 2:1, 1:4, and 4:1 mixtures.

Conversely, software-driven manual analysis provided better deconvolution results for the 1:10, 10:1, 8:1, and 1:6 mixtures. The GMID-X software struggled to determine the minor components of these mixtures, especially at 4 allele loci.

GMID-X occasionally had difficulties with genotype selection, which was attributed to the software not utilizing a Stochastic Threshold (ST). Dropouts (i.e. 11,F) were sometimes selected even when all of the alleles at a locus were above the ST used in the manual interpretation. In a few instances, alleles at a locus were below the ST, but no dropouts were selected as a possible genotype.

The percentage of alleles not called at 3 allele loci was 100% due to the use of the global cutoff filter, therefore it can be overcome by lowering this threshold. The global cutoff filter was 93% responsible for the alleles not called at 4 allele loci.

Choosing samples with a maximum number of heterozygote loci and minimum number of shared alleles allowed for effective deconvolution of 1:2 and 1:4 mixtures with the GMID-X software. Overall, the data do not appear to be affected by this approach.

FIGURE 1: Example of a mixture profile, 1:4 ratio of two components

FIGURE 2: Some alleles were not chosen to be part of the minor component when they were clearly present due to the software not utilizing an Analytical Threshold (AT) and Stochastic Threshold (ST) for the analysis. Instead, a mixture interpretation threshold was used as a global cutoff filter, with allele heights below 150 RFU not considered. This also caused problems with the minor component being pulled out properly. In order to overcome this issue, analysts can lower the mixture interpretation threshold (MIT) and reanalyze the data.

BOTTOM LINE:

- Overall, the mixture analysis tool within GMID-X was easy to use and has the potential to make the mixture interpretation process faster and easier for forensic DNA analysts; remembering that GMID-X (v1.3) is not currently considered an expert system for mixture analysis.

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