



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

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## Abstract

An internal validation of FSS-i3™ 4.2.2 Expert Software System was conducted to assist the Idaho State Police Convicted Offender DNA Database Laboratory in establishing the FSS-i3™ rule set and setting parameters. It also focused on evaluating the accuracy and reliability of FSS-i3™ and its comparison to GeneMapper®ID. This validation will impact the forensic community by reducing the Idaho State Police Convicted Offender DNA Database Laboratory bottleneck effect and resource requirements by decreasing the amount of time the analyst spends on DNA analysis.

## Introduction

To reduce the bottleneck effect on data interpretation and technical review, expert systems have been developed to replace the traditional manual system. Expert systems are recommended for use with single source DNA samples to save valuable time and increase throughput. FSS-i3™ is comprised of three different components: i-STress, i-STReam and i-ntegrity. This validation primarily focused on i-STress which is the core DNA interpretation tool of FSS-i3™. The i-STress module was designed to integrate with GeneMapper® ID, and it 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.

## Materials and Methods

### Computer Software Requirements

FSS-i3™ 4.2.2 Expert Software System  
GeneMapper® ID 3.2.1

### Preliminary Study Performed Prior to FSS-i3™ Validation

Stochastic Threshold Study: Purpose was to establish the ideal RFU value below which sister alleles show severe peak height imbalance.

- PowerPlex®16HS Multiplex
- Applied Biosystems® 7500
- Applied Biosystems® 3130xl Genetic Analyzer

### FSS-i3™ Validation Methods

.fsa files that had already been processed using PowerPlex®16HS were used for this validation.

A calibration set of 224 samples and a concordance set of 1,198 samples were used.

Both sets of data were used to assist in the establishing and fine tuning of the rule set and setting parameters.

Each time the rule set was changed, each of the data files in both data sets were reanalyzed.

The calibration data set included samples that consisted of known challenges such as stutter, heterozygous imbalance, pull up, minus A, spikes, tri-alleles, mixture, contamination, missing allele, missing locus, and off ladder alleles that included microvariants and +/- allelic ladders.

Allele calls, base pairs, and heights in relative fluorescent height (RFU) for each sample generated by FSS-i3™ were checked for concordance to the results generated by GeneMapper® ID.

