Internal Validation of FSS-i3™ 4.2.2 Expert Software System for use with Single Source PowerPlex® 16HS Multiplex DNA Samples

Dijana Coric, B.S[1, 2]; Cyndi Cunnington, M.S[2]; Season Seferyn, M.S.F.S[1]; Dr. Pamela Staton, Ph.D[1]

[1]Marshall University, Forensic Science Program, 1401 Forensic Science Dr., Huntington, WV 25701
[2]Idaho State Police, Forensic Biology Laboratory, 700 S. Stratford Dr., Meridian, ID 83642

Abstract

This validation focuses on establishing the FSS-i3™ rule set and settings parameters for the Idaho State Police Convicted Offender DNA Database Laboratory. A set of known data that had already been processed using the current system, GeneMapper® ID v. 3.2.1, was selected. The data consisted of a minimum of 200 calibration set samples and a minimum of 1000 concordance set samples obtained from an Applied Biosystems® 3130xl Genetic Analyzer. This validation also focuses on evaluating the compatibility and interaction between GeneMapper® ID and FSS-i3™, and the accuracy and reliability of FSS-i3™.

The validation illustrated FSS-i3™ is accurate, reliable and produces concordant results to those obtained using GeneMapper® ID 3.2.1. It was also determined that FSS-i3™ saves the analyst valuable time by eliminating the amount of samples and/or loci the analyst has to evaluate, and it narrows down the possible issue at that particular locus that was flagged for review. It was also demonstrated that FSS-i3™ is capable of analyzing single source DNA samples as well as, or better than, the current system in place, GeneMapper® ID. In the future, this validation can be expanded to establish a rule set and settings parameters for FTA single source samples which bypasses the quantification step.

Introduction

In October of 1998, the Federal Bureau of Investigation (FBI) launched its nationwide DNA database for NDIS participating law enforcement agencies. More than a decade later, the U.S. National DNA Index System (NDIS) database of the Combined

DNA Index System (CODIS) contains over 10 million DNA profiles and links all 50 U.S. states with the capability to search criminal DNA profiles. The use of DNA databases is considered a cost-effective method for reducing crime because a majority of crimes are committed by repeat offenders (Butler 435).

One of the highest labor efforts in the process of preparing DNA profiles for inclusion in a DNA database is the data interpretation stage. For many laboratories, data interpretation represents approximately 50% or more of the resource requirement to deliver final results for samples (Butler 424). To reduce the bottleneck effect on data interpretation, expert systems have been developed and implemented to replace the traditional manual system (Roby 17-19).

FSS-i3™ is comprised of three different components: i-STRess, i-STReam and i-ntegrity. The i-STRess module was designed to integrate with GeneMapper® ID, and it is considered the foundation of FSS-i3™. The i-STReam module evaluates two-person DNA mixtures and produces a best-fit major profile by using the peak height or area data and allele designations determined by i-STRess. Finally, the i-ntegrity module checks for potential sample to sample contamination within a batch by comparing all alleles designated in a sample to alleles in every other sample in the batch. This paper will primarily focus on i-STRess which is the core DNA interpretation tool of FSS-i3™ (Bill and Knox, 2005).

i-STRess interprets raw DNA data generated from the capillary electrophoresis instrument and identifies peaks, assigns alleles, ensures the data meets the laboratory defined criteria and describes the reasoning behind its decisions. It accomplishes these

tasks by applying a set of rules and filters established by the laboratory that imitate the analyst's decision making (Bill and Knox, 2005).

This validation focuses specifically on single source PowerPlex® 16HS multiplex DNA samples collected using data generated on the Applied Biosystems® 3130xl Genetic Analyzer and collection software, which is then passed on to GeneMapper® ID version 3.2.1 and finally imported and examined on FSS-i3™.

