B146 Improving Processing Efficiency for Forensic DNA Samples

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After attending this presentation, attendees will be familiar with several approaches used to reduce sample processing time by ~33% for databasing reference samples, including extraction normalization, fast Polymerase Chain Reaction (PCR), and quicker genetic analyzer detection while maintaining high-quality profiles similar to those observed using current methods (i.e., >90% full profiles using ³100rfu threshold with 100% concordant alleles, comparable inter-locus peak balance, PHR ³50%, stutter £20%, pull-up £20%, and no –A).

This presentation will impact the forensic science community by sharing methodologies to significantly improve sample-processing efficiency without the need to purchase new instrumentation or costly reagents. These improvements are robust and can be implemented in a variety of laboratory settings.

The goal of this project was to reduce processing time without incurring significant added costs via a multi-layered overhaul undertaken in the following order: (1) develop a quicker detection method for the 3130*xl* Genetic Analyzer using an alternative polymer/array length combination; (2) develop fast PCR protocols for various Short Tandem Repeat (STR) primer sets using shorter amplification parameters, low volume reactions, and standard non-fast thermal cyclers; and, (3) normalize an extraction process using the ChargeSwitch® Forensic DNA Purification Kit such that a small range of yields are consistently obtained, thereby eliminating the need for quantification and dilution prior to amplification.

First, alternative polymer and capillary array length combinations were evaluated and compared to the standard POP-4™/36cm array detection method employed for STR detection on a 3130xl Genetic Analyzer. Detection time for standard 4-dye and 5-dye STR amplification kits was reduced to ~25 minutes using POP-6™/22cm array by modifying numerous injection parameters. STR profile quality was evaluated via concordance, dropout, peak height, resolution, ILS sizing quality/migration, pull-up, –A, and background noise. Validation for 4-dye and 5-dye amplification kits consisted of ~200 samples per dye set and studies included precision, resolution, injection time, and comparison to POP-4™/36cm array detection. POP-6™/22cm array detection achieved 0.5bp precision for up to 250bp fragments and 1bp resolution, which are the advertised specifications for POP-4™/36cm array detection.

Next, four fast PCR reagents were evaluated using the Identifiler® primer set, including AmpliTaq® Gold Fast PCR Master Mix, KAPA2G™ Fast Multiplex PCR Kit, SpeedSTAR™ HS DNA Polymerase, and Type-it® Microsatellite PCR Kit. Profiles were evaluated via concordance, dropout, peak height, inter- and intra-locus peak balance, stutter, pull-up, –A, specificity, and background noise. In addition to profile quality, amplification time, reagent cost, and ease of PCR setup were also taken into account. Using these criteria, KAPA2G™ Fast Multiplex PCR Kit was selected to develop and validate fast protocols for the Identifiler®, Identifiler® Plus, and PowerPlex® 16 HS primer sets, using reaction volumes ranging from 3µl to 6µl and two non-fast thermal cyclers (384-well Veriti® and 96-well GeneAmp® PCR System 9700) with amplification times ranging from 43 to 51 minutes. Fast PCR validations consisted of ~200 samples and studies included sensitivity, reproducibility, precision, stochastic, comparison to standard PCR, and contamination. Sensitivity ranged from 0.1875ng to 3.0ng, but optimal DNA input ranged from 0.375ng to 1.5ng for each primer set and amplification volume.

Lastly, extraction normalization for the ChargeSwitch® Forensic DNA Purification Kit included a reduction in the quantity of magnetic beads used per sample and targeted final concentrations of ~0.42ng/µl to 1.63ng/µl for 3µl amplifications and ~0.21ng/µl to 0.83ng/µl for 6µl amplifications. As a result, the need for quantification and dilution of each sample prior to amplification was eliminated. It should be noted that this approach is only acceptable for reference samples (see Standard 9.4 of the 2011 Federal Bureau of Investigation (FBI) Quality Assurance Standards). Validation of the new process (normalized extraction plus fast PCR amplification and POP-6™ detection) included >200 samples and studies included reproducibility, precision, comparison to the current process, and contamination. Profiles were evaluated as described above and were as good as those obtained using the current process.

Implementation of this or a similar approach reduces start-to-finish processing time to approximately a single workday. Furthermore, results from these studies indicate that a significant reduction in sample processing time is possible without the need to purchase costly instrumentation or reagents and will enable a laboratory to decrease their number of instruments needed to maintain their sample throughput or, alternatively, to increase sample throughput by maintaining the same number of instruments.

Forensic DNA, Fast PCR, Quicker 3130xl Detection