Validation and Comparison of the AmpFℓSTR® Identifiler® Plus PCR Amplification Kit to Identifiler®, MiniFiler™, and Yfiler®

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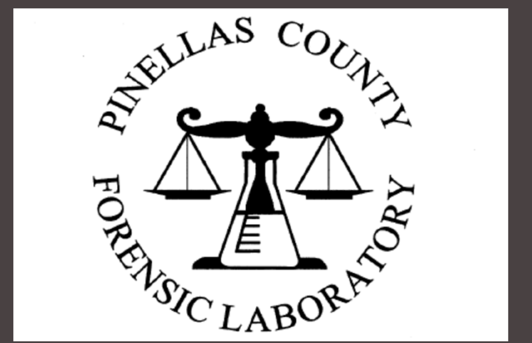

ABSTRACT

An internal validation of Applied Biosystems AmpFISTR® 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 establish that accurate and reliable genotypes were generated for analysis.

Recovery: To determine the amount of alleles recovered for each profile.

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.

Mixture: To reveal a sample's behavior containing two contributors.

Contamination: To examine all negative controls for possible contamination.

Ruggedness: To compare results between three thermal cyclers and two genetic analyzers to ensure consistency amongst instrumentation.

MATERIALS AND METHODS

Instrumentation:

- Applied Biosystems® PrepFiler® Automate Express System
  - Elution volume of 50µL
- Applied Biosystems® Quantifiler® Human DNA Quantification kit
- Applied Biosystems® 7500 Real Time PCR System
- GeneAmp® PCR System 9700 Thermal Cycler
- Applied Biosystems® 3130 Genetic Analyzer
- Applied Biosystems® 3130*xl* Genetic Analyzer
- GeneMapper® *ID-X* v1.3 software

Materials:

- Known positive controls, 9947A and 007
  - Mixed at ratios of 19:1, 9:1, 6:1, 4:1, 2:1, 1:1, 1:0, 0:1, 1:2, 1:4, 1:6, 1:9 and 1:19
- One single source sensitivity sample
  - diluted to 2.5, 1.25, 0.5, 0.25, 0.125, 0.063, 0.031, and 0.016
- One Applied Biosystems® Identifiler® Plus allelic ladder
- Twenty non-probative samples
  - Provided by Pinellas County Forensic Laboratory

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RESULTS

Figure 1

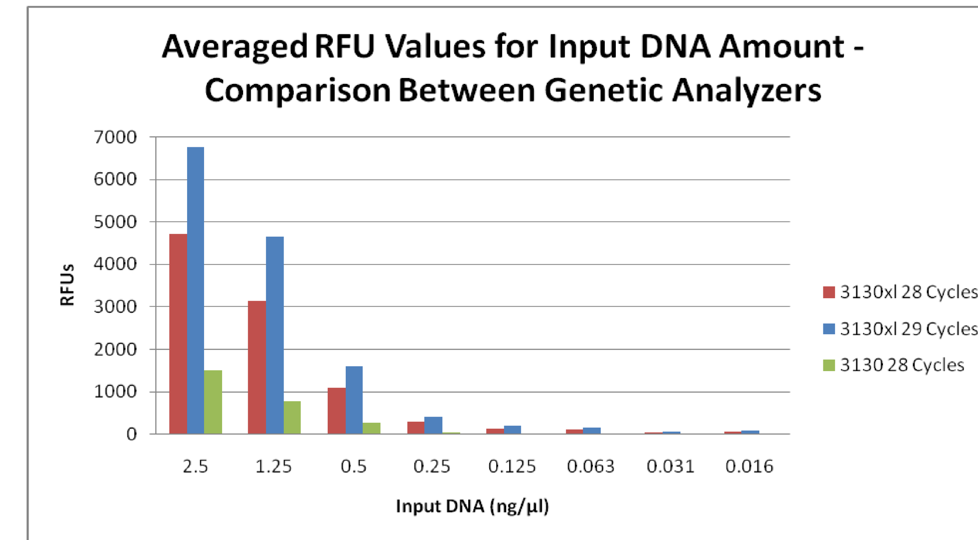


Figure 1 demonstrates the difference in sensitivity between the two instruments. A partial profile was detected down to 0.016 ng for samples run on the 3130xl. When the same samples were run on the 3130, a profile was only able to be detected down to 0.063 ng.

