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### **B70 Internal Validation and Comparative Analysis of the Promega® PowerPlex® Fusion and the Applied Biosystems® GlobalFiler™ Express Amplification Kits for Direct Amplification**

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After attending this presentation, attendees will better understand the key differences between the Promega® PowerPlex® Fusion and the Applied Biosystems® GlobalFiler™ Express Amplification Kits based on comparison and internal validation studies completed at the Department of Forensic Sciences. The evaluation focuses on the ability to amplify blood and saliva samples from FTA cards, as well as buccal swabs, in the same 96-well plate using a single cycle number and one optimal injection time.

This presentation will impact the forensic science community by explaining how the internal validation and comparison of Promega® PowerPlex® Fusion and the Applied Biosystems® GlobalFiler™ Express Amplification Kits will educate attendees on the performance of both Polymerase Chain Reaction (PCR) amplification kits. This comparison highlights the key differences in the kit protocols and overall results. The optimization of three different types of reference samples into a single cycle number and one optimal injection time will assist the laboratory with manual sample throughput until a fully automated workflow can be established.

In 2010, the Federal Bureau of Investigation (FBI) Combined DNA Index System (CODIS) Core Loci Working Group identified reasons for expanding the CODIS Core Loci in the United States. Among the reasons is a need to increase international data compatibility and the power of discrimination in missing person cases.

Both the Promega® PowerPlex® Fusion and the Applied Biosystems® GlobalFiler™ Express PCR systems have adapted to the extension of the CODIS Core Loci by increasing their multiplex reactions to 24 loci. The PowerPlex® Fusion Amplification Kit is a five-dye system that allows for the amplification and fluorescent detection of the 13 core CODIS (United States) loci and the 12 core European Standard Set loci. The GlobalFiler™ Express Amplification Kit is a six-dye assay that targets 21 autosomal STR loci, one Y-STR locus, and one Y insertion/deletion locus. Both the PowerPlex® Fusion and the GlobalFiler™ Express Amplification Kits have been approved for the National DNA Index System (NDIS). Therefore, the evaluation of both kits is important in determining the best fit for each individual laboratory as these are implemented for DNA testing.

Sensitivity, precision, concordance, reproducibility, and contamination studies were completed as part of the internal validation of the PowerPlex® Fusion and the GlobalFiler™ Express Amplification Kits using the Applied Biosystems® 3500 Genetic Analyzer. In addition, sample preparation, injection time, analytical threshold, and stochastic threshold were determined to optimize the protocol for analysis of blood and saliva on FTA® cards and buccal samples. The goal of the validation was not only to ensure that both kits produced reliable and robust results, but to also optimize a single thermal cycling parameter and one optimal injection time for all three types of samples.

The internal validation determined that for the PowerPlex® Fusion Amplification Kit, optimal results for blood and saliva on FTA® cards, as well as buccal samples, can be obtained with 26 amplification cycles and 12 second injections on the Applied Biosystems® 3500 Genetic Analyzer. Alternatively, for the GlobalFiler™ Express Amplification Kit, optimal results for these three types of samples can be obtained using 25 amplification cycles and 15 second injections on the Applied Biosystems® 3500 Genetic Analyzer. The ability to amplify and inject blood and saliva from FTA® cards and buccal reference samples on the same 96-well reaction plate will assist the laboratory with manual sample throughput until a fully automated workflow can be established.

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#### **GlobalFiler™ Express, PowerPlex® Fusion, Direct Amplification**