H20 Methods for Improvement of Allele Recovery With the GlobalFiler™ Assay

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After attending this presentation, attendees will have a better understanding of how the Polymerase Chain Reaction (PCR) cycle number, a 3500 instrument, and analysis parameters impact allele recovery with the GlobalFiler™ assay.

This presentation will impact the forensic science community by educating attendees of methods to improve their Short Tandem Repeat (STR) results with a challenged sample type.

Forensic casework samples, including bones, often have low DNA yields, degraded DNA, and PCR inhibitors. Newly developed STR genotyping kits including the GlobalFiler™ assay, are highly sensitive, robust to inhibitors and discriminating. This, combined with highly sensitive capillary Electrophoresis (CE) instruments, including the 3500 Genetic Analyzer, has resulted in useful STR profiles from previously untypeable forensic DNA samples; however, often, bone samples are still problematic and require modifications to both laboratory protocols and analysis procedures.

The GlobalFiler™ assay is the first 6-dye, 24-locus STR kit. It was designed with superior discrimination power, helping to enable forensic DNA labs to maximize information recovery and improve overall efficiency. The inclusion of ten mini-STRs maximizes results from degraded samples, such as bones. To increase the amount of useable information obtained with the GlobalFiler™ assay, forensics laboratories have options at multiple steps in the forensics workflow. During setup, the lab may increase the PCR cycle number from 29 to 30. Use of the 3500 CE instruments results in greatly increased sensitivity and reduced signal-to-noise ratios when compared with previous-generation CE instruments. Because of this, laboratories frequently use lower instrument and (dye-specific) Peak Amplitude Thresholds (PAT) to improve allele recovery.

To investigate the effects of PCR cycle number and reduced PAT on resulting STR profiles, three test sites processed both fresh and aged bone samples with commonly used sample preparation methods, followed by amplification with the GlobalFiler™ assay with 29 and 30 PCR cycles. For the majority of bone samples, less than 1ng DNA was added to the PCR reaction. The data were analyzed with both a 175 Relative Fluorescence Unit (RFU) threshold and a reduced PAT. This reduced PAT is specific to both the instrument and the dye channel.

The result of this analysis has been a comprehensive study comparing the effect of the PCR cycle number, PAT, and the 3500 CE instrument on the results obtained with compromised bone samples. As expected, the peak heights were increased by increasing the PCR cycle number. The effects of PCR cycle number and PAT on the number of alleles recovered wer substantial. The impact of these changes on the overall DNA profile and the potential analysis difficulties in distinguishing true signal from noise are also discussed.

DNA, Bone, GlobalFiler™