Validation of FSS-i^3 Version 4.2.1
PowerPlex 16, GeneMapperID 3.2, 3130xl Collection Software Version 3.0

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Introduction

This document provides an overview of the validation performed on FSS-i^3 version 4.2.1, for use with offender samples amplified with PowerPlex 16 and injected on a 3130xl using collection software version 3.0, and analyzed with GeneMapper ID version 3.2.

The DNA Profiling section of the Missouri State Highway Patrol Crime Laboratory analyzes convicted offender samples amplified with PowerPlex 16 and injected on a 3130xl using collection software version 3.0, with GeneMapper ID version 3.2. All samples are analyzed with a minimum threshold of 75 RFU for all colors, 20% heterozygous peak height ratio, the minimum peak height of the peaks within a triallelic pattern must be at least 50% of the maximum peak height, and a 20% stutter cutoff. There is one exception; a D5 10 allele that is not in the stutter position is acceptable at a 15% peak height ratio. Samples with profiles that do not meet the requirements may be redone to verify anomalies, and could be acceptable.

In 2005, Missouri became an all-felon state, with an instant backlog of over 100,000 samples. With the addition of new instrumentation, staff, and overtime, the anticipated seven-year backlog was completed in just over two years. Two analysts independently analyze all samples, and the final profiles are electronically compared for concordance before upload to CODIS. While analysis has not caused a dramatic bottleneck, the process takes time and the decision was made to evaluate an expert system. After attending the NIJ Expert System Testbed Project, FSS-i^3 was selected for possible validation.

Materials and Methods

All validation was done per the requirements of Appendix B of the DNA Data Acceptance Standards (Operational Procedures). Samples were identified based on the final paperwork from previously completed trays. Samples that are not entered into CODIS are highlighted on the printed plate record. Four trays with minimal highlighting were selected for calibration; thirteen trays were selected for concordance. Each tray has a maximum of 90 samples, with a total of 360 samples for the calibration set, and 1170 possible samples for the concordance check. Some additional samples, specifically chosen to challenge the system, were in separate projects. The rules were evaluated after each analysis and modified accordingly. All samples were analyzed with FSS-i^3 and GeneMapper ID, and the profiles obtained were compared to the profiles previously determined with GeneMapperID. The accuracy, precision, and reproducibility of FSS-i^3 were also examined.

Results

The two participating Criminalists received four days of training on the use of FSS-i^3. The calibration trays were analyzed using the rule set created during training. It was immediately clear that the high signal rule was set too conservatively, as many samples were being flagged for high signal. These profiles did not show pull up or split peaks, and were deemed acceptable. Offender samples are amplified directly from the 1.2 mm FTA paper punch, and it is not uncommon to have strong samples. The high signal rule was changed from 6000 RFU to 7000 RFU. Profiles obtained on the 3130xl tend to have very clean baselines, and with a proper spectral, pull up does not usually occur with peaks of 7000 RFU or below.

After further preliminary analysis, the degradation rule was also changed. It is very unusual to see signs of inhibition or degradation. Many samples do show imbalance across loci, which is reproducible and acceptable. The degradation rule calculates the ratio of the peak heights of the alleles at the lowest and highest molecular weight loci. The lower limit for the ratio was set at 0.5 initially, and changed to 0.2.

There were two discordant profiles in the calibration samples. The first was designated as a 5 at TPOX in the new GMID project, but was designated as a <6 with FSS-i^3 and the original GMID project. There is a virtual allele at 5 in TPOX, but the allele designation must be changed to <6 for import into CODIS. Both allele designations are equivalent.

The second discordant profile was due to analyst error. Both GMID projects had D5 designated as a 10, while the FSS-i^3 project had D5 designated as a 10, 11. This locus was flagged for Pref Amp AB, and the designation for the 11 allele should have been removed, as the peak height ratio was less than 20% in the stutter position.

The concordance trays were analyzed with the modified rule set, and the first concordance check was performed. One discrepancy was noted. There is a virtual allele at 17.2 in FGA, but the allele designation must be changed to <18 for import into CODIS. The allele designation was still marked as 17.2 in FSS-i^3, and was subsequently changed to <18 within the Multiplex Manager. The concordance samples were reanalyzed, and all profiles were concordant.