The expert system internal validation guidelines require that "at least 200 unique samples for calibration of the software be analyzed and a set of at least 1,000 unique samples for the concordance study of the software be analyzed with the current genotyping system" (Christen and Roby 14). The guidelines also state that different observed results or challenges be evaluated by FSS-i3™ during the validation (Christen and Roby 14-15).

Materials and Methods

A stochastic study was conducted prior to the FSS-i3™ validation to establish the peak height value below which sister alleles show severe peak height imbalance. It consisted of a serial dilution of amplified DNA that was set up using 10 different single source samples. The intensity and peak height ratios of the 10 different samples were analyzed and examined at different low level concentrations: 0.05ng, 0.1ng, 0.15ng and 0.2ng of template DNA. The variation in peak height ratio and peak intensity was observed. The peak height ratio versus average RFU (relative fluorescent unit) for 0.05ng, 0.1ng, 0.15ng and 0.2ng was plotted for both 3 seconds and 10 seconds capillary electrophoresis injections. The RFU value in which a majority of the peak height ratios

fell below the expected balance, 50% for PowerPlex® 16HS amplification kit, was observed and established as the stochastic threshold.

A set of known data that had already been processed using the Idaho State Police Convicted Offender DNA Database Laboratory's current system, GeneMapper® ID version 3.2.1, was selected. Each of the calibration and concordance set samples were reanalyzed with the current system, GeneMapper® ID, prior to analysis and comparison with FSS-i3™ because the preliminary studies done prior to the validation determined that the stutter % filter is to be increased to 20% filter for all loci, the stochastic threshold is to be 200 RFU, and the analytical threshold is to be decreased from 100 RFU to 75 RFU. The data sets consisted of a minimum of 200 calibration set samples and a minimum of 1000 concordance set samples obtained from an Applied Biosystems® 3130xl Genetic Analyzer. Additionally, there were numerous positive amplification controls, negative amplification controls, and reagent blanks included in the data sets.

The data sets were loaded into GeneMapper® ID Version 3.2.1 and the raw data was analyzed. Size standards were checked and edited for inconsistencies. Samples containing size standard issues or identifiable problems were removed from the data sets. The raw data from GeneMapper® ID was exported as a "RAW" data table. The RAW data table contained the peak size, height and area data for all peaks above the established peak amplitude threshold.

Rule sets used in previous validations of FSS-i3™ and the FSS-i3™ rule set recommendations was implemented as a starting point for i-STRess processing.

The calibration set consisted of known challenge samples. The challenges that were included in the calibration data set included: >20% stutter, locus peak amplitude imbalance, artifacts: pull up, shoulder (+A and -A), spikes, peaks: tri-allelic patterns, mixture, contamination, missing allele, missing loci and off ladder alleles that included microvariants and above/below allelic ladders.

After adjusting the rule set accordingly, by using the calibration data set results, the concordance data sets were then processed using i-STRess. The ladders were called automatically, and the rule set was applied. Results were then generated and those samples that were flagged for review were analyzed.

Results

Figure 1: Stochastic study with peak height ratio for PowerPlex® 16HS reactions using 0.05, 0.1, 0.15 and 0.2ng of template DNA at 3kV 10 seconds.

