B87 Evaluation of a Modified DNA Extraction Approach for Improved Short Tandem Repeat (STR) Recovery From Severely Degraded Skeletal Elements

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After attending this presentation, attendees will learn best practices for processing DNA extracts from degraded skeletal remains where the goal is to increase the purity and concentration of extracts for the purpose of improving the likelihood that more complete STR profiles will be generated.

This presentation will impact the forensic science community, particularly laboratories working on missing persons cases, by presenting a new way to purify and enrich skeletal remains extracts for nuclear DNA (nucDNA) while minimizing the amount of Polymerase Chain Reaction (PCR) inhibitors that co-purify with the DNA.

Degraded skeletal remains generally contain limited quantities of nucDNA and thus, DNA-based identification efforts often target the mitochondrial DNA (mtDNA) control region due to the relative abundance of intact mtDNA as compared to nucDNA; however, in many cases the discriminatory power of mtDNA is inadequate to permit identification and STR analysis becomes essential. Unfortunately, commercial STR kits such as the AmpFlSTR® Yfiler® PCR Amplification kit, AmpFlSTR® MiniFiler™ PCR Amplification Kit, and PowerPlex® 16 HS PCR Amplification Kit require input DNA quantities greater than what is typically extracted from highly degraded bone samples. As a result, amplification is generally unsuccessful when following the manufacturer's recommendations.

In 2013, a Low Copy Number (LCN) Yfiler® protocol was adopted by the mitochondrial DNA section working on remains from unaccounted-for individuals from past conflicts at the Armed Forces DNA Identification Laboratory (AFDIL). This LCN protocol requires an increased amount of Taq polymerase and more cycles during PCR amplification. To be reported, an allele must be observed above stochastic threshold in at least two out of three or more independent amplifications. This protocol has increased the success rate for generating four or more alleles (the minimum number of alleles needed to report out a sample) to 40%. Unfortunately, in many cases, STR profiles remain incomplete.[1]

Currently, 0.2g to 0.25g of bone powder is digested with a demineralization buffer (0.5 M EDTA and 1% N-lauroylsarcosine) and proteinase K; concentrated with an Amicon® *Ultra-4* Centrifugal Filter Unit and purified twice with the MinElute® PCR Purification kit. Preliminary optimization experiments started with increasing the amount of bone powder to 0.5g or 1.0g (two or four times in amount) which resulted in a non-linear increase in DNA yield. Therefore, one possible explanation for the low DNA yield observed with the addition of increased amounts of bone powder is the binding competition between DNA and humic acid for the silica membrane of the MinElute® column. The goal of this project was to increase STR typing success rates by increasing the DNA concentration from bone extracts by finding a way to limit the effects of humic acid binding competition.[2,3]

Presented here is a protocol where multiple 0.2g to 0.25g bone powder samples were extracted, pooled, and concentrated in a way that limited humic acid competition for silica. The extracts were quantified and LCN Yfiler® amplified before and after pooling. Results show that pooling three or four extracts produces the most significant increase in alleles reported. This can also be seen in the table below.

Number of Extracts Pooled	Cases with Equal or Better Results	Total Number of Cases	Success Rate
Two	7	14	50%
Three	3	4	75%

Four	8	9	88.9%

References:

1. K.A. Sturk, M.D. Coble, S.M. Barritt, J.A. Irwin. Evaluation of modified Yfiler® amplification strategy for compromised samples, *Croat. Med. J.* 50 (2009) 228–238.
2. Irwin JA, Just RS, Loreille OM, Parsons TJ. Characterization of a modified amplification approach for improved STR recovery from severely degraded skeletal elements. *Forensic Sci Int Genet*. 2012 Sep;6 (5):578-87.
3. Harry, M., B. Gambier, Y. Bourezgui, and E. Garnier-Sillam. "Evaluation of Purification Procedures for DNA Extracted from Rich Organic Samples: Interference with Humic Substances." *Analusis* 27.5 (1999): 439-41.

LCN STR Amplifications, Skeletal Remains, Pooling