



# Internal Validation and Comparative Analysis of the PowerPlex® Fusion and the GlobalFiler™ Express Amplification Kits for Direct Amplification



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## ABSTRACT

In 2010, the FBI CODIS Core Loci Working Group recommended expansion of the CODIS Core Loci in the United States (Hares, 2012). Reasons given for expanding the CODIS Core Loci from the usual 13 loci to 24 loci included the need for international data compatibility and increased power of discrimination in missing person cases. Both Promega PowerPlex® Fusion and Applied Biosystems® GlobalFiler™ Express PCR systems have incorporated these changes.

Sensitivity, precision, concordance, reproducibility and contamination studies were performed as part of an internal validation study of these systems performed on an Applied Biosystems® 3500 Genetic Analyzer. The goal of this study was to validate that both commercial kits produce reliable and robust results, in addition to identifying a single thermal cycling parameter and single injection time for both systems using FTA cards (blood and saliva) and buccal samples.

## INTRODUCTION

Direct amplification eliminates the need to perform DNA extraction and DNA quantification of reference samples. Two of the most novel direct PCR kits are the PowerPlex® Fusion and the GlobalFiler™ Express Amplification Kits. These kits were developed as part of the effort to extend the Combined DNA Index System (CODIS) core loci. The expansion allows an increase in international data compatibility and discrimination power to aid in missing person cases. It also reduces the likelihood of adventitious matches due to the rapid increase in number of profiles stored in National DNA Index System (NDIS).

The internal validations were necessary before implementing a procedure for the analysis of blood and saliva samples on FTA cards, as well as buccal swabs. The side-by-side validation allowed Department of Forensic Sciences to properly evaluate the advantages and disadvantages of both kits to choose the one better suited for their needs.

## VALIDATION STUDIES

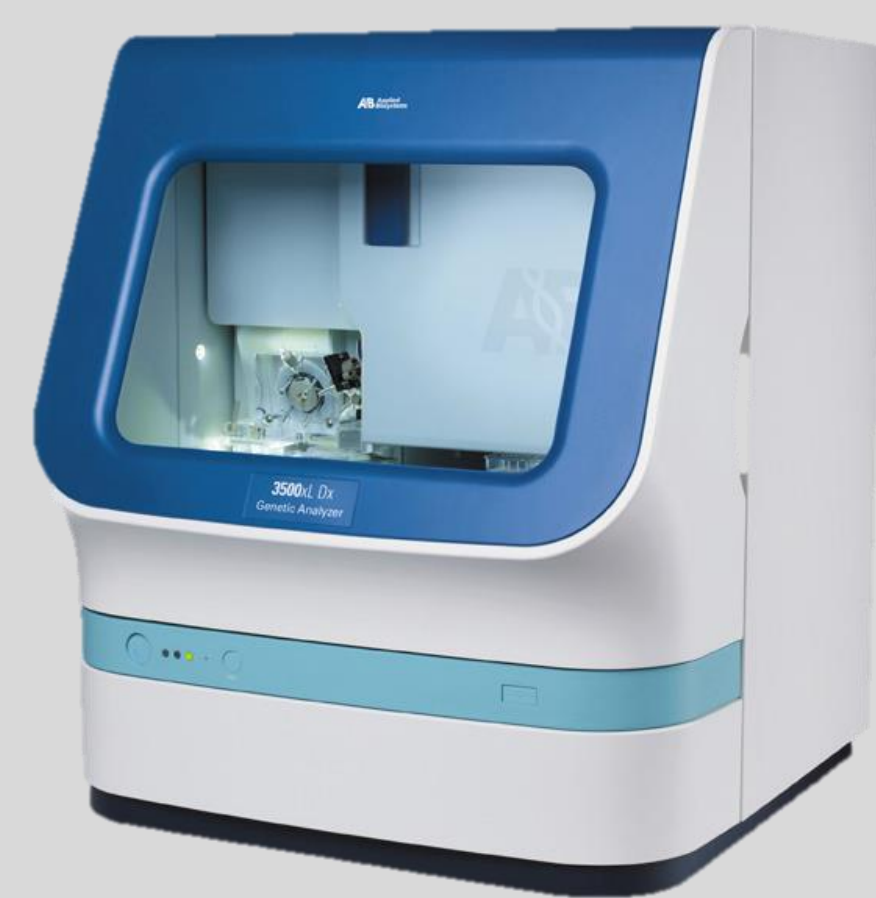
- Sensitivity
- Precision
- Concordance\*
- Reproducibility
- Contamination\*
- Injection Time
- Analytical Threshold
- Stochastic Threshold

