

## **METHODS FOR IMPROVEMENT OF ALLELE RECOVERY WITH THE GLOBALFILER ASSAY**

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Bone samples are one of the most difficult sample types encountered in forensics laboratories. The extracted DNA is often low quantity and degraded, making it difficult to obtain useful STR profiles. Recent short tandem repeat (STR) genotyping kits including the GlobalFiler™ assay are highly sensitive, robust to inhibitors and discriminating. The GlobalFiler™ assay contains all of the markers included in the major global databases, including 10 mini-STRs, designed to deliver optimized results with even highly degraded samples. These features of the GlobalFiler™ assay, combined with highly sensitive capillary electrophoresis instruments including the 3500 Genetic Analyzer has resulted in useful STR profiles from previously untypeable forensic DNA samples. However, bone samples are often still problematic because of DNA degradation and low levels of DNA. For these reasons, modifications to both laboratory protocols as well as analysis procedures may be required.

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 signal to noise ratios when compared with previous generation CE instruments. Laboratories frequently use lower instrument and dye specific calling thresholds to improve allele recovery.

To investigate the effects of PCR cycle number and reduced calling thresholds on resulting STR profiles, three test sites processed both fresh and aged bone samples with commonly used sample preparation methods and followed up with amplification with the GlobalFiler™ assay with 29 and 30 PCR cycles. The data from both PCR cycles was analyzed with both a 175 RFU threshold and a lower threshold, specific to both the instrument and the dye channel. The result of this analysis has been a thorough study comparing the effect of PCR cycle number, calling threshold and 3500 CE instrument on the results obtained with challenging bone samples. The number of alleles recovered and peak heights obtained were improved with these modifications. The impact of these changes on the overall DNA profile and potential analysis difficulties in distinguishing true signal from noise with compromised samples are also discussed.

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