Figure 2

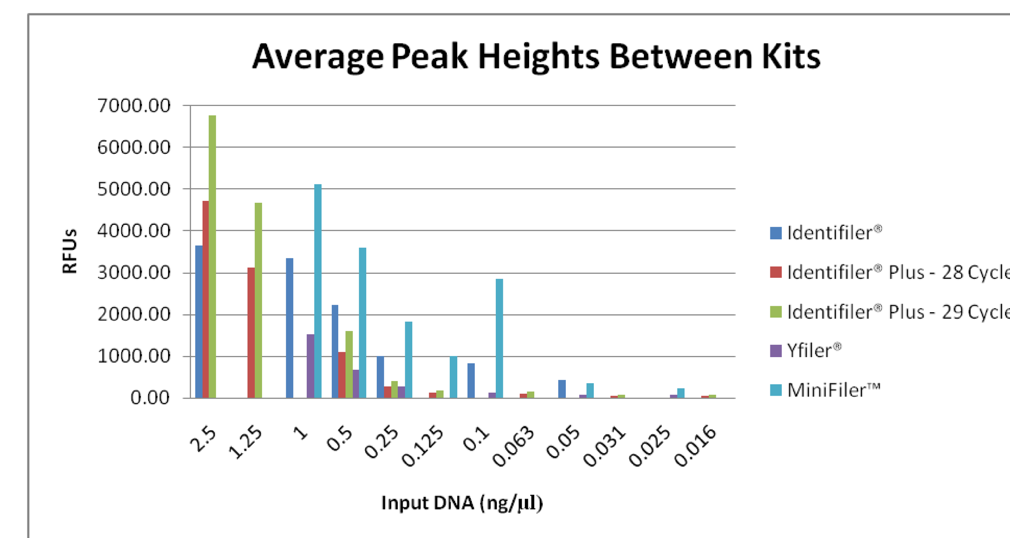


Figure 2 shows the average peak values at each DNA concentration for the 4 amplification kits. When a trend analysis was performed, MiniFiler was determined to be the most sensitive, followed by Identifiler Plus at 29 cycles, Identifiler, Identifiler Plus at 28 cycles, and Yfiler.

Figure 3

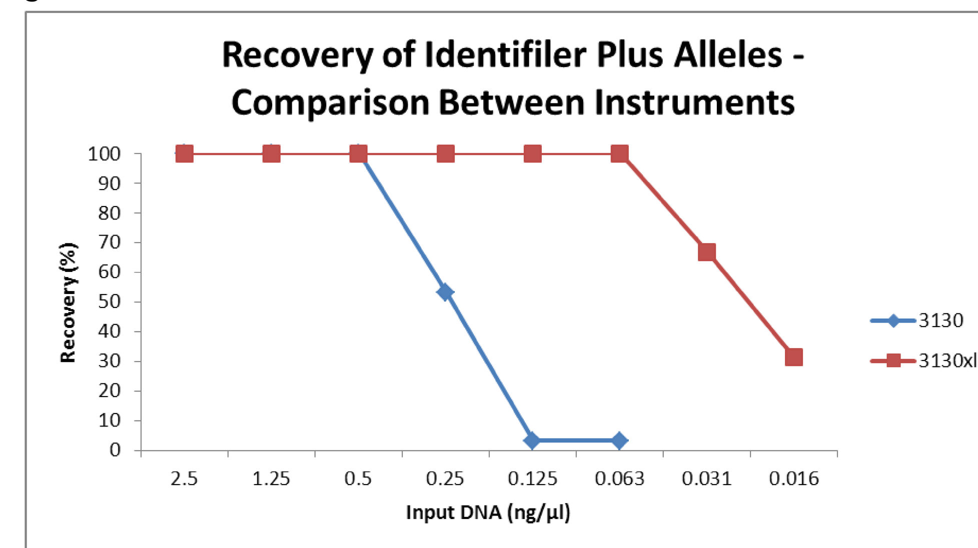


Figure 3 re-iterates the increased sensitivity of the 3130xl genetic analyzer when compared to the 3130. A full profile was able to be recovered down to 0.063 ng on the 3130xl, while a full profile was only able to be obtained down to 0.5 ng on the 3130.

