B133 The Effect of Using an Extra Polymerase Chain Reaction (PCR) Cycle With GlobalFiler® When Amplifying Skeletal Samples

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After attending this presentation, attendees will understand the advantages and disadvantages of altering the suggested number of PCR cycles as a means to increase Short Tandem Repeat (STR) success for low template and/or degraded bone samples.

This presentation will impact the forensic science community by providing insight into the benefits and cautions when increasing the PCR cycle number using a common STR amplification kit in an attempt to improve the DNA typing results from low-template and/or degraded skeletal samples for human identification.

Using STRs to identify human remains has its challenges. By their nature, bone samples often contain low amounts of DNA template. Depending on the length of time and types of conditions they were exposed, bones are also often highly degraded and/or inhibited. When DNA degrades, it breaks into increasingly smaller fragments which may lead to amplification failure of the longer STR markers (>250bp). In addition, the low amount of DNA template (<100pg) available for amplification also often negatively affects STR results due to stochastic effects, such as allele and/or locus dropout or drop-in, allele imbalance, and increased stutter. A common approach to improving STR results from low-template samples (low copy number typing) is to increase the number of PCR cycles, but this method may also result in more complicated STR profiles.

In this study, various bone and tooth samples (N=24) were harvested from cremated, embalmed, buried, and decomposed human remains to compare the effects of increasing the number of PCR amplifications from 29 to 30 using the GlobalFiler® DNA Amplification Kit. Each bone was sanded, chipped, and cleaned with a wash series of 15% bleach, water, and ethanol before being powdered in a 6700 SPEX® freezer mill. DNA was extracted from 100mg of bone powder by following the recommended bone protocols using the QIAamp® DNA Investigator Kit or the PrepFiler® BTA Forensic DNA Extraction Kit (2x 50mg powder per sample – pooled elutes). The quantity and quality of each sample was determined using the Quantifiler® Trio DNA Quantification Kit, amplified using the GlobalFiler® PCR Amplification Kit (29 or 30 cycles), and detected using the 3500 genetic analyzer.

The results of this study demonstrated that when the number of PCR cycles was increased from 29 to 30, the number of alleles detected and the peak heights increased significantly (p <0.05). Although improvement in STR results was observed in almost all samples, the most notable improvement was observed in samples with the lesser DNA template (<0.05ng); however, no notable improvement in average heterozygote peak height balance was observed. While adding another PCR cycle increased the number of alleles detected and the average peak height, an increase in PCR artifacts was also observed in STR profiles generated using 30 cycles; however, this increase was minimal as only seven drop-in alleles, six off-ladder peaks, and one event of pull-up were observed in a total of 48 amplifications.

Overall, this research has shown that regardless of the DNA extraction method used in this project, consistently more alleles were recovered from bone and tooth samples with the addition of an extra PCR cycle using the GlobalFiler® PCR Amplification Kit with minimal adverse STR artifacts.

Skeletal Samples, Short Tandem Repeats, Low Copy Number