A16 Validation of the PowerPlex®18D Direct Amplification System

Kelly M. Borycki, BS, Marshall University Forensic Science Center, 1401 Forensic Science Drive, Huntington, WV 25701; Jennifer Bas, MFS, Las Vegas Metro Police Department Crime Lab, 5605 West Badura Avenue, Suite 120B, Las Vegas, NV 89118; and Justin Godby, MFS, and Pamela J. Staton, PhD, Marshall University Forensic Science Center, 1401 Forensic Science Drive, Huntington, WV 25701*

After attending this presentation, attendees will better understand the capabilities of direct PCR amplification for processing forensic DNA profiles.

This presentation will impact the forensic science community by showing the reliability of a new technology that can potentially save a significant amount of time and money during the production of forensic DNA profiles. It will also provide other laboratories that are considering adopting direct amplification with a general internal validation scheme.

As the role of forensic DNA analysis has grown, there has been a significant increase in the number of samples that forensic DNA laboratories receive for analysis. Many states are moving toward, or have already adopted, legislation that would require a DNA sample to be collected from all arrestees, drastically increasing the throughput requirements of many laboratories. In order to maintain efficiency and prevent a sample backlog, it is imperative to reduce the time and cost associated with forensic DNA testing.

Direct PCR amplification significantly reduces the time required for DNA analysis by eliminating both DNA extraction and quantification on single source blood or buccal stain samples. Promega's direct PCR amplification chemistry, PowerPlex® 18D (PP18D), further decreases the time required to produce a final profile by using shorter thermal cycling - approximately one-half required by traditional amplification chemistries. With this kit, database or reference samples can be processed, reviewed, and uploaded into CODIS in as little as one day.

Due to the lack of a purification step when using PP18D with FTA® punches, the chemistry has been designed to overcome most types of inhibition that would commonly be encountered with this sample type. An additional advantage to the PP18D kit is the amplification of two non-CODIS, highly discriminating penta-nucleotide repeat unit markers that can increase the level of discrimination associated with profiles generated using this amplification system.

This study sought to validate the PowerPlex® 18D direct amplification kit for use with single-source FTA® card samples. Samples were collected on the Whatman® EasiCollect™ device. One 1.2mm punch was taken using a Harris manual punch and amplified for 27 cycles on an Applied Biosystems GeneAmp® PCR System 9700 thermal cycler. Half reaction volumes (7.5µL water, 2.5µL 5X Master Mix, 2.5µL 5X Primer Pair Mix) were used to further reduce the cost associated per sample. Fragment separation was performed using Applied Biosystems 3130xl Genetic Analyzers. Data generated was analyzed using the Applied Biosystems GeneMapper® ID-*X* software v1.1.1. When applicable, quantification was performed using the Quantifiler® Human DNA Quantification kit and the Applied Biosystems 7500 Real-Time PCR System.

PP18D was determined to yield full profiles from samples with concentrations as low as 0.4ng/µL. The kit was shown to be precise at all 18 loci with 99.7% certainty. Known/non-probative samples that were collected from employees were compared to known profiles, and all samples (85 of 85) were concordant. Seven samples were amplified and run on different instruments and different days to test reproducibility and were found to be concordant. Other common sample types (cotton swabs, Omni® swabs, and extracted DNA) were also tested and yielded full profiles.

Based on the findings of this study, PP18D for processing database samples, as well as troubleshooting single-source casework samples, will be incorporated. Future studies will be done to determine the feasibility and reliability of performing direct PCR amplification on non-FTA® card samples once the lysis buffer from Promega is released for commercial use.

Direct Amplification, Validation, Efficiency

 ** Presenting Author*