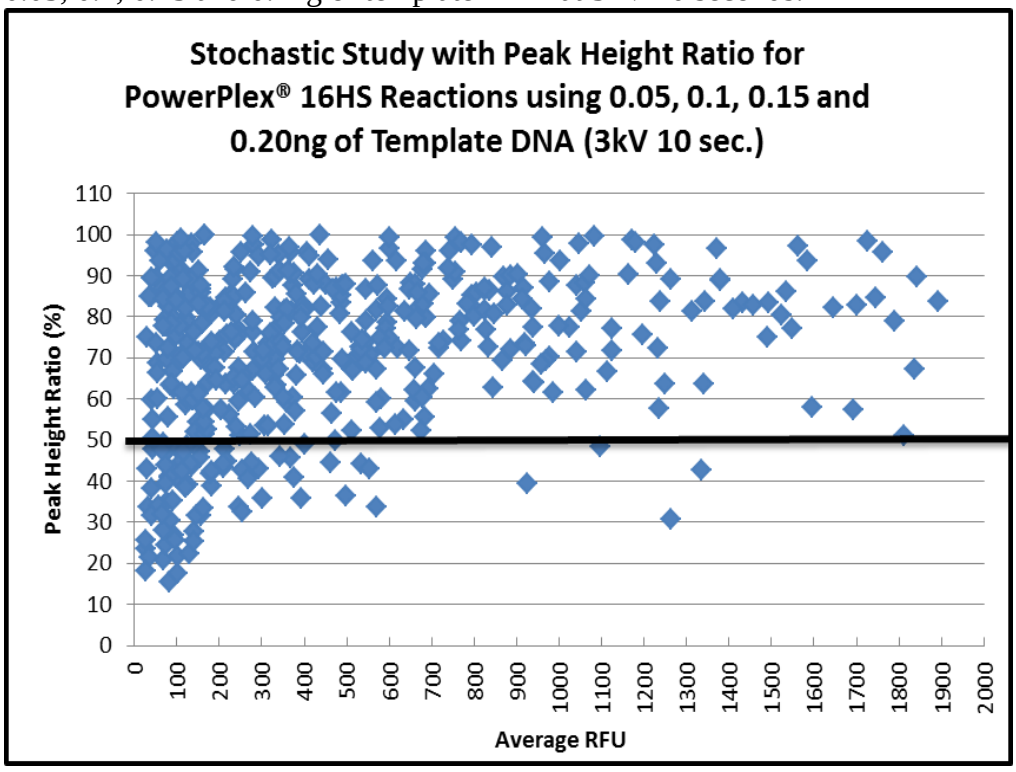
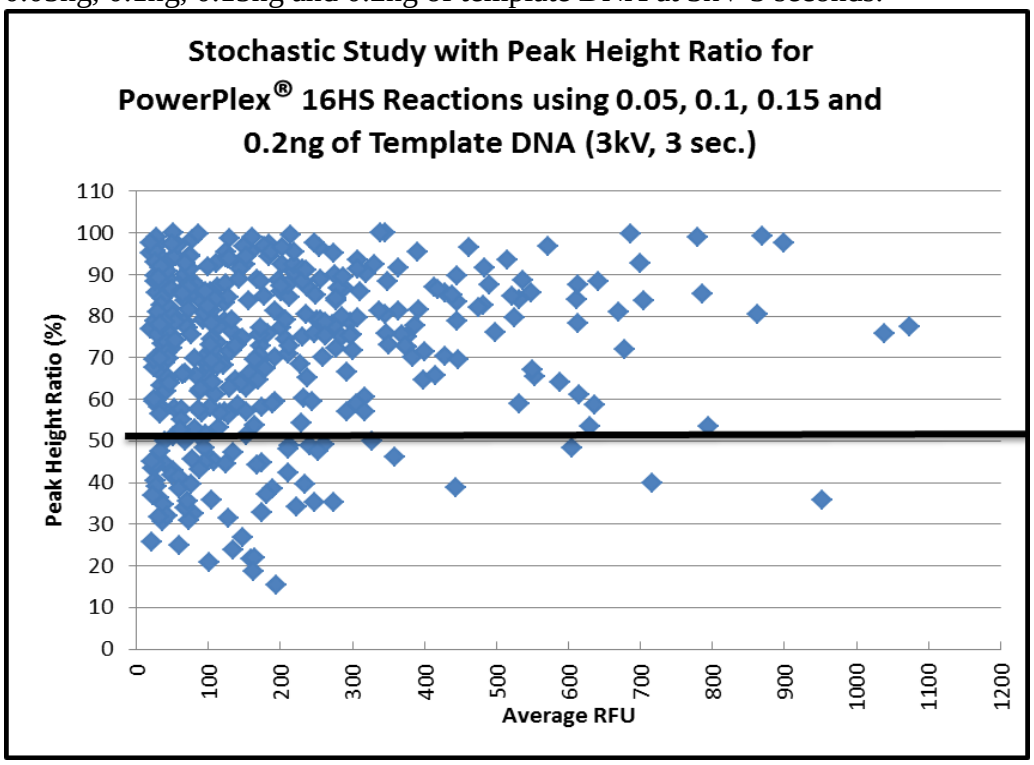