\*Not discussed in this poster

## METHODS AND INSTRUMENTATION

### Materials and Reagents:

- FTA Cards (blood and saliva)
- Buccal Swabs
- Prep-n-Go™ Buffer
- SwabSolution™

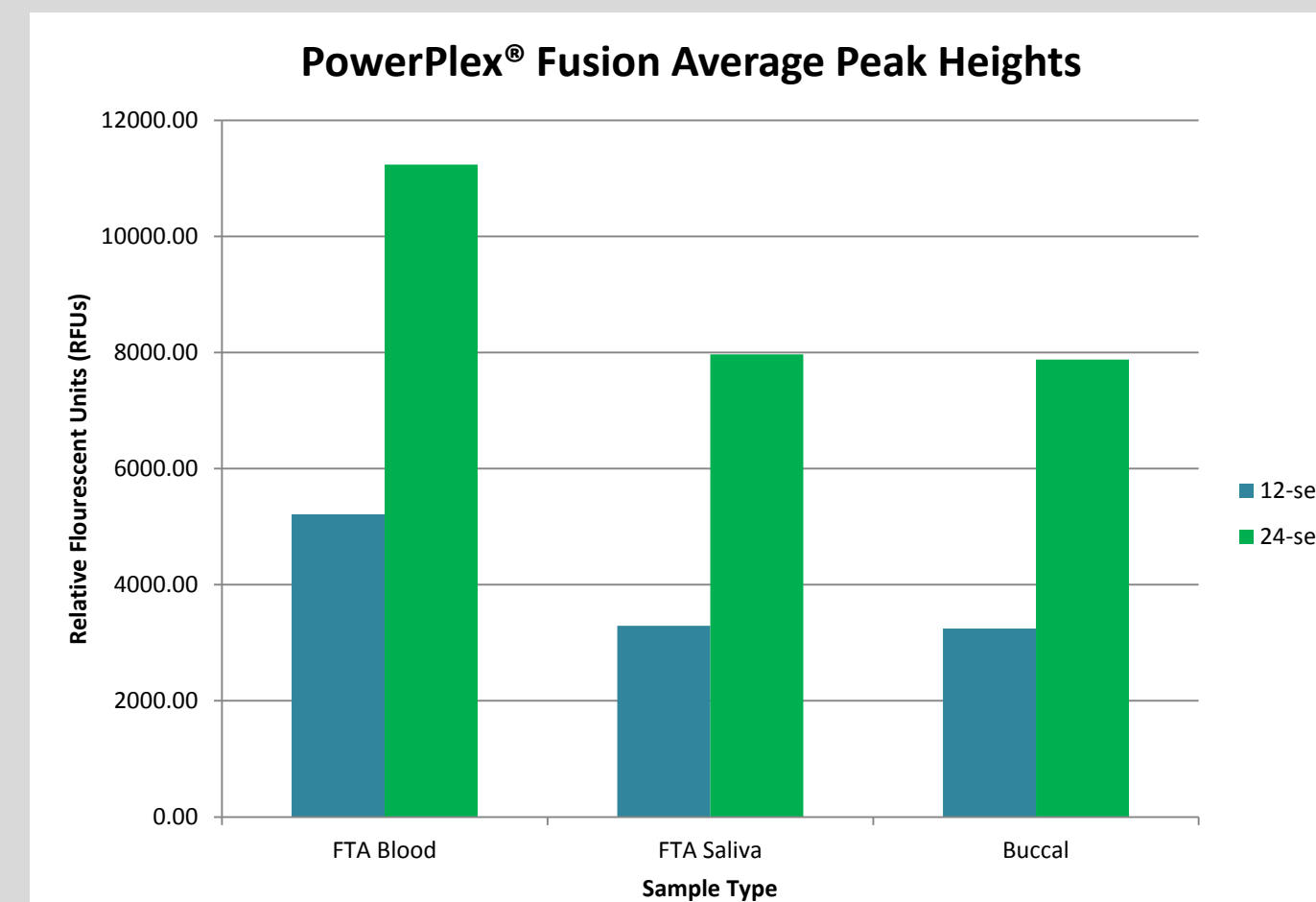