Figure 4

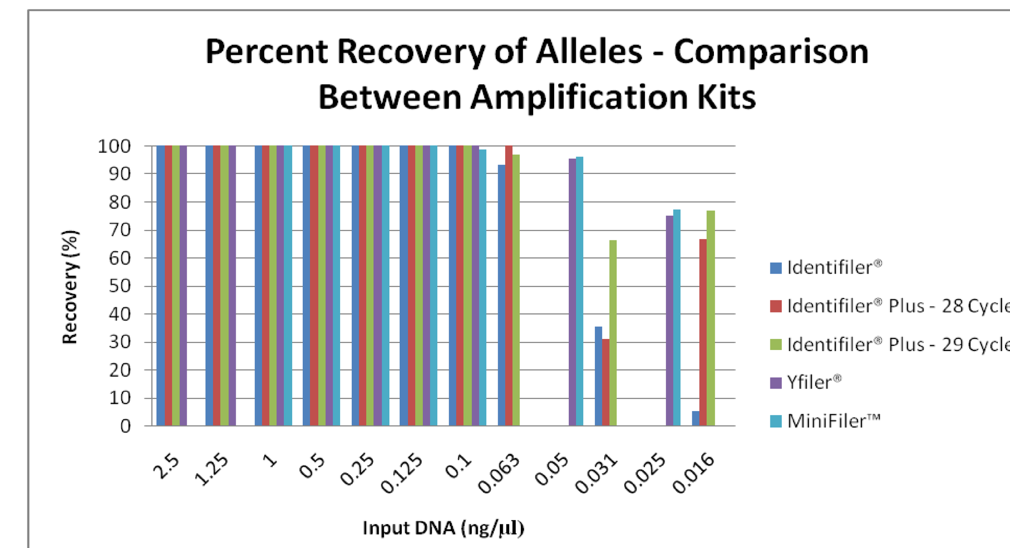
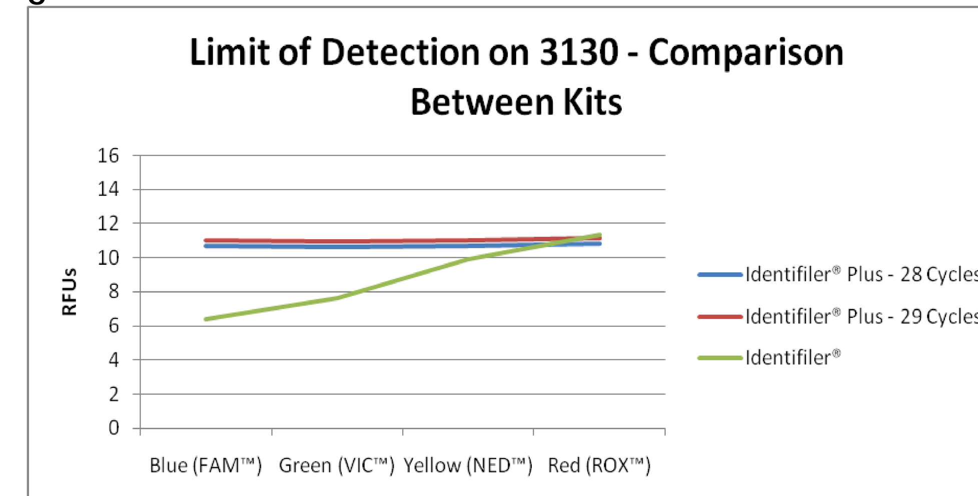


Figure 4 compares the recovery results for ID+ to the recoveries calculated for the other kits in previous validations. The recovery for ID+ seems greatly improved over Identifiler.

