THE BENEFIT OF USING THE QIAGEN MINELUTE® PCR PURIFICATION KIT FOR POST PCR CLEANUP ON LOW LEVEL DNA SAMPLES

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This study was conducted to demonstrate the benefits of including the Qiagen MinElute® PCR Purification Kit into the analysis scheme on forensic samples containing low quantities of DNA.

The study was designed to evaluate the similarities and differences in capillary electrophoresis signal detection when using the Qiagen MinElute® PCR Purification Kit on amplified DNA obtained from commonly used short tandem repeat (STR) commercial amplification kits. The STR amplification methods used in this study were the Applied Biosystems' AmpfℓSTR® Profiler Plus® kit, Cofiler® kit, Identifiler® kit, Minifiler™ kit, and the Yfiler® kit, and Promega's PowerPlex® 16 system, PowerPlex® Y system, and the PowerPlex® S5 system.

The Qiagen MinElute® PCR Purification Kit uses a silica membrane to bind DNA fragments ranging in size from 70 bp to 4 kb. While the DNA is bound to the membrane, impurities such as unwanted primers, salts, enzymes, unincorporated nucleotides, dyes, oils, and detergents flow through the column. Removal of these impurities ensures that more DNA is injected during the electrokinetic injection on the instrumentation, thus increasing the fluorescent signal intensity.

All single source samples were extracted using a standard organic extraction method and quantitated using the Applied Biosystems Quantifiler® Human DNA Quantification Kit on an Applied Biosystems 7500 Real-Time PCR System. Serial dilutions were prepared from DNA extracts at the following concentrations: 1.0, 0.5, 0.25, 0.125n, 0.0625, 0.03125, 0.015625, and 0.0078 ng and amplified with each STR multiplex following the manufacturer's specifications using an Applied Biosystems GeneAmp® PCR 9700 thermal cycler. A portion of the amplified DNA from these dilutions was purified using the Qiagen MinElute® PCR Purification Kit. Both purified and non-purified samples from each of the dilutions were separated and detected using the Applied Biosystems 3130*xl* Genetic Analyzer. The data were analyzed using GeneMapper® ID Software v3.2 using a threshold of 75 rfu. The fluorescent signals from both purified and non-purified samples were compared for all eight STR multiplexes to assess the change in fluorescent signal, stutter ratio, heterozygosity, and baseline noise.

Preliminary results of low level samples have shown increased signal levels after clean up using the Qiagen MinElute® PCR Purification Kit. This has consistently been shown during preliminary trials using Applied Biosystems' AmpfℓSTR® Profiler Plus® kit, Cofiler® kit, and Identifiler® kit. Results for the dilution series using all of the above listed amplification kits will be presented in the poster.

Crime laboratories have seen an increase in the submission of requests for analysis on evidentiary items with low quantities of DNA. This study demonstrated that the Qiagen MinElute® PCR Purification Kit consistently increased the fluorescent signal with the eight evaluated commercial STR amplification kits. This purification kit can be integrated into the laboratory process with little effort for method validation and at minimal cost. Integration of the Qiagen MinElute® PCR Purification Kit into the DNA analysis procedure is a simple, cost effective method that can be easily implemented by a crime laboratory to increase the overall sensitivity of their DNA analysis methods.