B115 The New Kit on the Block: Optimization of the QIAGEN® Investigator® 24plex GO! Kit for Direct Amplification

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After attending this presentation, attendees will understand how to achieve a high first-pass success rate when performing direct amplification of 1.2mm punches from buccal samples collected on non-treated paper with the QIAGEN® Investigator® 24plex GO! Kit. Attendees will also understand that a high first-pass success rate is achievable with reduced reaction volumes in addition to the standard manufacturer's protocol.

This presentation will impact the forensic science community by demonstrating the feasibility of direct amplification with the QIAGEN® Investigator® 24plex GO! Kit for the processing of reference samples collected on non-treated matrices. Attendees will also be presented kit characteristic data including average peak heights and inter- and intra-color balance values.

Recent trends in sample processing for databasing and paternity purposes have moved toward direct amplification systems. DNA samples can be collected and stored on non-treated matrices, such as the Bode Buccal DNA Collector™ until sample testing is required. QIAGEN's® recently released direct amplification kit encompasses the Combined DNA Index System (CODIS) core loci, the European standard set markers, and additional loci including a Y-chromosomal Short Tandem Repeat (Y-STR) and quality sensors.

This presentation will describe the studies performed with the Investigator® 24plex GO! Kit to obtain optimal results from samples collected utilizing the Bode Buccal DNA Collector™, a non-treated matrix, at varying reaction volumes: 20µl (Full Reaction), 10µl (Half Reaction), and 5µl (Quarter Reaction). A total of 100 (n=100) self-collected samples, approximately 1.5 years old at the time of testing, were utilized in this experiment. These 100 samples were stored in a controlled micro environment (~20°C-25°C and <10% humidity). Slightly aged samples were chosen as they may be more representative of a routine databasing sample rather than a fresh sample collected a few days prior to testing.

This presentation will display the optimized procedures for cell lysis, reaction mix components, thermal cycling parameters, and 3500xL injection conditions. The manufacturer's recommended procedure for "other papers" or non-treated matrices did not include a cell lysis step. The use of this procedure resulted in a poor success rate with very few samples exhibiting called alleles. A modified procedure was developed in order to perform a direct amplification procedure with a 1.2mm punch from a non-treated matrix. 2µl of QIAGEN's® Investigator® STR GO! Lysis Buffer (designed for a swab protocol) was added to a 1.2mm punch contained in a 96-well reaction plate. After ensuring that the punch was submerged in the liquid, the reaction plate was placed on a heat block set at 95°C for five minutes in order to dry the punch. Failure to incubate the sample and dry the punch resulted in DNA profiles with a ski slope, and the complete dropout of the "S" internal quality sensor indicating inhibition.

The volumes of the amplification reaction mix components followed the manufacturer's recommendations for the full (20µl 27 cycles) reaction. Each component was proportionally reduced to create the half (10µl 26 cycles) and quarter (5µl 25 cycles) reaction mixes.

Capillary electrophoresis setup and run parameters were optimized to achieve consistent, reliable, and reproducible results. The manufacturer's recommendation indicated to add 12µl of formamide/Internal Lane Standard (ILS) mix containing 12µl of formamide and 0.5µl of BTO550 ILS per sample. This resulted in low overall ILS peak heights (~300-500 Relative Fluorescence Units (RFUs)). Multiple samples failed as relatively low pull-up peaks (~100-200 RFUs) caused significant sizing issues resulting in uninterpretable data. Optimization parameters, including the use of additional ILS and a standard amplification product dilution, will be discussed during the presentation.

These optimized procedures resulted in a first pass success rate of 93% for a full reaction (20µl). The highest first pass success rate (99%) was observed when utilizing the half reaction (10µl) protocol. In the full reaction, the "S" quality sensor displayed RFU values, on average, slightly higher than the "Q" quality sensor. In the half reaction, the opposite was observed.

Direct amplification of reference samples utilizing QIAGEN's® Investigator® 24plex GO! Kit can provide a time-efficient method for obtaining complete genetic profiles with a high first pass success rate. This presentation will demonstrate methods to increase direct amplification feasibility by decreasing overall costs per sample through the use of reduced volume reactions.

Direct Amplification, QIAGEN®, Buccal Sample

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