### Instrumentation:

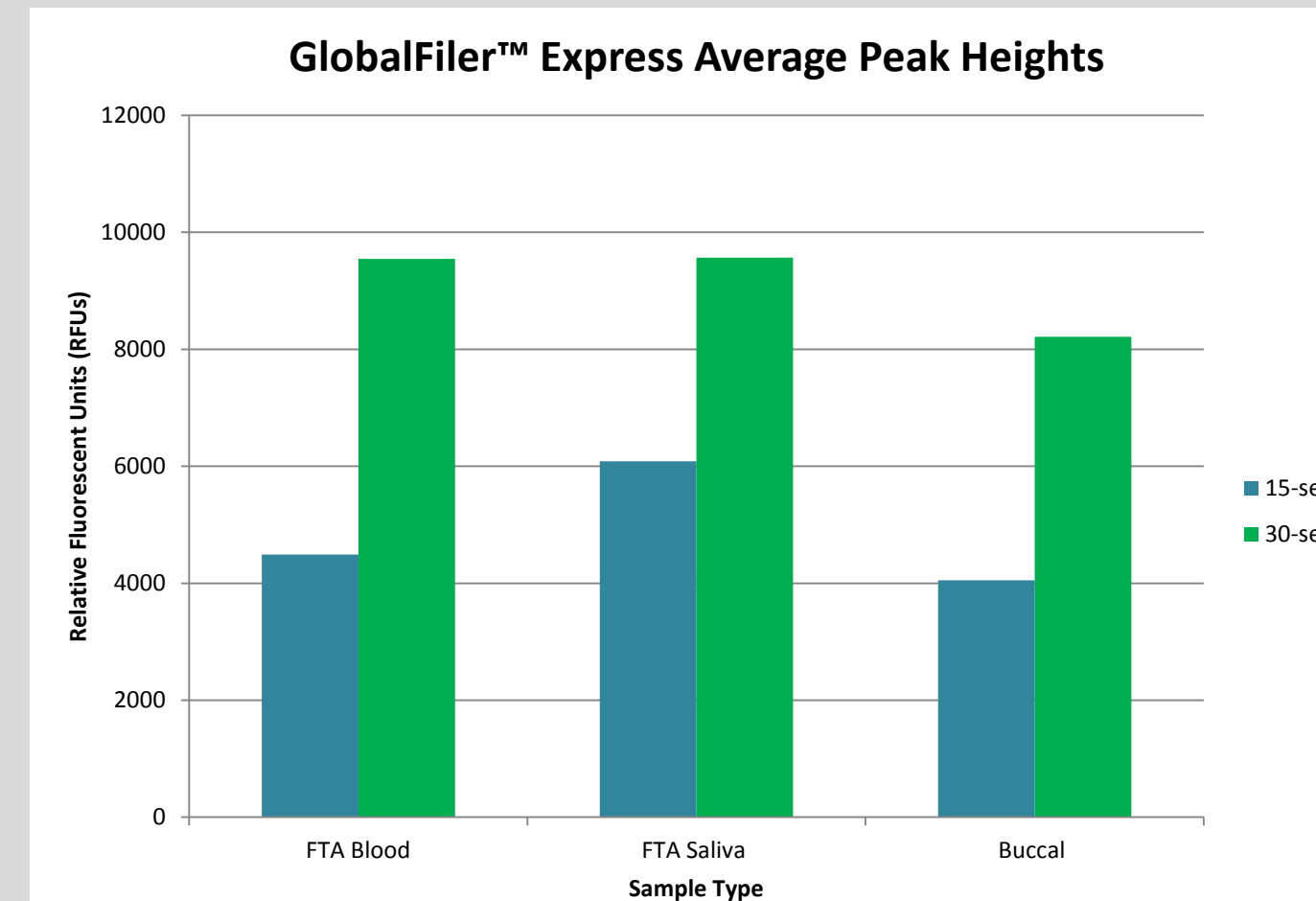
- Applied Biosystems® GeneAmp® PCR System 9700
- Applied Biosystems® 3500 Genetic Analyzer
- GeneMapper® ID-X Software Version 1.4