## Results

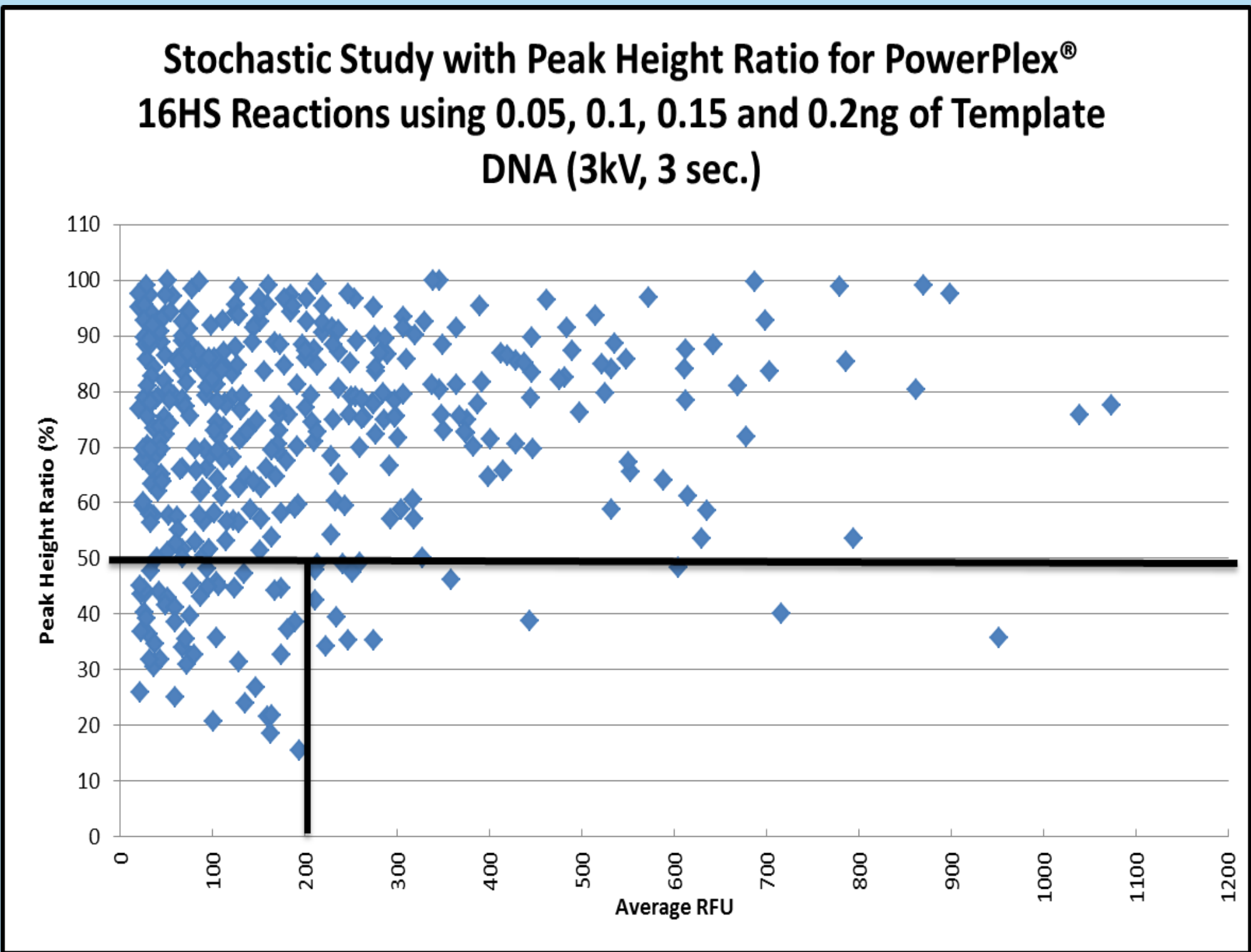


Figure 1

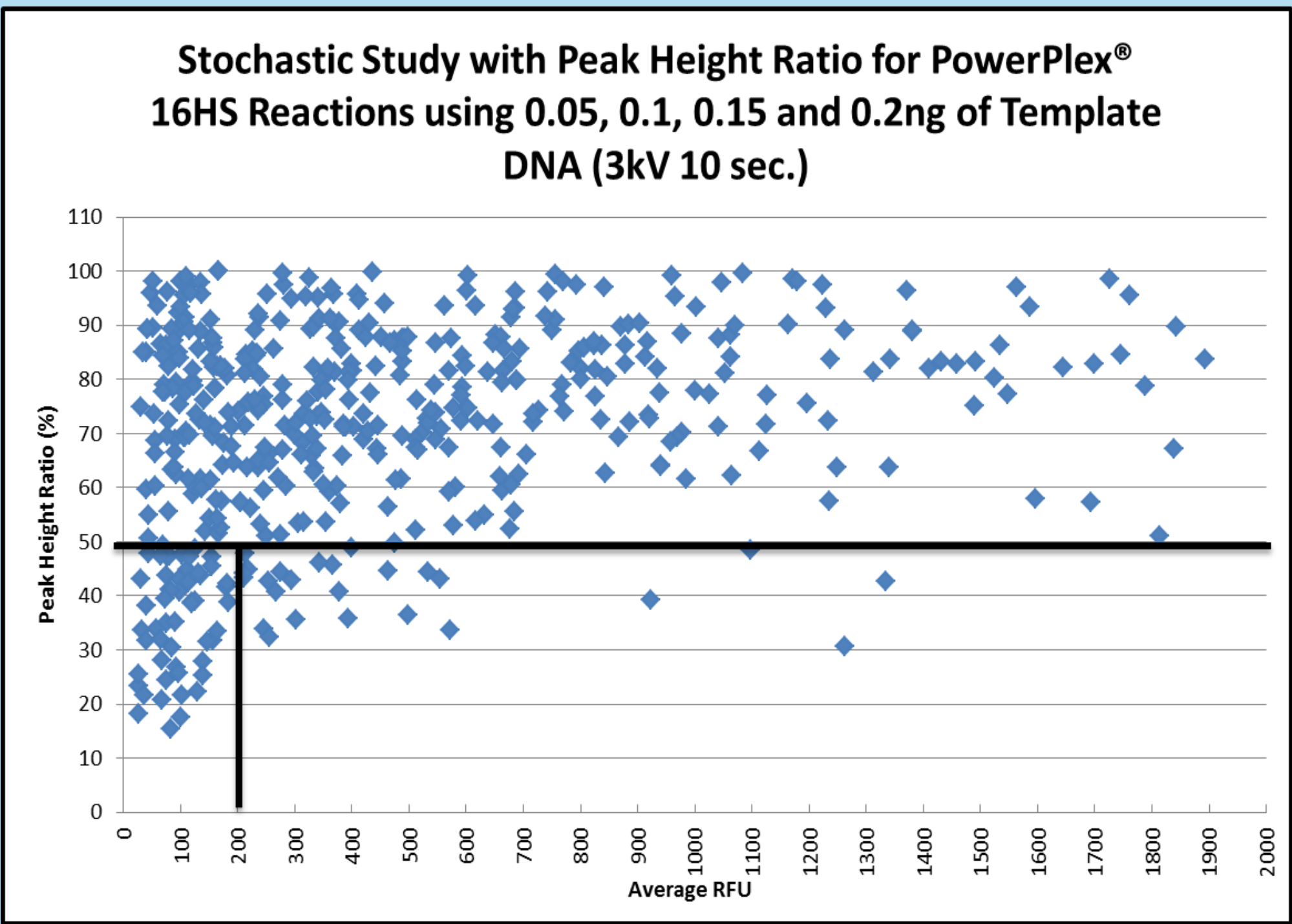


Figure 2

Table 1: Summary of Calibration 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).

## Results

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: 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 2 <sup>nd</sup> 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%.

Changes to the FSS-i3™ rule set and settings parameter were made throughout not only the calibration set but also the concordance set.

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

## Conclusions

FSS-i3™ requires several steps in its process flow:

- GeneMapper® ID generates raw data.

- Data is then passed along to the i-STress component of FSS-i3™ for interpretation.

- Interaction between the two applications was evaluated and determined to work very well with few issues.

- Few issues dealt with establishing and using the FSS-i3™ system.

FSS-i3™ is accurate, reliable, and produces concordant results to those obtained using GeneMapper®ID.

Capable of analyzing single source DNA samples as well as, or better than, the current system in place, GeneMapper®ID

Advantages of FSS-i3™:

- Saves valuable time
- Backlog reduction
- Narrows down what the possible issue could be

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