Figure 2: Stochastic study with peak height ratio for PowerPlex® 16HS reactions using 0.05ng, 0.1ng, 0.15ng and 0.2ng of template DNA at 3kV 3 seconds.



Calibration and Concordance Set Summary

Table 1: Summary of Calibration Set Results

Calibration Set:	
# of samples tested	224
# of negative control lanes	13
# of positive control lanes	8
# of reagent blank control lanes	14
# of ladder lanes	21
# of loci marked for review	400
# of possible loci	4,144
% of loci passed as acceptable without review	90.35%
% of loci marked for review	9.65%

The number of loci flagged for review includes challenges located in the control lanes

(224 samples + 35 controls * 16 loci = 4,144 loci).

Table 2: Summary of Concordance Set Results

Concordance Set:	
# of samples tested	1,198
# of negative control lanes	15
# of positive control lanes	19
# of reagent blank control lanes	19
# of ladder lanes	40
# of loci marked for review	1,251
# of possible loci	20,016
% of loci passed as acceptable without review	93.75%
% of loci marked for review	6.25%

The number of loci flagged for review includes challenges located in the control lanes

(1,198 samples + 53 controls * 16 loci = 20,016 loci).

Table 3: Five samples for each required challenge and FSS-i3™ performance on each sample and their specific challenge.

Challenge Type	**Plate and Sample Identification**	**Did FSS-i3™ correctly flag the challenge?**
>20% Stutter	010312CRC:ID3000003937	Yes
	010312CRC:ID3000003956	Yes
	010312CRC:ID3000003977	Yes
	012412CRC:ID3000004057	Yes
	022712CRC:ID3000003788	Yes
Locus Peak Amplitude Imbalance	112012RLN:ID3000000900	Yes
	112012RLN:ID3000000959	Yes
	112012RLN:ID3000006415	Yes
	010312CRC:ID3000003939	Yes
	010312CRC:ID3000003955	Yes
Pull Up	112012RLN:ID3000001010	Yes
	112012RLN:ID3000001042	Yes
	052813JLC2:ID3000010433	Yes
	103012RLN:ID3000004514	Yes
	112712JLC:ECQ	Yes
Tri-allelic Patterns	112012RLN:ID3000001073	Yes
	022712CRC:ID3000003862	Yes
	103012RLN:ID3000005343	Yes

	022513JLC:ID3000007815	Yes
	031813JLC2:ID3000008421	Yes

Contamination	010312CRC:ID3000003968	Yes
	010312CRC:RB	Yes
	012412CRC:Reamp. RB1	Yes
	012412CRC:Reinj. RB	Yes
	011513JLC:ID3000007140	Yes

Mixture	010312CRC:ID3000003968	Yes
	010312CRC:RB	Yes
	012412CRC:Reinj. RB	Yes
	052013JLC2:ID3000010078	Yes
	011513JLC:ID3000007140	Yes

Missing Allele	112012RLN:ID3000006406	Yes
	112012RLN:ID3000006466	Yes
	022712CRC:ID3000003870	Yes
	012412CRC:ID3000004087	Yes
	052813JLC2:ID3000010404	Yes

Missing Loci	112012RLN:ID3000006406	Yes
	022712CRC:ID3000003870	Yes
	012412CRC:ID3000004079	Yes
	012412CRC:ID3000004087	Yes
	103012RLN:ID3000004426	Yes

