A70 Evaluation of PowerPlex® 18D and PowerPlex® 21 With Buccal Samples Collected on Non-Treated Paper

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After attending this presentation, attendees understand how to achieve a high first pass success rate when performing direct amplification of 1.2mm or 2mm punches from buccal samples on non-treated paper with PowerPlex® 18D and PowerPlex®21.

This presentation will impact the forensic science community by demonstrating the feasibility of two new direct amplification kits for the processing of reference samples, exploring the feasibility of increasing the size of the standard punch from 1.2mm to 2mm for direct amplification.

The efficiency of reference sample processing for databasing and paternity purposes has been greatly increased by the development of direct amplification systems. DNA samples can be collected and stored on non-treated matrices, such as the Bode Buccal DNA Collector™, until sample processing is required. The Promega Corporation has recently released two direct amplification kits, PowerPlex® 18D and PowerPlex® 21 for samples collected on both treated and non-treated collection paper. This presentation will describe the studies with each amplification system to obtain optimal results from samples collected with the Bode Buccal DNA Collector ™.

Direct amplification of reference samples eliminates the need for the time-consuming extraction and quantification steps encountered in routine processing. Eliminating these steps will save time, but it can also lead to additional amplification reactions and costs as normalization does not occur. Cellular deposition varies from individuals resulting in either excessive or inadequate samples. Eating, drinking, medicine intake, health status, and non-cooperation during sample collection will all affect the number of cells deposited onto the collection paper. This variation in cellular deposition can either lead to over or under amplification if the procedure is not optimized. Optimizing the amplification parameters to account for sample variation reduces re-sampling and re-amplification, while increasing the processing efficiency of the laboratory.

Evaluation studies optimized procedures for sampling, cell lysis, thermal cycling parameters, spectral calibration, and ABI Prism® 3130 Genetic Analyzer injection parameters for both PowerPlex® 18D and PowerPlex® 21.

These optimized parameters with PowerPlex® 18D resulted in a first pass success rate of >86% for a full 25µl reaction using a single 1.2mm punch. The remaining 14% failed internal quality standards due to either over amplification or capillary electrophoresis issues (spikes, migration, or ILS problems). Complete profiles were obtained from all one hundred (n=100) samples tested without re-sampling or re-amplification.

In addition to displaying the first pass success rate for a full reaction with PowerPlex 18D, this research demonstrated the first pass success rates for 1.2mm samples (n=100) in a 12.5µl half reaction incorporating and eliminating the 20-minute lysis incubation step.

The optimized procedures for obtaining a high first pass success rate for both full reaction (25µl) and half reaction (12.5µl) will be discussed when utilizing a single 1.2mm punch with the PowerPlex® 21 amplification system. When dealing with an aged sample, or a sample with poor cellular deposition due to one of the causes previously mentioned, a 2.0mm punch may produce better results. This presentation will display results for sample processing with 2.0mm punches with PowerPlex® 18D and PowerPlex® 21, instead of the standard 1.2mm punch. Increasing the punch size can increase the chances of obtaining a complete profile using the same parameters utilized on high-quality samples.

Direct Amplification, PowerPlex® 18D/21, Half Reaction

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