NYC Office of Chief Medical Examiner, Department of Forensic Biology
Internal Validation of GeneMarker HID software

Executive Summary

SoftGenetics (SG) GeneMarker HID STR Analysis software (version 2.8.2) was tested by the NYC Office of Chief Medical Examiner, Department of Forensic Biology to determine its reliability for use for casework. The internal validation studies were performed following the Scientific Working Group DNA Analysis Methods (SWGDAM) guidelines on such testing along with some additional testing. Data from Promega's PowerPlex Fusion validation, which was run on two 3130xl instruments that were determined to be the least and most sensitive*, and analyzed in GeneMarker HID software, was used for most studies. Additional analyses were performed on relevant samples in house as needed. The ability of the software to correctly call alleles was determined in the Accuracy section by comparing the developed profiles of the positive controls and NIST SRM 2391c standards to profiles provided by Promega and NIST. Concordance between the previous amplification system (Identifiler 28 cycles) and analysis software (GeneMapper ID 2.4.1) was determined by comparing allele calls of previously developed Identifiler profiles to profiles developed using PowerPlex Fusion and GeneMarker HID, at loci where comparisons can be made. Once concordance had been established, sensitivity and mixture studies were evaluated. The GeneMarker HID software settings were determined during both Promega's PowerPlex Fusion validation and GeneMarker HID validation. (See "Settings Evaluation" write up). Some settings were changed in relation to the study performed. Such changes are explained in the sectional summaries to which they apply. In summary, the GeneMarker HID software validation demonstrated that it is reliable for use in casework, and can be further shown in each study's conclusion.

**Refer to STRmix™ validation for instrument sensitivity data*

Precision: Based on the base pair sizes derived from injections analyzed with GeneMarker HID, the assignment of alleles in the PowerPlex Fusion Allelic Ladder is precise on average by 0.14bp with GeneMarker HID, with the greatest standard deviation of 0.1 for both instruments. These values are well below the minimum value of 1 base that is necessary in differentiating two different alleles.

Accuracy, Reproducibility/Repeatability: The assignment of alleles in the positive control and NIST SRM 2391c standards A-D was determined to be accurate and was reproducible and repeatable over multiple injections and multiple users when analyzed with GeneMarker HID. Complete profiles were detected for all the samples and results were concordant with expected profiles.

Contamination: The resulting baseline is reproducible over multiple injections of negative controls and known samples when analyzed with GeneMarker HID. Some dye and non-specific artifacts were detected for the negative control samples in GeneMarker HID, but the artifacts were editable. One peak was present in one amplification negative control at D7S820. This result was not reproducible. Numerous pull-up and non-specific artifacts were detected for the known samples in GeneMarker HID but the artifacts were editable. The results obtained from GeneMarker HID analysis showed concordance between runs, with the exception of one amplification negative mentioned above.

Known/Non-Probative: Allele assignments in GeneMarker HID are mostly concordant to GeneMapper ID at loci where comparisons could be made The main difference between the analyses is the number of edits, which are prominent with the PowerPlex Fusion samples analyzed with GeneMarker. The most frequent edit was pull-up, which was seen more in GeneMarker than GeneMapper ID. Dye artifact and primer front edits were also commonly seen in GeneMarker at the 100 bp or less sizing fragment area of the dye lanes.

With the analysis of the non-probative and mock evidence data, additional true minor alleles were detected when the samples were amplified in PowerPlex Fusion and analyzed with GeneMarker versus when the samples were amplified in Identifiler and analyzed in GeneMapper ID This difference is most likely due to the increased sensitivity of the PowerPlex Fusion kit, a lower analytical threshold, and higher amplification input amount of 750pg used during the PowerPlex Fusion validation. Analysis of the non-probative and mock evidence data showed twenty-one distinct instances of gained allele calls with PowerPlex Fusion analyzed with GeneMarker, fourteen of which were true alleles while the remaining eight were artifacts in stutter positions that were not able to be edited. There were two instances of the loss of an allele.