Microvariant Allele	112012RLN:ID3000000851	Yes
	112012RLN:ID3000000876	Yes
	112012RLN:ID3000006414	Yes
	022712CRC:ID3000003851	Yes
	031813JLC2:ID3000008377	Yes

Above/Below Allelic Ladder	022712CRC:ID3000003852	Yes
	103012RLN:ID2001003477	Yes
	052812JLC2:ID3000010440	Yes
	112712JLC:ID3000006127	Yes
	061813JLC2:ID3000011651	Yes

Spikes	032112RLN:ID3000004153	Yes

Shoulders (+A and –A)	012412CRC:ID3000004064	Yes
	052813JLC2:ID3000010437	Yes
	052813JLC2:ID3000010410	Yes
	052813JLC2:ID3000010429	Yes
	052813JLC2:ID3000010444	Yes

Table 4: Different rule changes made after analysis of certain data files.

Data Files Analyzed in Concordance Set	**FSS-i3™ Rules Altered**
010813JLC	N/A
011513JLC	Main peak filter % was changed from 12% to 9.5% and the main peak filter will operate on the 2nd main allele.
022513JLC	N/A
030413JLC	N/A
121712JLC	Pull up threshold was changed from 40% to 35% and the sizing tolerance was changed from +/- 0.3 bp to 0.35 bp.
032112RLN	Off ladder rule was changed from 0.51 bp to 0.495 bp. Peak morphology upper limit was changed from 0.15 to 0.175.
061813JLC2	Main peak filter % was changed from 9.5% to 0% and the main peak filter is set at a flat RFU value of 75 RFU.
052013JLC	Minus A sizing tolerance was changed from +/- 0.2 bp to 0.3 bp.
052013JLC2	Changed positives tab in the scientific settings from *P to P* to represent any value that begins with a P rather than ends with a P.
052413JLC2	N/A
051313JLC	N/A
051313JLC2	N/A
040113JLC2	N/A
041513JLC2	N/A
031813JLC2	Changed minus A threshold from 15% to 5%.

After the rule changes were made and implemented then each of the individual data files were reanalyzed to ensure consistency and concordance with the new rule set.

Discussion

Based on the data accumulation for determining stochastic threshold for single source samples for both GeneMapper® ID and FSS-i3™ analysis which is summarized in Figures 1 and 2; the Idaho State Police Convicted Offender DNA Database Laboratory decided to implement a stochastic threshold of 200 RFU.

FSS-i3™ Expert Software System's purpose is to store knowledge on how to respond to a particular result or situation. And when a challenge is presented, "use the stored knowledge in the program to respond with an explanation" (Roby 17). This validation demonstrated that FSS-i3™ is capable of correctly identifying samples that require editing and flagging them for review. However, in some instances FSS-i3™ cannot and did not always provide a correct reasoning or explanation because FSS-i3™ contains only rules to solve most commonly encountered problems. Nonetheless, FSS-i3™ still recognizes that there is an issue that needs to be addressed with a particular sample, so FSS-i3™ flags that particular sample for review, but it may not always provide an accurate or detailed enough reasoning behind what the possible issue could be. In these cases, an analyst is needed to make the final decision. A few of those encountered situations are discussed in the following paragraphs.

According to the FSS-i3™ version 4 User Guide, the pull up rule fires if a point of data matches another designated allele in base pair or bp size, and the matching peak must be less than the threshold percentage of the designated allele height. It is important to consider that sample data from a 96 well plate might have a few pull up rules fire even if the allele, off ladder allele, etcetera is not pull up but indeed a true allele. The reason

for this occurrence is because there are overlapping alleles that occur in the same bp positions in different loci (i.e. often observed in the TPOX locus and sometimes D21 locus). This can be observed in both the calibration and concordance set data used in this validation.