Melissa Schwandt, from Promega, offered to examine the rule set to make sure there were no other obvious problems. Ms. Schwandt discovered a mistake in the Advanced Ladder Settings, within the Multiplex Manager. The minimum RFU was set at 45 RFU, rather than the necessary minimum of 75 RFU. The setting was changed to 75 RFU.

Ms. Schwandt also observed that the low heterozygote rule had been turned off. This was done with the expectation that other rules, such as Signal to Noise or Pref Amp, would still identify undesignated alleles. A triallelic sample with two alleles at or near threshold and one allele below threshold may not be flagged with the low heterozygote rule turned off. After further consideration, the rule was turned back on, with a minimum threshold of 100 RFU. All calibration and concordance samples were reanalyzed with the newest modifications to the rule set.

Once the rule set was finalized, the concordance samples were analyzed with ladders and controls labeled by type. All profiles were concordant. While looking over notes from the initial training session, it was noted that the FSS recommends analyzing with all samples labeled as “Sample”, including ladders. GeneMapper ID analyzes ladders labeled as “Ladder” differently than if all are labeled as “Sample”. The concordance samples were reanalyzed with all samples labeled as “Sample”, including ladders and controls, and all profiles were concordant.

During analysis with FSS-i^3, the raw data is available with GeneMapper ID. It can be beneficial to have the ladders labeled as ladders, so that alleles are designated in the GeneMapper project. The laboratory will have to decide how to label samples when a final analysis procedure is developed.

A second analyst independently analyzed the concordance samples with FSS-i^3. This was the final concordance check. Profiles were exported from the original GeneMapper ID projects, which were independently analyzed by two analysts. The manual GeneMapper ID profiles were compared to the original profiles. The FSS-i^3 profiles, with samples labeled by type and as samples, were also compared to the original profiles. Finally, the FSS-i^3 profiles from the second analyst were compared for concordance.

Each profile that was designated as “Accept” was concordant with the previously obtained profile. Samples that were flagged as “Edit/Reject” were also concordant at all loci that were not deemed unacceptable. This demonstrates that the system is very accurate when designating allele calls.

During analysis, FSS-i^3 generates audit files, one of which is named “RAW designations”. This table includes the sample name, peak height, peak area, and allele call for each locus. One RAW designation table was compared to the raw output file created by GeneMapperID, and demonstrated complete concordance at over 21,000 data comparisons. This demonstrates that the system is very precise when using the output table from GeneMapperID to size and then designate alleles.

Each tray was analyzed three times with FSS-i^3, and most profiles were concordant. All discordances, due to analyst error, were resolved with reanalysis. Concordant results from three independent analyses indicate that the system generates reproducible profiles.

Discussion

The expert system has been thoroughly tested and shown to produce acceptable, correct profiles with minimal human intervention (see Table 1). There were many expansion proposals in Missouri this legislative session, and it is necessary to have the profiling system as efficient as possible in anticipation of such change. It is expected that the expert system will save time during analysis, and aid in minimizing the risk of error. The validation has been approved by NDIS, and all Criminalists in the section will be trained on FSS-i^3. As more samples are run through the program, it may be possible to adjust the rule set to further minimize the number of samples that need review.

During the validation process, the CODIS import parameters were changed at the state level to allow for the entry of unchanged virtual allele designations. The profiles are modified upon import to SDIS, to allele designations that are allowable at NDIS. This change should reduce the amount of analysis time spent on samples that are otherwise acceptable.

Version 4.2.2 was release during the validation of version 4.2.1. This new version will be fully validated and submitted for approval as time permits, with the goal of using version 4.2.2 for all convicted offender samples.

Table 1: Concordance Check Details

13 full trays of 90 samples each	1170 possible profiles
- 2 bad injections	1168 possible profiles
- 91 samples flagged	1077 profiles classified as Accept
53 samples in separate projects	53 possible profiles
- 53 samples flagged	0 profiles classified as Accept
1168 samples + 53 samples	1221 possible profiles
91 samples + 53 samples flagged	144 samples flagged
1077 samples Accept/ 1221 total	88% Accept (92% Accept, excluding separate projects)