A15 Internal Validation of AmpFISTR® Identifiler® Plus Amplification Chemistry on Applied Biosystems' ABI PRISM® 310 Genetic Analyzer

Tiffany D. Bard, BS, 611 22nd Street, Apartment 322B, Huntington, WV 25703; and Cara E. Lupino, MS, Rhode Island Department of Health, 50 Orms Street, Providence, RI 02904*

After attending this presentation, attendees will have acquired a deeper understanding of how well Identifiler® Plus amplification kit advances the interpretation process. Nine studies reveal the superiority of this amplification kit when run on a 310 genetic analyzer.

This presentation will impact the forensic science community with a greater understanding of the results Identifiler® Plus can bring to the laboratory. Identifiler® Plus achieves greater sensitivity, faster amplification time, and increased interpretation of mixtures.

An internal validation of the Identifiler® Plus amplification kit was conducted to assist the Rhode Island Department of Health Forensic Science Laboratory's transition from a two kit amplification system (Profiler® Plus /COfiler®) to a one kit system increasing turnaround time and decreasing consumable usage. Since this is a small laboratory with strict budgeting, transitioning to Identifiler® Plus will increase throughput, save money by reducing consumable usage, and assist in relieving the backlog of cases.

The Identifiler® Plus amplification kit includes the 13 core CODIS markers, Amelogenin, and an additional two markers to increase the level of discrimination of the final profile as compared to Profiler® Plus/COfiler®. The new chemistry of this kit combines the Ampli®Taq Gold DNA Polymerase with a Master Mix reagent and reduces the overall kit package by two tubes and one box. Identifiler® Plus also provides this laboratory with a 5-dye amplification system and faster amplification time. The new improvements to the PCR thermal protocol remove about an hour of the amplification time. The combination of a one kit system paired with the new PCR protocol increases the laboratory's turn-around time and allows cases to be processed faster and more efficiently.

Samples were amplified with Identifiler® Plus on a GeneAmp® PCR System 9700 Thermal Cycler using a 29 cycle thermal protocol. Nine studies including sensitivity, precision/reproducibility, concordance, mixture, known and non-probative evidence samples, match criteria, contamination, denature, and stutter percentage were performed on two Applied Biosystems PRISM® 310 Genetic Analyzers and generated data was analyzed using GeneMapper *ID* v3.1.0.

The sensitivity study assessed the range of DNA concentrations that could produce reliable typing results. A precision/reproducibility study was performed to determine if accurate and reliable genotypes could be reproduced over consecutive days. All alleles were evaluated to determine if they fell within the recommended ±0.5bp window. The concordance study assessed the ability of Identifiler® Plus to accurately reproduce the NIST SRM 2391b reference samples. A mixture study was performed to examine the behavior of a sample containing two contributors comparing the resulting profiles to previous ones typed in Profiler® Plus/COfiler®. Match criteria and contamination studies were completed to determine if the positive and negative controls were working properly; positive controls had expected profiles and negative controls contained no contamination. A denature study was evaluated to determine if there was a significant difference between denaturing/chilling the samples before placement on a genetic analyzer and not denaturing/chilling. Lastly, an intra-laboratory stutter study was performed to evaluate if stutter remained under the 15% - 20% threshold.

Sensitivity study results indicated 0.5ng was the ideal input of target DNA to amplify at 29 PCR cycles with Identifiler® Plus. The sizing precision for each allele demonstrated the accuracy and reliability of sizing the Identifiler® Plus amplicons, even when several maximum/minimum values exceeded the recommended ±0.5bp window. Results obtained during the concordance study demonstrated intra-laboratory concordance when NIST SRM samples are amplified with Identifiler® Plus on multiple genetic analyzers. Mixture results showed a major contributor could be extracted at every marker with Identifiler® Plus when amplifying a target input of 0.3ng in samples containing a mixture of two individuals' DNA at ratios 10:1 and 1:10.

The methods utilized in the known and non-probative casework samples study were successful in the recovery of at least a partial DNA profile in concordance with Profiler® Plus/COfiler®. With proper lab technique and appropriate DNA target amount, extraction positives and amplification positives produced the correct profile with few extraneous peaks, and no contamination of any negative sample occurred during the validation. For the denature study, the step of 95°C denaturation and a snap-cooling step on an ice block prior to placing the samples on the instrument for separation caused the reduction of peak heights as compared to samples that were not denatured and snap-cooled prior to run.

Internal Validation, AmpFISTR® Identifiler® Plus Amplfication Kit, ABI PRISM® 310 Genetic Analyzer

 ** Presenting Author*