FSS-i3™ does not have a +4 or –8 stutter rule; instead either an extra allele rule (if there are more than 2 potential alleles in that locus) or the Preferential Amplification AB rule will fire. The Preferential Amplification AB rule will fire if there are only 2 potential alleles in that locus and -8 or +4 stutter is one of the potential alleles. In that scenario, FSS-i3™ considers the -8 or +4 stutter as a true allele and places it in the major designation panel alongside the other true allele, yet it still recognizes that the peak height imbalance is significant and therefore the Preferential Amplification AB rule fires. In either scenario, once the analyst looks at the peak they can determine if the extra allele or the much less intense allele (if the Preferential Amplification AB rule is fired) by simply looking to see if the allele is one core repeat unit longer than the main allele or 2 core repeat units shorter than the main allele.

As stated in the FSS-i3™ version 4 User Guide, the n-Peak (minus A) rule will fire if a non-designated peak occurs at the n-peak position of a designated allele. The n-peak must be above the threshold percentage and within a laboratory determined sizing tolerance for the minus A rule to fire. In the scenario that the minus A peak is also considered a rare (microvariant) by FSS-i3™ then the minus A rule will not fire because FSS-i3™ considers that peak an allele. If this scenario occurs then an extra allele rule will fire (if there are more than 2 potential alleles in that locus) or the Preferential

Amplification AB rule will fire (if there only 2 potential alleles in that locus) in conjunction with the rares rule. The analyst can easily determine that the "allele" considered by FSS-i3™ is actually minus A by looking to see if the allele peak is approximately 1 base pair away from the main allele.

According to the FSS-i3™ version 4 User Guide, the off ladder rule will fire if a designated allele falls outside of the given tolerance of its virtual ladder template peak. The off ladder rule can be used to identify alleles that approach the edge of the acceptable window set in the advanced ladder tab, and could represent a shift in the ladder template. The rule will fire if the data strays from its ladder allele between the rule tolerance and the advanced ladder tolerance. If the alleles have strayed past the advanced ladder tolerance then the off ladder rule will not fire and the alleles will not be designated, instead the extra peak and potential signal: noise rule will fire to indicate that there is an issue. In that case, an analyst can overlay the individual peaks (potential true alleles) to see if the peaks fall off the ladder and the analyst can also do a calculation using the ladder template base pairs and the individual peaks base pairs in question to determine the amount of deviation. Both these techniques can be used to verify that the peaks in question are most likely true alleles, but they just stray too far from the advanced ladder template for FSS-i3™ to assign them allele designations and fire the off ladder rule.

FSS-i3™ flags for review problematic samples by applying a set of rules and filters established by the laboratory that imitate the analyst's decision making (Bill and Knox, 2005). Therefore FSS-i3™ eliminates large amount of samples or to be more specific, loci, that the analyst has to look at and evaluate. It also narrows down what the

possible issue could be at that particular locus that was flagged. However, as illustrated in the previous few examples, FSS-i3™ will not always be able to correctly identify the issue at hand but it will be able to identify that there is an issue with that sample and alert the analyst. Though FSS-i3™ may not be able to directly pinpoint the issue at hand with a problematic sample, it is able to recognize samples that are outside its rules and/or in which there is a possible alternate judgment and alert the analyst.

Throughout the analysis of the concordance set data files, some changes were made to the rule set and settings parameters after certain data files were analyzed as can be observed in Table 4. Each time the rule set was changed, each of the data files in the concordance set were reanalyzed and checked for concordance. The reasoning behind the individual changes throughout the concordance set study is discussed in the following paragraphs.

The off ladder sizing tolerance was changed from 0.51 bp to 0.495 bp after analyzing a plate that had numerous true alleles that were being called off ladders in GeneMapper® ID. With the off ladder sizing tolerance set at 0.51 bp, several samples were not flagged in FSS-i3™ for review with the off ladder rule because the allele(s) deviation from the virtual ladder fell outside of the sizing tolerance or right at/below the tolerance therefore it was not flagged for review. The sizing tolerance was lowered to 0.495 bps to compensate for those few samples and each one of them was flagged for review with the off ladder rule being fired.

