A80 An Evaluation of Direct PCR Amplification

Reena Roy, PhD, Penn State Univ, Forensic Science Program, 325 Whitmore Lab, University Park, PA 16802; and Daniel E. Hall, BS, Penn State Univ, Forensic Science Program, 107 Whitmore Laboratory, University Park, PA 16802*

After attending this presentation, attendees will understand the concept of direct amplification from nine different substrates. Attendees will also appreciate the method that allows the scientist to generate autosomal and Y-STR profiles from substrates without the cumbersome steps of extraction and quantitation.

This presentation will impact the forensic science community by informing the forensic DNA analyst of a faster, less expensive approach to obtaining DNA profiles from bodily fluids using eight commercially available amplification kits.

The objective of this research was to generate complete autosomal STR profiles from body fluids using direct amplification and various commercially available STR amplification kits. Attempts were also made to detect the Y-profile from male body fluids using 1.2mm punches and the AmpFℓSTR® Yfiler™ kit following direct amplification.

STR analysis of blood and buccal samples is often used in the fields of forensic biology and genetics for casework, paternity testing, and convicted-felon DNA profiling. A primary advantage of direct amplification without purification of DNA is the high throughput of databank samples. Direct amplification of DNA stored on various types of substrates reduces the time required to obtain a DNA profile, thus reducing cost and increasing efficiency.

FTA® cards are suitable for criminal offender DNA database samples and casework reference samples. The preservative in these cards contains proprietary chemicals to protect DNA molecules from nuclease degradation, and to protect the host matrix from bacterial growth. DNA from biological samples deposited on FTA® paper and similar commercial storage devices has been found to be stable for a period of several years when stored at ambient temperature. A primary advantage of FTA® paper and similar substrates is their ability to provide consistent results without quantification, and the procedure can be automated.

Direct amplification kits contain improved PCR buffer cycling protocols that can overcome inhibitors during PCR amplification steps. Most collection media for storing dried body fluid samples contain chemicals that are capable of lysing cells (which may contain PCR inhibitors) to preserve DNA within a sample. Using devices without lysing chemicals, such as the Bode DNA Collector, requires an additional lysis step, or else amplification quality may be poor and allelic dropout may occur. Some of the amplification kits listed above have been optimized with enhanced reagents for direct amplification. However, it may not be cost effective for crime laboratories to use these direct amplification kits. Kits such as the PowerPlex® 16 System and the AmpFℓSTR® Yfiler™ Amplification Kit require the addition of polymerase in the reaction.

The following nine collection media were used for this study: proPRIME*i* Indicating Micro, 705 Micro, Blood Direct #1 and #2, Collection Card, CEP swab from FITZCO, EasiCollect from Whatman, FTA Indicating Micro, and Bode DNA Collector. Blood from two deceased individuals and saliva from three living donors were used in this study. The three single-source saliva samples and two single-source blood samples were deposited on each of the 45 collection devices.

A 1.2mm punch of each of the 45 substrates containing one body fluid was amplified with PowerPlex® 18D, PowerPlex® 16 HS, PowerPlex® 16, and PowerPlex® 21 Systems from Promega Corporation, and AmpFℓSTR® Identifiler® Direct, Identifiler® Plus, and Identifiler® PCR Amplification Kits from Applied Biosystems following each manufacturer's recommended conditions. Similarly, 1.2mm punches of the substrates containing male body fluids were amplified with AmpFℓSTR® Yfiler™ PCR Amplification Kit.

Results from the eight kits mentioned above were compared. Both blood and saliva samples appeared to yield complete DNA profiles. Two different reaction volumes were attempted with substrates that yielded complete profiles from single source samples, the first using the manufacturer's recommended volume, and the second using half of the reaction volume suggested in the protocol. For some of the substrates, thermal cycling conditions were modified as necessary to generate complete DNA profiles.

Another goal of this research was to demonstrate that direct PCR amplification can be applied to commercially available kits not intended for direct amplification. The results indicate that it is possible to do so.

Direct Amplification, STR, Y-STR

 ** Presenting Author*