Validation and Comparison of the AmpFSTR® Identifiler® Plus PCR Amplification Kit to Identifiler®, MiniFiler™, and Yfiler®
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ABSTRACT
An internal validation of Applied Biosystems AmpFSTR® Identifiler® Plus (ID+) amplification kit was conducted to assist the Pinellas County Forensic Laboratory in improving the turnaround time of their caseload and low copy DNA interpretation. The results determined that this kit would aid in turnaround time and increase sensitivity. The Identifiler® Plus kit was also compared to Identifiler®, MiniFiler™, and Yfiler® amplification chemistries in order to determine the full capabilities of the kit as well as any improvements or regressions that may be present.

INTRODUCTION
Seven validation studies were performed to comply with the FBI quality assurance standards. They were as follows:

• Accuracy: To analyze how Identifiler® Plus amplifies known evidence samples.
• Precision/Reproducibility: To analyze how Identifiler® Plus amplifies known evidence samples.
• Linearity/Range: To determine the sensitivity and ideal range of amplifiable DNA to serve as a target to produce a reliable profile with limited stochastic effects.
• Recovery: To determine the amount of alleles recovered for each profile.
• Ruggedness: To compare results between three thermal cyclers and two genetic analyzers to ensure consistency amongst instrumentation.
• Contamination: To examine all negative controls for possible contamination.

MATERIALS AND METHODS

Instrumentation:
• Applied Biosystems® PrepFiler® Automated Express System
• Applied Biosystems® Quantifiler® Human DNA Quantification kit
• Applied Biosystems® 7900 Real-Time PCR System
• GeneAmp® PCR System 7900 Thermal Cycler
• Applied Biosystems® 3130 Genetic Analyzer
• Applied Biosystems® 3130v Genetic Analyzer
• GeneMapper® ID-X v1.3 software

Materials:
• Known positive controls, 9047A and 007
• Mixes at ratios of 1:1, 1:6, 1:6.1, 4:1, 2:1, 1:1.0, 0:1, 1:2, 1:4, 1:6, 1:9, and 1:19
• One single source sensitivity sample
• Diluted to 2:1, 2:5, 2:125, 0.025, 0.063, 0.031, and 0:016
• One Applied Biosystems® Identifiler® Plus allelic ladder
• Twenty non-probable samples
• Provided by Pinellas County Forensic Laboratory

REFERENCES
• Applied Biosystems, AmpFSTR® Identifiler® Plus PCR Amplification Kit User Guide. Part Number 4404211. Rev. D. Printed 02/01

RESULTS

Figure 1: demonstrates the difference in sensitivity between the two instruments. A partial profile was detected down to 0.018 ng for samples run on the 3130. When the same samples were run on the 3130x, a full profile was only able to be detected down to 0.038 ng.

Figure 2: shows the average peak values at each DNA concentration for the 4 amplification kits. When the trend analysis was performed, MiniFiler was determined to be the most sensitive, followed by Identifiler® Plus at 20 cycles, Identifiler, Identifiler Plus at 20 cycles, and Yfiler.

Figure 3: demonstrates the increased sensitivity of the 3130xl genetic analyzer when compared to the 3130. A full profile was able to be recovered down to 0.125 ng on the 3130xl, while a full profile was only able to be attained down to 0.5 ng on the 3130.

Figure 4: re-iterates the increased sensitivity of the 3130xl genetic analyzer when compared to the 3130. MiniFiler appears to have the best overall recovery, however, it also contains only 9 loci while ID and ID+ contain 15 loci.

Figure 5: displays the LODs for ID+ at 28 and 29 cycles and Identifiler at 28 cycles for samples run on the 3130. The intra-color balance for Identifiler is visibly skewed to the right, with a difference of approximately 0.5 ng for samples run on the 3130.

Figure 6: provides a comparison between Amplification kits. The 0.125ng – 0.5ng range is ideal for questioned and low copy DNA samples. The 0.5ng - 1ng range is ideal for mixture samples and the 1ng - 2ng range is ideal for full profile samples.

CONCLUSIONS
• The Accuracy Study determined that all known samples resulted in correct profiles.
• The Precision/Reproducibility Study determined characteristic errors inherent to sizing method. The third injection was slightly higher than the manufacturer’s recommended value of 0.15 bp. Multiple ladders should be run and spaced across the plate to ensure allele sizing within the ±0.5 bp window.
• The Recovery Study showed full allele recovery down to 0.063 ng. The 3130xl demonstrated better recovery than the 3130, but this may have been the result of the time delay between runs. MiniFiler appears to have the best overall recovery, however, it also contains only 9 loci while ID and ID+ contain 15 loci.
• The Linearity/Ranged Study revealed low baseline noise and extraneous peaks with an amplification target of 0.5ng or less. Even though Identifiler Plus has been advertised as more sensitive than Identifiler, the results from this study demonstrate that the sensitivity is relatively the same, if not lower than Identifiler.
• The Ruggedness Study resulted in higher peak heights when coupled with 29 PCR cycles, but those samples showed preferential amplification.
• The Contamination Study found one instance of contamination, which resulted from intra-lab contamination and not the kit reagents. All other negative controls showed clean results.

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