The peak morphology rule originally had an upper limit threshold set at 15%. If the peak morphology ratio falls above the upper limit threshold this indicates that the

peak has height but no significant area which could be indicative of a spike or pull up. The reasoning behind setting the upper limit at 15% was based on previous validations of the GeneMapper® ID / FSS-i3™ for the 3130xl instrument. An analyzed plate flagged a few samples for review with the peak morphology rule which did not need to be flagged as spike or pull up because those samples had allele(s) that were true alleles, not spikes or pull up. Adjusting the peak morphology upper limit to 17.5% eliminated these samples being flagged for review, but still properly flagged the spike in the concordance plate 032112RLN.

The main peak filter was originally set at 12% and using the 2nd main allele at a locus as the filter reference point. After analyzing plate 011513JLC, there were a couple of loci on 2 different samples that should have been flagged for review but were not because the 12% main peak filter eliminated them from being considered. During the calibration set analysis, the 12% main peak filter worked perfectly and did not indicate there to be any issues. The main issue with a few of the loci that were filtered out in plate 011513JLC and not flagged for review was that one of the loci had 3 alleles which could potentially indicate a tri-allele pattern or mixture. That is vital and FSS-i3™ definitely needs to be able to recognize that extra allele and flag that locus for review. Decreasing the main peak filter to 9.5% main peak filter (still using the 2nd main allele as a filter reference point) solved that particular issue. After analyzing several more concordance set plates, it was determined it would be more cautious to change the main peak filter from 9.5% to 0% and setting the main peak filter at a flat 75 RFU value. Setting the main peak filter as a flat RFU value instead of using a percentage is important to databasing

laboratories with high levels of heterozygote imbalance such as the Idaho State Police Convicted Offender DNA Database Laboratory.

The pull up threshold was decreased from 40% to 35%, and the sizing tolerance was increased from 0.3 bps to 0.35 bps after analyzing plate 121712JLC. One of the samples in the plate had a pull up artifact that was flagged for review by FSS-i3™ with the Preferential Amplification AB rule firing, but the pull up rule did not fire alongside the Preferential Amplification AB to signify that the allele is not a true allele but is a pull up artifact. The reason FSS-i3™ did not also fire the pull up rule is because the point of data that matched the designated allele had a 0.35 bp deviation but the sizing tolerance was set to +/- 0.3 bps. The sizing tolerance was changed to +/- 0.35 bps and the situation was remedied. The threshold was decreased from 40% to 35% to eliminate a few extra pull up firings that occur regularly at TPOX in a few of the samples; when in fact it is not pull up but a true allele with overlapping issues. It does not eliminate all the unnecessary extra pull up firings that occur regularly at TPOX because of overlapping issues but it decreases the abundance of them. Also, increasing the sizing tolerance allows the pull up rule to catch a majority of the pull up artifacts found throughout various data, but it will not catch all the pull up because some will deviate past +/- 0.35 bp. However, FSS-i3™ still flags that particular peak and/or allele with the extra peak, extra allele (if there are more than 2 potential alleles in that locus), or Preferential Amplification AB (if there only 2 potential alleles in that locus). Either way, FSS-i3™ will flag that particular locus in that sample for review or the analyst will be able to determine whether it is pull up or not.

The minus A rule's sizing tolerance was changed from +/- 0.2 to +/- 0.3 and its threshold was decreased from 15% to 5%. After analyzing a couple of concordance data sets, it was determined that a majority of Idaho State Police Convicted Offender DNA Database Laboratory minus A is at a low threshold at approximately 5%. Since the Idaho State Police Convicted Offender DNA Database Laboratory does not usually encounter many minus A in reference samples using DNA IQ extraction methods, not many were found in the analysis of the calibration data sets to properly establish the minus A rule. But after analyzing several more plates in the concordance study, a few minus A incidences were found and using this information the minus A rule set was able to be established with more confidence.

