Evaluation of a novel approach to low-copy number (LCN) DNA methodologies for generation of short tandem repeat (STR) profiles

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After attending this presentation, attendees will be familiar with the relative impact of different approaches to low-copy DNA analysis on stochastic effects as compared to increased Polymerase Chain Reaction (PCR) cycle number protocols.

This presentation will impact the forensic science community by assessing alternative methods for analyzing low template DNA from evidentiary material that will allow for the generation of more complete STR profiles. With increased allelic recovery in addition to decreased stochastic events as compared to other LCN methods, an improved protocol for LCN samples will increase the ability of forensic laboratories to generate useful profiles.

Producing genetic profiles from samples with 100pg or less of template DNA, also known as LCN samples, is difficult using standard PCR methods. Modified sample preparation protocols which increase the cycle number used for amplification up to 34 cycles have been developed to improve profile recovery from these sample types; however, increasing the PCR cycle number produces allelic imbalance and drop out as well as other stochastically induced events, such as increased stutter ratios. Increasing the number of PCR cycles can result in either more complex mixtures due to the amplification of trace DNA that would not otherwise be detected or in the amplification of trace DNA contaminants introduced during sample processing that would not otherwise have been detected. These effects can complicate the interpretation of resulting STR profiles. The method assessed in this study seeks to increase the percent recovery of a genetic profile as compared to standard PCR methods without producing the increased stochastic effects observed with increased cycle number methods.

Dilutions of extracted reference quality DNA were prepared so that the maximum input volume for amplification (in this case 17.5µl) would result in the addition of the following amounts of DNA: 245pg, 122.5pg, 85.8pg, 42.9pg, 30.0pg, 15.0pg, 10.5pg, 5.3pg, 3.7pg, and 1.8pg. Autosomal STR profiles were developed from each dilution in the series by four different assay methods (five replicates each). Samples in treatment group one were amplified following the manufacturer's recommended protocol with 30 amplification cycles. Samples in treatment group two were amplified following the manufacturer's recommended protocol with the addition of four extra amplification cycles (34 amplification cycles total). Samples in treatment group three followed the manufacturer's recommended protocol with 30 amplification cycles except that 50µl of DNA extract was dried down and reconstituted in 17.5µl TE-4, thus resulting in a greater overall input of DNA. Samples in treatment group four followed the manufacturer's recommended protocol with 30 amplification cycles except that 50µl of DNA extract was added to a centrifugal filter and concentrated in 17.5µl TE-4, thus resulting in a greater overall input of DNA. All samples were amplified using an Applied Biosystems® GeneAmp® PCR

System 9700 using a Promega® PowerPlex® 16 HS System and analyzed using capillary electrophoresis on an Applied Biosystems® 3130 Genetic Analyzer. Resulting genetic profiles were compared based on percent recovery of donor alleles, stutter ratios, and allelic balance, as well as for allelic drop in.

Full DNA profiles were consistently obtained with as little as 15pg template DNA for dried down samples. Samples at the same initial concentration, 0.30pg/µl, only yielded 35.5%, 45.8%, and 29% of donor alleles for standard samples, increased cycle number samples, and samples concentrated with filters prior to amplification, respectively. When compared to samples with the same input of template DNA prepared following the other methods, dried down samples did not show significant increased stutter nor was any peak height imbalance or allelic drop-in observed; however, increased stutter, peak height imbalance, and drop-in was observed in those samples that underwent increased PCR cycle number. Several replicates of dried down samples even produced full profiles with only 10pg template DNA. Resulting data illustrates that concentrating extracts prior to amplification by drying them down is a method well suited for use in forensic biology laboratories when attempting to develop genetic profiles from low copy number samples.