## RESULTS

### Sensitivity/Injection Study

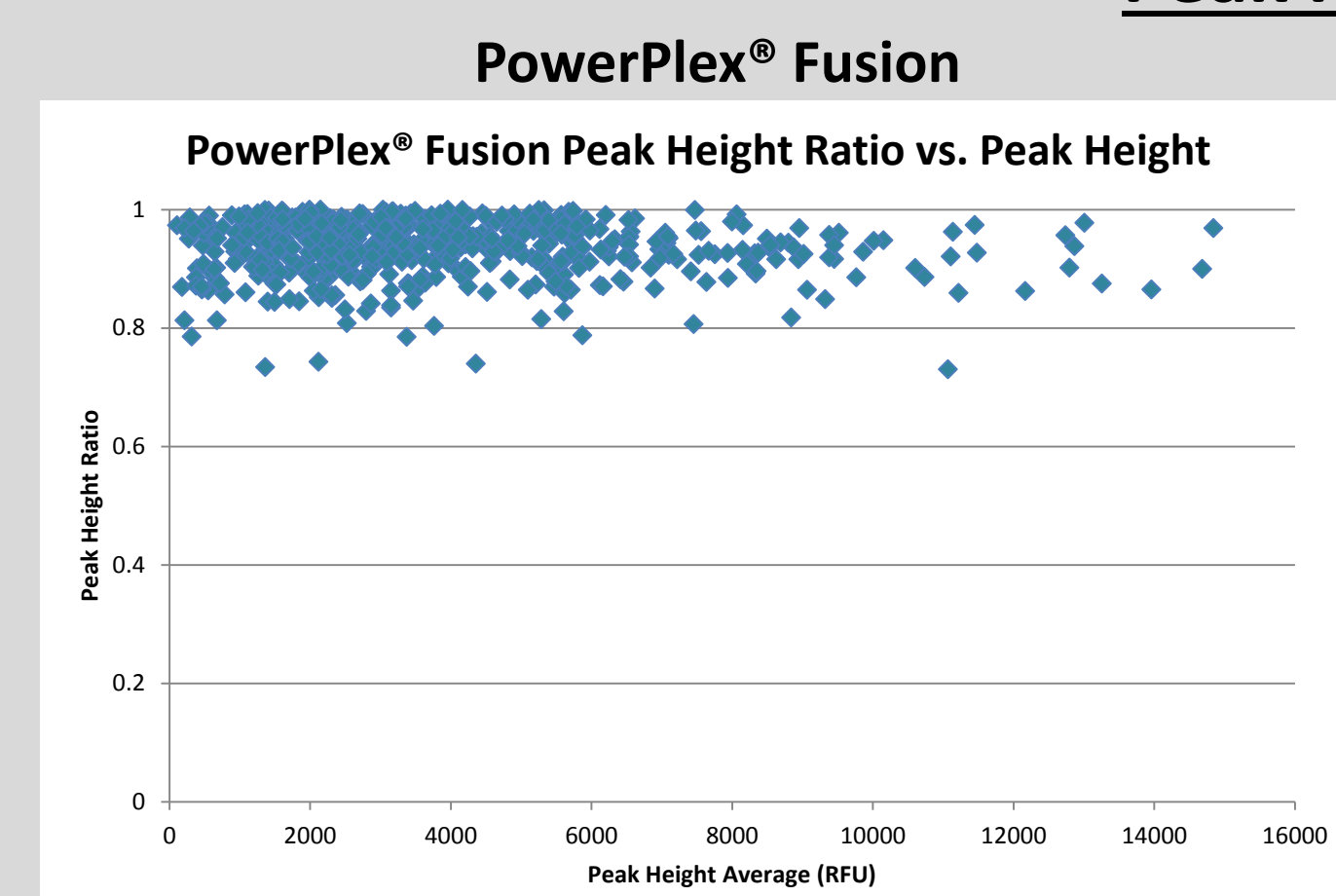


Average peak height comparison between 12- and 24-second injections (26 PCR cycles).