Sensitivity: Allele calls for peaks belonging to the profile were detected for samples close to the target quant amount (750pg-100pg) and decreased for lower quant samples (75pg-3.25pg) in the GeneMarker HID software. A minimum of at least 50% of the profile was obtained down to 25pg. Average peak heights for all loci were above the analytical threshold of 50 RFU down to 30pg.

An increase in editable artifacts was observed overall with GeneMarker HID. This could be due to the difference in proprietary peak detection algorithms between the two software systems. The majority of the editing were classified as pull-up or labeled dye artifacts that may be data/amplification set-related. Adjustments to the input amount and analysis range can alleviate the amount of editing across the sample.

Mixtures: The alleles detected during analysis of two-person, three-person and four-person mixtures were all consistent with the individual contributors to each mixture, with the exception of editable artifacts. For two person mixtures, at least 50% of unique minor alleles were detectable for all ratios at 750pg and 500pg. A minimum detection of 68% of the total alleles was observed across all input amounts and ratios for three person mixture samples. A minimum detection of 66% of the total alleles was observed across all input amounts and ratios for four person mixture samples. This value could be artificially inflated due to allelic sharing amongst contributors across all loci.

Settings Evaluation Study:

After evaluation of both the Pull-up Correction and Saturation Repair settings, it was determined that these settings will not be used during analysis due to the peak height adjustments being made by the software, and the possible negative effects on STRmix™ analysis.

After evaluation of various global filters it was noticed that almost half of the lost alleles could be recovered by going down to a 3% filter, while still greatly reducing the number of artifacts. However, after beginning work on the STRmix™ validation it was determined the Global Max filter and Min Heterozygote Imbalance (%) filter could not be used during analysis since the filters were removing stutter peaks which STRmix™ needs to properly analyze the sample and causing an elevated stutter variance value. Therefore, a lower input amount of 525pg was decided upon to achieve fewer artifacts. Samples amplified with a 525pg input amount achieved the same accuracy and comparable peak height ratios as samples amplified with a 750pg input amount.

Additional GeneMarker analysis settings, such as “Auto Panel Adjustment”, “Auto Range (frame)”, “Spike Removal”, “Superior Baseline Subtraction”, “Smooth”, and “Auto Select Best Ladder” achieved accurate and concordant results and were incorporated as default analysis settings.

Addendum to the Powerplex Fusion Validation, Genemarker HID v2.8.2 Analysis Software Validation, and STRmix Validation

Global filter and DNA input amount evaluation

A large number of artifacts were observed using a 50 RFU analytical threshold. Therefore, a Global Filter evaluation was conducted. Lowering the input amount to 500pg and diluting the instrument matrix to make it less saturated were not successful in removing these artifacts. A global filter was evaluated to determine if artifacts could be reduced without excessive loss of data. Analysis of a portion of the samples run on both Athena and Newton was performed. A Global Max filter was applied during the run wizard, as well as a Min Heterozygote Imbalance (%) filter within the panel settings across all the locations. These samples were analyzed with a 10% filter, 8% filter, 5% filter, 4% filter, and 3% filter set in both locations. After evaluation it was noticed that almost half of the lost alleles could be recovered by going down to a 3% filter, while still greatly reducing the number of artifacts. Therefore, for all evidence and exemplar samples, it was decided that a filter of 3% would be applied for the Global Max filter during the run wizard and across all locations within the panel for the Min Heterozygote Imbalance (%) filter.

However, after beginning work on the STRmix® validation it was determined that the Global Max filter and Min Heterozygote Imbalance filter could not be used during analysis since the filters were removing potential low-level stutter peaks which STRmix® needed to properly analyze the sample. Therefore, to be able to remove both filters, a lower input amount of 525pg was decided upon to achieve fewer artifacts. This is justified because samples amplified with a 525pg input amount achieved the same accuracy and comparable peak height ratios as samples amplified with a 750pg input amount.

The final decision for Powerplex Fusion @ 29 cycles stands as follows:

Global Max Filter:	0%
Min Heterozygote Imbalance Filter:	0%
Optimum DNA Input Amount:	525pg

Based on sensitivity studies and mixture studies, the minimum DNA Input Amount has been set at 37.5pg.