A79 Development of a Direct Amplification Method for Exemplar and Pseudo-Exemplar Reference Samples Using Identifiler® Plus

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The goal of this presentation is to explore methods of direct PCR amplification for processing exemplar and pseudo-exemplar reference samples.

This presentation will impact the forensic science community by evaluating methods of direct PCR amplification that eliminate the need for purification and quantitation, greatly reducing the time and cost for analysis, offering DNA analysts a more effective means of processing reference samples.

Pilot studies comparing the AmpFℓSTR® Identifiler® Direct PCR Amplification Kit and AmpFℓSTR® Identifiler® Plus PCR Amplification Kit were conducted to determine which amplification system best fits the needs of the Office of Chief Medical Examiner of New York City. The goal of these experiments was to establish a standard protocol that will account for variability of DNA amounts among reference samples.

Identifiler® Direct is a PCR amplification kit optimized for amplifying blood samples spotted onto FTA® cards that have not been extracted or quantified. Use of non-FTA® collection methods, according to Applied Biosystems (ABI), requires pretreatment with Prep-and-Go Buffer™. To potentially bypass this step, various sampling techniques and thermal cycling parameters were tested. The ABI GeneAmp® PCR System 9700 thermal cycler and the ABI 3130xl Genetic Analyzer were used for amplification and separation, respectively, and all data was analyzed with GeneMapper® ID v3.2.1. Initial results showed that partial profiles were obtained for buccal samples and non-FTA blood cards. These profiles displayed many N-bands. Due to the additional time required to process samples using the Prep-and-Go Buffer™, other less costly amplification kits were then considered.

Another method of direct PCR amplification utilizes Identifiler® Plus, which is optimized to overcome inhibition. The chemistry of the kit enables unpurified extracts to be directly amplified. Buccal swabs and blood spotted onto Whatman® non-FTA® paper were incubated in 0.2% Tween® 20, 0.1mg/mL Proteinase K, and 2.4% Trehalose in TE^{-4} for 30 minutes at 56°C followed by five minutes at 99°C. An aliquot of neat extract was directly amplified using a half reaction of Identifiler® Plus (5.0µL Reaction Mix, 2.5µL Primer Set). Experiments with various cutting sizes, extraction volumes, aliquots for amplification, and thermal cycling parameters were conducted using samples containing a wide range of DNA. In order to generate results for all samples, the 29-cycle protocol was implemented. In addition, the final elongation time was increased to 60 minutes to minimize N-bands observed at Amelogenin only. Full profiles were obtained for all samples tested with only 30% of the samples needing an additional injection on the 3130xl Genetic Analyzer at lower parameters due to oversized peaks.

Reproducibility, sensitivity, and stability were also evaluated. A large number of different donors were tested, each in duplicate on different days and using different instruments. To decrease the amount of DNA collected, donors were instructed to abbreviate the amount of time they swabbed their mouths. More controlled sensitivity studies with known amounts of DNA were also performed. In order to compromise buccal specimens, swabs from previously tested donors were stored under accelerated aging conditions. Although for most samples, protocol adjustments were unnecessary, in a few cases with very low yields of DNA, the amplification aliquot volume was increased.

Methods were also optimized for pseudo-exemplar samples such as bottles, cans, cups, straws, cigarette butts, and chewing gum. One uniform protocol that encompassed all of these sample types was developed with the exception of different-sized cuttings for each substrate. Preliminary results showed that over 98% of all samples yielded full profiles, with 15% requiring a second injection at either a higher or lower parameter on the 3130xl Genetic Analyzer.

In brief, implementing a short extraction step followed by direct amplification with Identifiler® Plus proved to be a cost-effective method to profile true and pseudo exemplar samples in a single day or less. For the overwhelming majority of samples, full profiles were generated using standard parameters. For a select number of samples, an additional injection on the 3130xl Genetic Analyzer with more or less sensitive parameters and/or increased template volume for amplification was required. In both cases, the initial STR results could be evaluated to accurately predict the additional step(s) needed to preserve both time and cost.

Direct Amplification, Exemplar, DNA Analysis

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