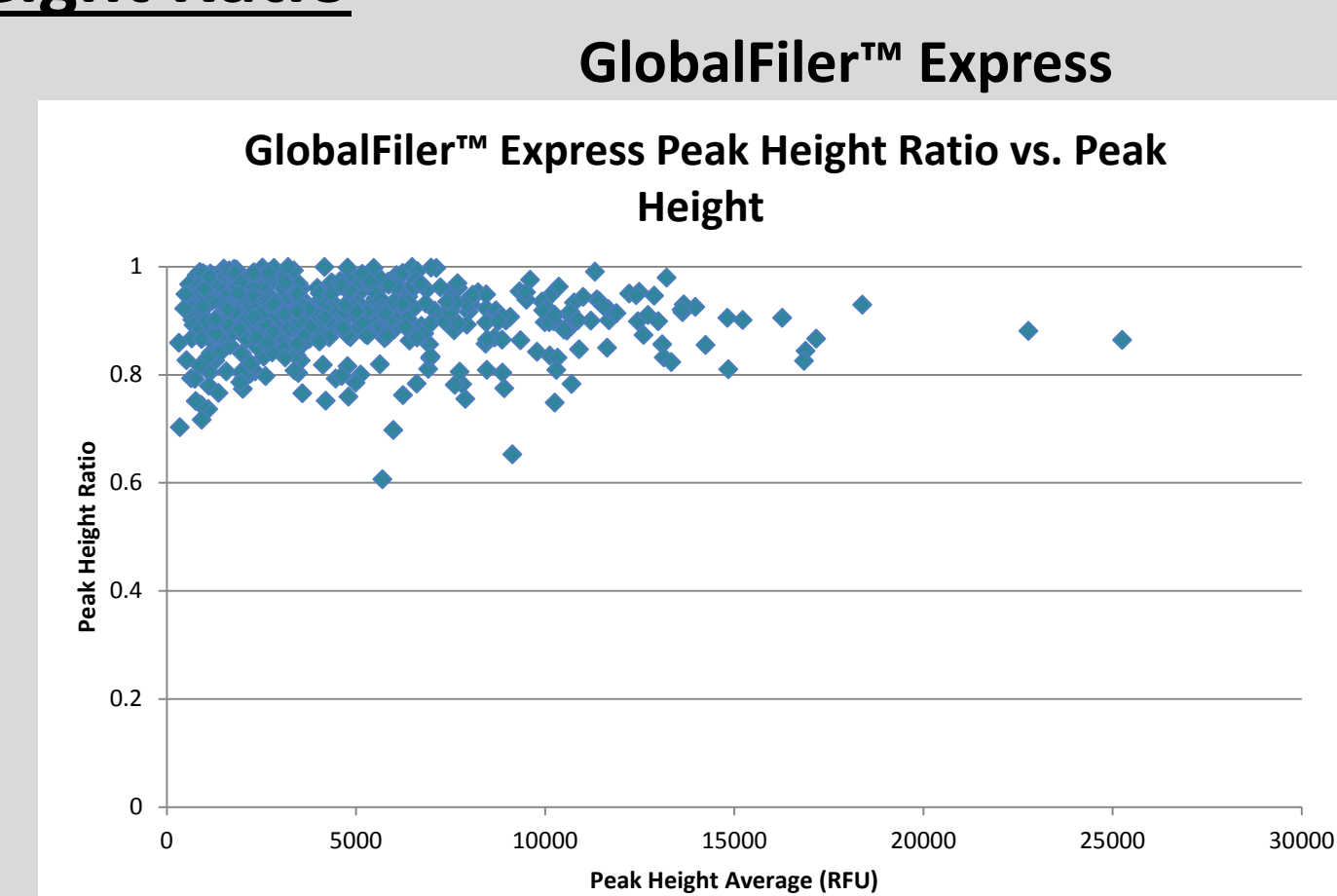


Average peak height comparison between 15- and 30-second injections (25 PCR cycles).

