B65 Purification of Low Quality Human Remains Extract Using Centri•Sep Columns

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The goal of this presentation is to present to the forensic community a method for the removal of PCR inhibitors and other low molecular weight components from challenged human bone DNA extracts. The relative ease and efficacy of this technique facilitates incorporation into laboratory procedures for achieving reportable STR profiles from low quality samples.

When utilizing DNA techniques to identify human remains from mass disasters, the forensic analyst is exposed to many challenges; foremost of which is the compromised quality of recovered samples. The bones of mass disaster victims have often been subjected to various destructive environmental conditions causing degradation of nuclear DNA and introduction of PCR inhibitors. Since success in achieving complete STR profiles is severely affected by both degradation and inhibition, amplification may yield only alleles from the smaller STR loci if any loci are detectable at all. Partial profiles can pose a problem in creating statistically significant matches for identification purposes. This presentation will impact the forensic community and/or humanity by demonstrating how the use of Centri•Sep columns has proven successful in the purification of inhibited and degraded human remains samples, resulting in reportable profiles for samples that otherwise would have failed to yield a result.

To assist in identification efforts, The Bode Technology Group has incorporated the use of Centri?Sep columns (Princeton Separations) for pre-PCR purification of challenged DNA extracts from human bones. Centri?Sep allows for equilibration of multiplex STR profiles by averting preferential primer binding of smaller oligonucleotides and avoiding concentration of inhibitors. Although Centri?Sep columns were originally designed for post-sequencing dye terminator clean-up, the column's ability to remove 98% of salts and low-molecular-weight impurities increases the ratio of larger DNA fragments. The hydrated gel matrix in the column efficiently separates large molecules from small molecules via size exclusion chromatography. During this process, the centrifugal force promotes flow of the extract through a stationary matrix where some molecules will be retained within extremely small porous beads; while larger molecules consequently filtrate through the column to become the eluant. Use of Centri?Sep columns has proven successful in the purification of inhibited and degraded human remains samples, resulting in reportable profiles for samples that otherwise would have failed to yield a result.

The Bode Technology Group will present several examples of samples that yielded imbalanced and partial profiles when first amplified with the Applied Biosystems AmpF*l*STR® Identifiler® Kit (targeting between 1.0-1.5 ng template per reaction), yet after purification with the Centri•Sep column, high partial or full profiles were obtained under similar amplification conditions. Additionally, the electropherograms presented will depict cleaner profiles consisting of greater balance among all loci.

PCR Inhibitors, Bone Samples, Centri•Sep Columns

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