VALIDATION OF DIRECT PCR AMPLIFICATION FROM FABRICS USING POWERPLEX® Y23

Amanda Dargay, B.S. and Reena Roy, Ph.D., Pennsylvania State University

The goal of this research was to generate DNA profiles from saliva deposited on different fabric substrates. Each substrate containing saliva was amplified under various conditions. Some samples were treated with purification reagents prior to amplification with the Powerplex® Y23 amplification kit, while others were left untreated. DNA profiles generated were compared for concordance within and between the substrates.

Saliva samples (100µl) from male donors were deposited on cotton and cotton-polyester fabric. The substrates containing the body fluid was left at room temperature and dried overnight. Some of these samples were also left for approximately one week at room temperature. Each substrate containing dried saliva was punched using a 1.2mm Harris Micro-Punch®. The first batch of substrates was not treated with any washing reagent. A second batch of punches was treated with Prep-n-Go™ buffer as recommend by the manufacturer of the solution. PunchSolution ™ buffer was added to the last batch of punches, and left to dry as recommended by the manufacturer.

Amplification reagents from PowerPlex® Y23 amplification kit were added directly to all of the punches in all three batches. Amplification parameters were varied in this study in order to detect complete Y-STR DNA profiles from all substrates. All reactions were performed in either 12.5 or 6.25µl reaction volumes.

Analysis of the amplified products was performed by capillary electrophoresis injection on the Applied Biosystems 3130xl Genetic Analyzer. The generated data were analyzed using GeneMarker® HID Software Version 2.6.0 from SoftGenetics®.

Y-STR profiles were successfully obtained from all of the substrates containing the saliva samples. The optimal PCR amplification parameter was observed to be 27 cycles for saliva.

Concordant profiles were generated between the substrates treated with or without washing reagents. Both 6.25µl and 12.5µl reaction volumes yielded complete profiles.

In the forensic science community, direct PCR amplification could have an important impact. This research study demonstrates that crime scene samples, such as fabrics containing body fluids, can be amplified directly. The result of this research indicates that the samples can be processed faster using this method. The procedure also allows for decreased reagent consumption and consequently, reduces cost. The concordance profiles were obtained with or without washing the substrates. Therefore, implementing the use of direct PCR amplification using a minimum amount of body fluid would be valuable in the forensic science community.