### Peak Height Ratio

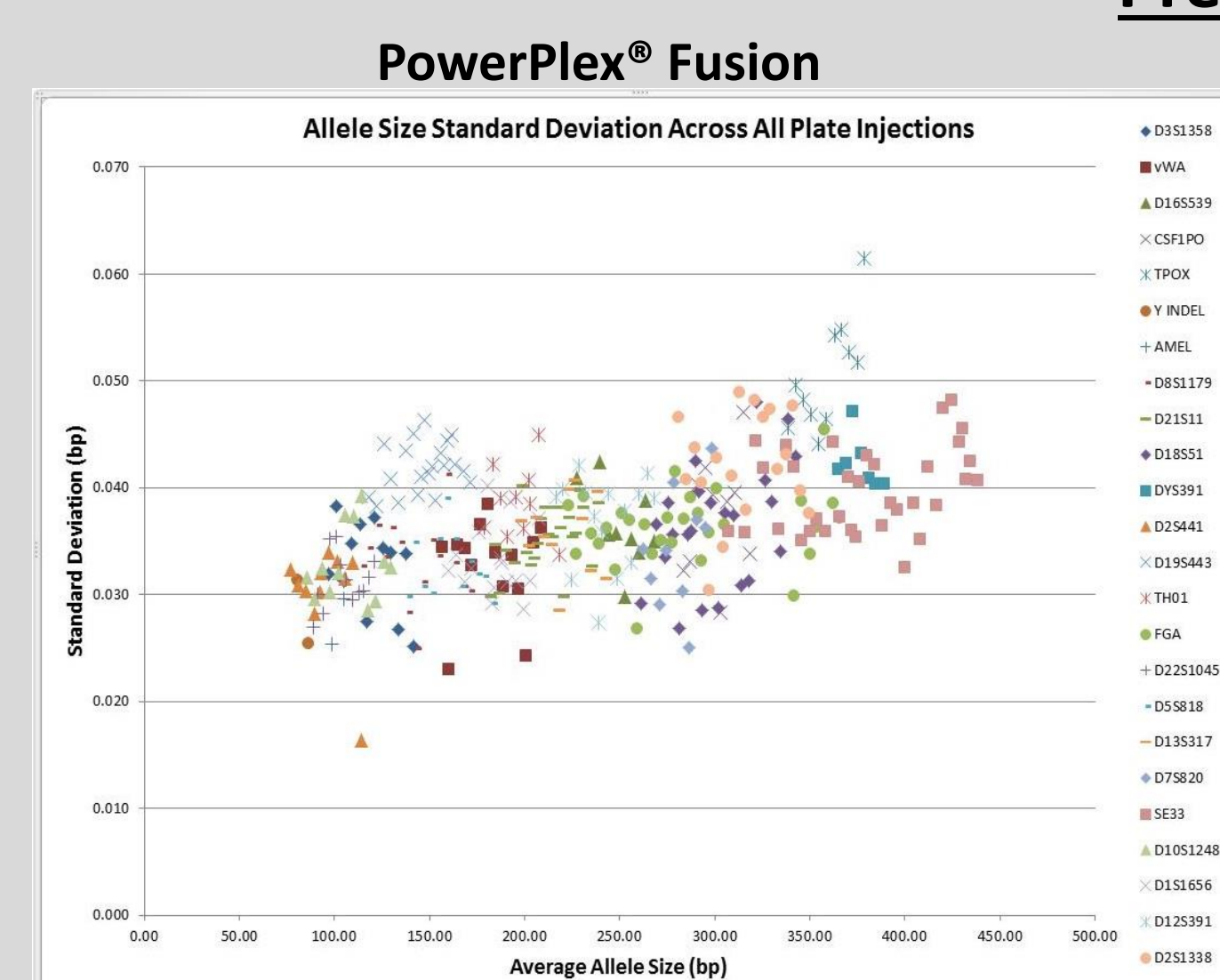


Peak height ratio over corresponding average peak heights of all samples processed at 26 cycles with a 12 second injection. Lowest peak height ratio observed: 73%.

