Internal Validation of GeneMapper®*ID-X* for use in Forensic Casework

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ABSTRACT

Due to the implementation of robotic equipment to extract, quantitate, amplify, and detect forensic DNA samples, the bottleneck of forensic DNA analysis has shifted to data interpretation. There is now a need for computer software that maximizes efficiency and encompasses the resources needed for DNA analysis. Applied Biosystems' GeneMapper®*ID-X* is one of the software systems capable of reducing this bottleneck and providing a suite of tools to assist in single source and DNA mixture interpretation.

GeneMapper®*ID-X* v1.2 (GM*ID-X*) was validated for use with questioned casework samples and a foundation to utilize the expert system capabilities for known casework samples was established. Original GeneMapper®ID v3.2.1 (GMID) validation data was analyzed with GM*ID-X* and the results were compared. The GM*ID-X* software was able to produce accurate, reliable, reproducible and concordant results to those obtained using GMID. It was also the goal of this project to find a software system that can aid in performing interpretation of DNA mixtures. Therefore, the mixture deconvolution tool portions of both GM*ID-X* and NicheVision Forensics, LLC ArmedXpert™ were evaluated for their use in deconvoluting two and three-person DNA mixtures.

INTRODUCTION

The discontinuation of the Applied Biosystems™ 3100 System Genetic Analyzers, GM*ID-X*'s new mixture analysis tool and the piqued interest in the implementation of expert systems into forensic laboratories led PBSO to purchase GeneMapper®*ID-X* v1.2. This validation was intended to test the robustness, reliability and sensitivity of the GM*ID-X* software and its concordance with GMID to demonstrate that it is suitable for use in forensic casework with PowerPlex®16 and the PBSO's AB™ 3130x*l* A and AB™ 3130x*l* B.

All algorithms in GeneMapper®*ID-X* are unchanged from GeneMapper®ID v.3.2.1 (GMID). This, along with similar user interfaces, will allow for a smooth transition for analysts from GMID to GM*ID-X*. Furthermore, with the capability to analyze both .fsa and .HID sample files GM*ID-X* can act as a long term software system for laboratories looking to maintain current analysis protocols while providing flexibility for future advancements in technology. GM*ID-X* not only allows for the streamlining of data analysis, it also qualifies as an expert system by assisting in the manual review of single source samples.

Both GM*ID-X* and the recently released ArmedXpert™ software program from NicheVision Forensics, LLC contain new mixture analysis tools meant to assist analysts in deconvoluting two and three-person mixtures and help provide a laboratory with a common platform from which to interpret DNA mixtures.

MATERIALS AND METHODS

- .fsa files from previously generated data using PowerPlex®16 and two 3130x*l*s were used for this validation
- During each study allele calls, basepairs, and heights in relative fluorescent units (RFU) for each sample generated by GM*ID-X* were compared to the results given by GMID
- The studies conducted were as follows: (1) Known Samples and Stutter; (2) NIST Samples/Concordance; (3) Reproducibility; (4) Non-Probative casework samples; (5) Precision; (6) Signal to Noise; (7) Sensitivity and Analytical Threshold Studies and (8) Mixture Studies

RESULTS AND DISCUSSION

Known Samples and Stutter Studies:

- Known samples were concordant for 29/31 known samples compared to the data analyzed using GMID
- The first non-concordance could be attributed to a lower calling threshold, while the second was due to a failure in Size Quality
- Generally, the recommended stutter %'s for PBSO with GM*ID-X* were less than those published by the Promega Corporation

Sensitivity and Analytical Threshold Studies:

- 3130x*l* A: PHR fell below 60% with greater frequency at ≤0.125ng for all three injection times and all three sample sets
- 3130x*l* B: PHR fell below 60% with greater frequency at ≤0.25ng for Samples 1 and 2, and ≤0.5ng for Sample 3
- Dropout occurred with more frequency beginning at 0.125ng

Signal to Noise Study:

- Average background noise for 3, 5, and 10 second injections were 7.69, 8.81, and 11.65 RFUs, respectively

Precision Study:

- Data demonstrated GM*ID-X* is capable of analyzing data within the ±0.5bp allele binning requirements
- All data concordant with previous validation of GMID

Table 1 3130x*l* A and B Precision Study data showing number of ladders evaluated and average standard deviation

Genetic Analyzer	Total # of Ladders	Average STDEV
3130x*l* A	29	0.036
3130x*l* B	163	0.051

Expert System Evaluation:

- New Analysis Summary tab in GM*ID-X* expedites review by separating passing samples from those that require further review
- Quality control and profile comparison features aid in assessment of controls and staff profiles
- New PQVs and colored marker headers to highlight challenged loci and enhanced artifact peak labeling
- Tracks edits to streamline technical review

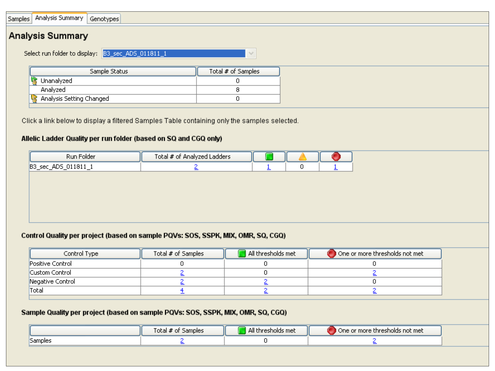

Figure 1: GM*ID-X* Analysis Summary tab

Non-Probative Casework Samples Study:

- Non-probative samples were non-concordant for 7/17 samples compared to the data analyzed using GMID
- Non-concordances are most likely due to:
  - GMID/*ID-X* filtered out an allele as stutter when other did not
  - Differences in minimum calling threshold between the validations
  - Refined interpretation guidelines from original 2008 guidelines
- GM*ID-X* software performed reliably

NIST and Reproducibility Samples Studies:

- All NIST and reproducibility DNA profiles were as predicted
- All data was concordant to previously obtained data with GMID

Mixture Study:

- For all three mixture sets, major-minor contributors were easily distinguishable beginning at the 3:1 and 1:3 ratios
- Mixture profiles were non-concordant 10/35 samples
  - One of the three reasons listed above

Mixture Analysis Tools Comparison:

- Performed to determine ease of use and establish workflows for GM*ID-X* and ArmedExpert™ mixture analysis tools
- Both analysis tools aid in reducing the time-consuming interpretation of complicated mixture data
- Both analysis tools can compute RMP, CPI/CPE and likelihood ratio statistics

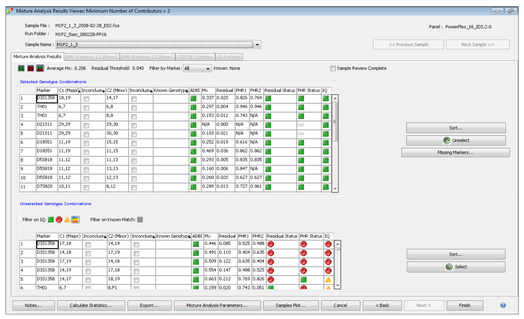

Figure 2: GM*ID-X* Mixture Analysis Results Viewer

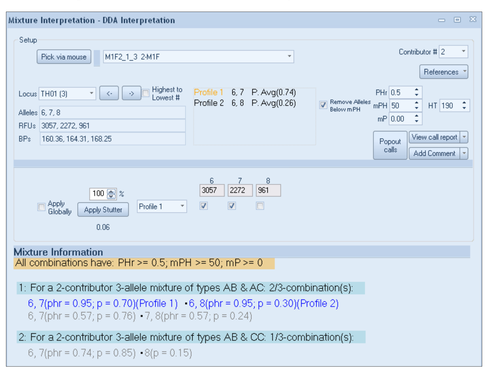

Figure 3: ArmedXpert™ Mixture Interpretation Window

CONCLUSIONS

- The GeneMapper®*ID-X* v 1.2 software was able to produce accurate, reliable, reproducible and concordant results to those obtained using GeneMapper®ID v3.2.1.
- The precision studies showed that GM*ID-X* can provide precise and concordant results.
- The known, NIST and non-probative samples showed concordant data to both published data as well as previously analyzed data using GMID.
- For the user defined settings available in *GMID-X*, a minimum peak calling threshold of 50 RFU is recommended for both AB™ 3130x*l* Genetic Analyzers A and B.
- Any non-concordances found between GM*ID-X* and GMID could be attributed to a cause unrelated to the software program.
- Throughout the validation, *GMID-X* v1.2 performed as expected.
- GM*ID-X* is suitable for use in forensic casework with PowerPlex®16 and PBSO's AB™ 3130x*l* A and B instruments.
- GM*ID-X* has been validated for use with casework samples and the foundation for its validation for use as an expert system for known single source samples has been established.
- Both the mixture tools in GM*ID-X* and ArmedXpert™ proved to be effective in aiding in mixture deconvolution.

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