Conclusions

The FSS-i3™ Expert Software System process requires several steps that involves GeneMapper® ID generating data and passing it along to the i-STRess component of FSS-i3™ for interpretation. The Raw Export Table created in GeneMapper® ID will capture the peak size, height and area data for all peaks above the established peak amplitude threshold and that corresponding information will be imported into FSS-i3™ for proper analysis. Therefore the process designed must ensure that GeneMapper® ID and FSS-i3™ interact compatibly and seamlessly. The process flow between the two applications was evaluated during this validation, and it was determined to work very well with few issues. The small issues that did arise dealt with establishing and using the system. Once the analyst gets more comfortable with the systems functions and intricacies, the system is extremely efficient and saves the analyst

valuable time. FSS-i3™ not only eliminates a vast amount of samples or to be more specific, loci, that the analyst has to look at and evaluate, but it also narrows down what the possible issue could be at that particular locus that was flagged, while sample plots can be viewed using GeneMapper® ID if necessary.

FSS-i3™ generates two different results: a profile can be passed as acceptable without review or flagged for human review. It is vital that the samples being passed as acceptable without review are correct. Out of the 224 samples and 35 controls that were analyzed during the calibration set and the 1,198 samples and 53 controls that were analyzed in the concordance set, the finalized data for both the calibration and concordance set was checked against the original data set to check for concordance. All the samples passed as acceptable without review yielded correct results.

This validation illustrated that the use of FSS-i3™ Expert Software System is accurate, reliable and produces concordant results to those obtained using GeneMapper® ID 3.2.1. FSS-i3™ is capable of analyzing single source DNA samples as well as, or better than, the current system in place, GeneMapper® ID 3.2.1. In the future, this validation can be expanded to establish a rule set and settings parameters for FTA single source samples which bypasses the quantification step.

References

- Butler, John M. *Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers*. Burlington: Elsevier Academic, 2005.

- Bill, M. and Knox, C (2005) *FSS-i3™ Expert Systems.* The Forensic Science Service, United Kingdom and Promega Corporation.

- Forensic Science Service Ltd. *FSS-i3 Version 4 User Guide*. Forensic Science Service, 2007.

- Frappier, R., et al. (2008). *Improving forensic DNA laboratory throughput: Enhanced data analysis and expert systems capability*. Forensic Magazine, 25-31.

- Marshall University Forensic Science DNA Laboratory. *National Institute of Justice (NIJ) Expert System Testbed Project Demonstrations*. Huntington: 2007.

- Palsson, B., et al. (1999). *Using quality measures to facilitate allele calling in high throughput genotyping*. Genome Research, 1002-1012.

- Roby, R. and Christen, A.D. (2007) *Validating Expert Systems: Examples with the FSS-i3 Expert Systems Software*. Profiles in DNA, 13-15.

- Roby, R.K. et al. (2005) *The National Institute of Justice's Expert Systems Testbed Project.* In: Proceedings of the Sixteenth Symposium on Human Identification, Promega.

Acknowledgements

I thank Cyndi Cunnington, Idaho State Police Forensic Biology Unit Technical Leader, Rylene Nowlin, ISP Forensic Scientist, Gina Mann, ISP Forensic Scientist, Jodie Carney, ISP Forensic Scientist, and Tommie Quinney, ISP Forensic Scientist, for all their assistance and guidance throughout this validation. I would also like to thank Season Seferyn of the Marshall University Forensic Science DNA Laboratory, and Dr. Pamela Staton, Marshall University topic advisor, for their help and support. This project was supported by Award No. 2009-IJ-CX-K11 awarded by the National Institute of Justice,

Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication/program/exhibition are those of the author(s) and do not necessarily reflect the views of the Department of Justice.