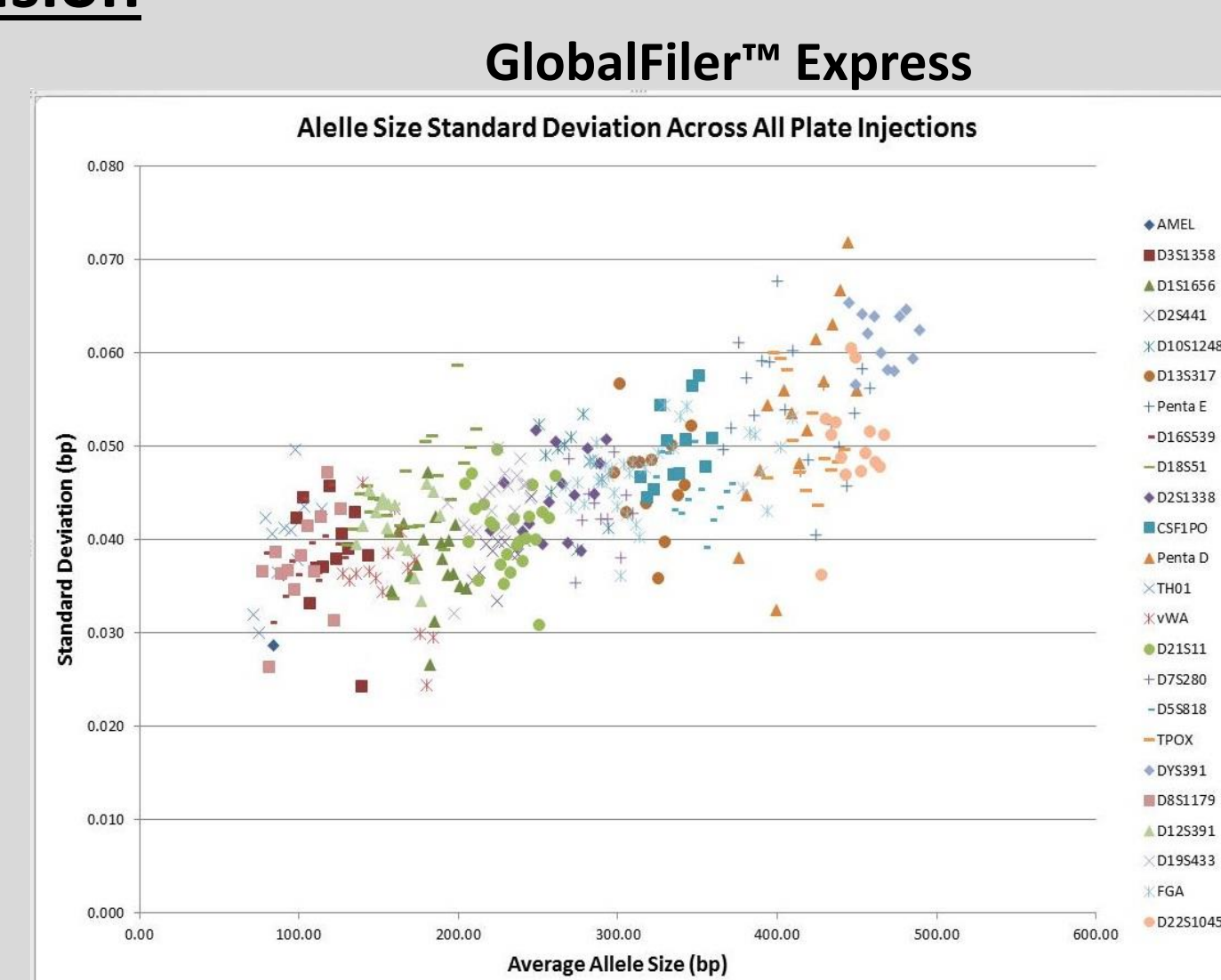


Peak height ratio over corresponding average peak heights of all samples processed at 25 cycles with a 15 second injection. Lowest peak height ratio observed: 60.6%.

### Precision



The following loci have the highest standard deviations in the PowerPlex® Fusion amplification kit: D22S1045, DYS391, and Penta E and Penta D.



The following loci have the highest standard deviations in the GlobalFiler™ Express amplification kit: SE33, DYS391 and TPOX.

**Note:** Additional statistics were compiled to ensure that alleles fall within the +/- 0.5 base pair window set by the GMID-X software. The data indicated that no markers and/or alleles within injections or across injections exceed a standard deviation of 0.15 base pairs. Therefore, both amplification kits are precise and only 1 allelic ladder is required per 3 injections during capillary electrophoresis setup.

### Analytical and Stochastic Thresholds

All samples were analyzed using the recommended analytical and stochastic thresholds set during the validation. The minimum peak height ratios expected for PowerPlex® Fusion and GlobalFiler™ Express are 70% and 50%, respectively.

PowerPlex® Fusion 26 Cycles – 12 Second Injection		
Min Peak Height Ratio	Analytical Threshold (RFU)	Stochastic Threshold (RFU)
70%	100	150

GlobalFiler™ Express 25 Cycles – 15 Second Injection		
Min Peak Height Ratio	Analytical Threshold (RFU)	Stochastic Threshold (RFU)