Figure 5



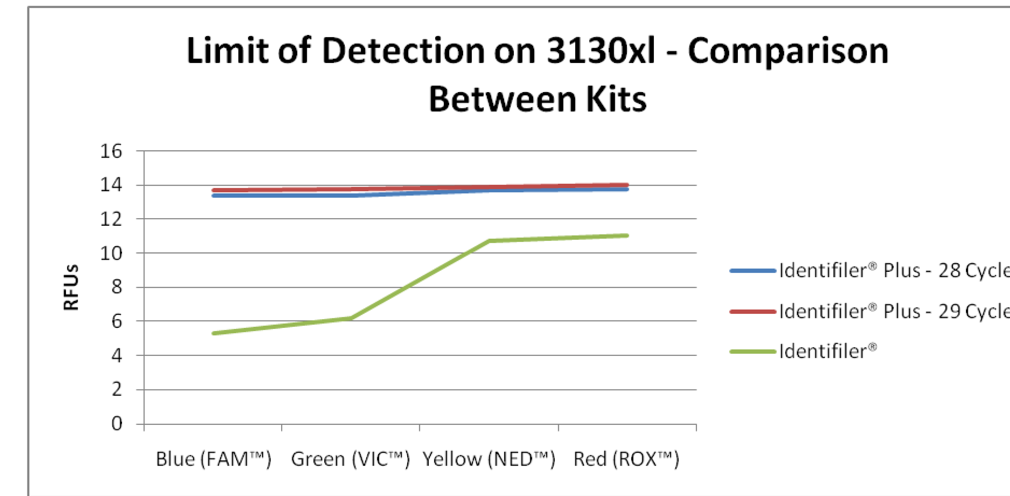
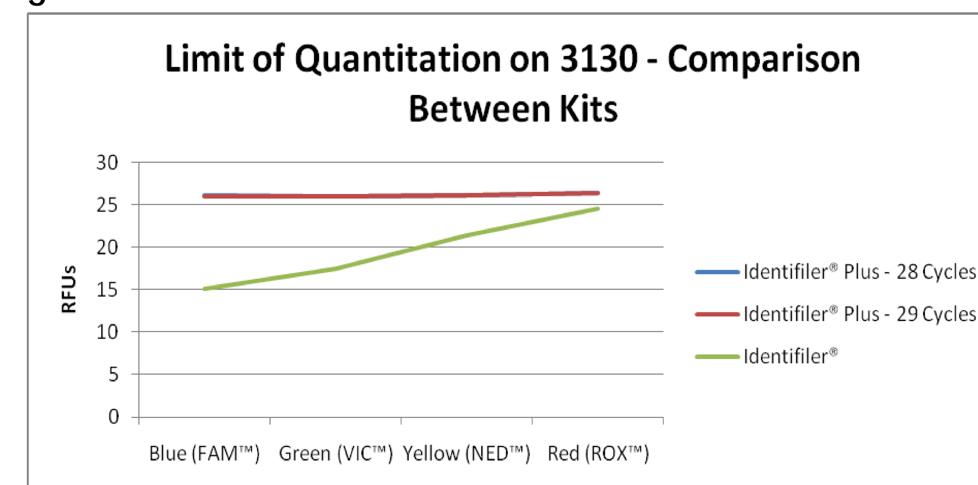
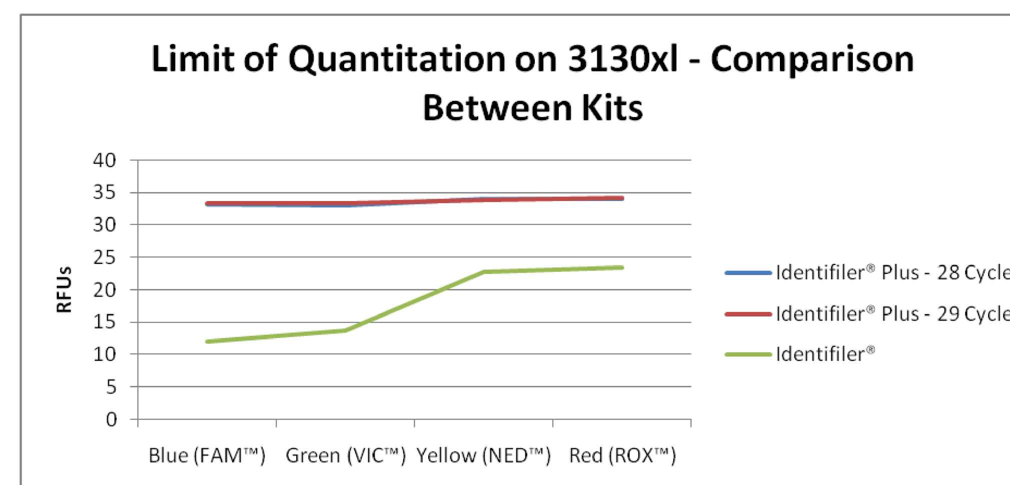


Figure 5 displays the LODs for ID+ at 28 and 29 cycles and Identifiler at 28 cycles for samples run on the 3130. The intra-color balance for Identifiler is visibly skewed to the right, with a difference of approx. 4 bp between dye channels. In contrast, the LODs for ID+ are very well-balanced for both cycle numbers. This indicates that while the differences in sensitivity between ID and ID+ are small, with ID being slightly more sensitive, there has been a significant increase in overall intra-color balance for ID+.

Figure 6





When the LOQs were calculated for both kits, the difference between 28 and 29 cycles of ID+ becomes negligible, as shown in Figure 6. The same results were seen for samples run on the 3130xl.

DISCUSSION

- 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 ±0.45 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/Range 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 Mixture 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.
- The Ruggedness Study showed no difference between the peak heights of samples amplified with the three thermal cyclers. The LODs and LOQs calculated for ID+ were higher than that of ID, but it is believed that the manufacturer sacrificed sensitivity for better overall peak height balance.

CONCLUSIONS

- Accuracy Study: All Identifiler® Plus profiles generated were concordant to the previous Identifiler® profiles provided. The results showed that with an appropriate DNA target amount, extraction positives and amplification positives produce the correct profile with few extraneous peaks.
- Precision/Reproducibility and Concordance Studies: Results fell within the manufacturer's recommended range of 0.15 bp.
- Recovery Study: Recovery of ID+ is improved from ID for sensitivity samples. All mixture samples demonstrated recovery of at least 85%.
- Linearity/Range Study: The 0.125ng – 0.5ng range is ideal for questioned and known samples. A target of 0.3ng at 28 PCR cycles is optimal.
- Mixture Study: Samples amplified for 28 cycles were deconvoluted down to a 1:4/4:1 ratio, while samples amplified for 29 cycles were deconvoluted down to a 1:9/9:1 ratio.
- Contamination Study: The results showed that with proper lab technique, contamination of reagent blanks, amplification blanks, or run negatives should not occur.
- Ruggedness Study: Samples run at different times as well as on different thermal cyclers and 3130 instrumentation were consistent with expected results and with each other.

ACKNOWLEDGMENTS

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