50%	70	140

## PROTOCOL OBSERVATIONS

### PowerPlex® Fusion:

- Incubation 70°C for 30 min
- Reagents need to thaw before use
- Uniform reaction mix
- DNA Control 2800M: needs dilution
- Approximate 90 min amplification

### GlobalFiler™ Express:

- Incubation room temp for 20 min
- Reagents ready for use
- Non-uniform reaction mix
- DNA Control 007: No dilution needed
- Approximate 30 min amplification

## CONCLUSIONS

Both amplification kits produce robust and reliable results. The following parameters are recommended for the Department of Forensic Sciences:

### PowerPlex® Fusion

26 cycles with a 12 second injection for blood and saliva on FTA cards, as well as buccal swab samples. Amplification will involve one 1.2mm punch of saliva or blood on FTA cards and 2 µL of swab DNA. If drop-out or single peaks below the stochastic threshold are observed, these samples may be improved with a 24 second injection.

### GlobalFiler™ Express

25 cycles with a 15 second injection for blood and saliva on FTA cards, as well as buccal swab samples. Amplification will involve one 1.2mm punch of saliva or blood on FTA cards and 3 µL of swab DNA. If drop-out or single peaks below the stochastic threshold are observed, these samples may be improved with a 30 second injection.

## ACKNOWLEDGMENTS

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### Disclaimer:

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