Y3 Evaluating a DNA Extraction Procedure for Skeletal Remains

Jacob D. Nead, James Creek, PA 16657; Christina Neal, MS, Signature Science, Charlottesville, VA 22911; David Russell, MS, Signature Science, Charlottesville, VA 22911*

Learning Overview: After attending this presentation, attendees will better understand the impact of detergents and proteinase on the efficacy of DNA recovery from skeletal remains.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing results that can be used as proofs of concept to inform laboratories on future steps toward the optimization of bone demineralization buffers and procedures with increasing efficiency.

Skeletal remains are an important source of evidence in a wide variety of forensic cases, including missing persons, mass tragedies, mass graves, and burned victims.[1] The development of an extraction protocol of DNA from skeletal remains would allow a laboratory to expand the range of evidence samples that can be analyzed in-house and thus broaden the range of casework that a laboratory can process. Due to the nature of skeletal evidence, mainly its persistence in harsh environmental conditions, this type of biological evidence often exhibits low-quantity and degraded DNA.[1] The goal of a bone extraction procedure is to recover high-quality DNA while limiting contamination of the sample with exogenous DNA and Polymerase Chain Reaction (PCR) inhibitors.

As part of an internship project, a literature search was conducted to identify the best path forward for the extraction of DNA from bone. Current literature offers a diverse catalog of extraction procedures with varying degrees of efficiency, effectiveness, sensitivity, and downstream application. The proposed procedure includes sample decontamination, total demineralization of the bone powder, concentration of the sample, and purification of the extract.[2] The general workflow of the proposed method is primarily adopted from the Armed Forces DNA Identification Laboratory (AFDIL) procedure, first published in 2007 and more recently expanded upon in a 2019 study.[3,4] Extracts were quantitated using the Investigator® Quantiplex® Pro kit, PCR amplified using the GlobalFiler™ kit, genotyped via the SeqStudio™ genomic analyzer, and analyzed with GeneMapper® ID-X software v1.6.

The goal of this study was to evaluate the efficacy of the proposed method and to consider the optimization of several aspects of the process. Focus was placed on the recovery of nuclear DNA for its diverse downstream applications and high power of discrimination. The method was evaluated based on DNA recovery, consistency of results, inhibition indices, degradation metrics, contamination, and Short Tandem Repeat (STR) profile quality. The effect of adding a detergent to the demineralization reaction was studied by comparing Ethylenediaminetetraacetic Acid (EDTA) buffers with and without a 1% concentration of the detergent, N-laurylsarcosine.[5] Additionally, various volumes of proteinase K spike-in were compared to assess its effect on DNA recovery.[3,4,6] Yield was observed in relation to variable sample input masses.

The method exhibited consistent recovery of DNA from skeletal samples with evidence of high reproducibility and average yields well above the target PCR input for amplification. Inhibition was observed to be negligible; however, the samples did exhibit elevated degradation indices. Degradation was further observed in the STR profiles with signal intensities decreasing from smaller targets to larger loci. The addition of N-laurylsarcosine as a detergent to the demineralization step was generally associated with improved DNA recovery. Similarly, the addition of 200µL of proteinase K was supported as the optimal volume of spike-in at inputs of 100 and 250 milligrams of bone powder. A preliminary trend was observed supporting greater DNA yield with larger sample inputs.

Future studies may investigate increasing DNA recovery to match input requirements for more diverse analyses such as Single Nucleotide Polymorphism (SNP) genotyping and whole genome sequencing. Additional consideration may be given to alternative detergents such as Sodium Dodecyl Sulfate (SDS) to evaluate the impact on the efficiency of DNA recovery. Several recent studies have also provided evidence of potential alternatives to the use of proteinase K as a protein denaturant. Trypsin, another broadly specific protease, has been shown as one alternative.[7] In another publication, the enzyme clostridiopeptidase A exhibited significant reduction in time required to totally demineralize bone samples.[8]

Reference(s):

1. Jon Davoren et al. Highly Effective DNA Extraction Method for Nuclear Short Tandem Repeat Testing of Skeletal Remains from Mass Graves. *Croatian Medical Journal*. 48: (August 2007): 478-485.
2. Procedures for Preparation and Extraction of Calcified Tissue Samples. *FBI DNA Procedures Manual,* (2015).
3. Odile Loreille et al. High Efficiency DNA Extraction from Bone by Total Demineralization. *Forensic Science International: Genetics.* 1 (March 2007): 191-195.
4. Suni Edson. Getting Ahead: Extraction of DNA from Skeletonized Cranial Material and Teeth. *Journal of Forensic Sciences.* 64 (July 2019): 1646-1657.
5. Nadin Rohland and Michael Hofreiter. Comparison and Optimization of Ancient DNA Extraction. *BioTechniques*. 42, no. 3 (March 2007): 343-352.
6. Stijn Desmyter and Christine De Greef. A More Efficient Extraction Method of Human Bone Resulting in Improved DNA Profiling. *Forensic Science International: Genetics.* 1 (August 2008): 24-25.
7. Piotr Skowron et al. An Alternative for Proteinase K-Heat-Sensitive Protease from Fungus *Onygena corvina* for Biotechnology: Cloning, Engineering, Expression, Characterization and Special Application for Protein Sequencing. *Microbial Cell Factories*, 19, no. 1, (June 2020): 135.
8. Richard Li. Application of Proteinases for DNA Isolation of Bone Specimens. *National Criminal Justice Reference Service*, (July 2009).

DNA Extraction, Skeletal Remains, Evaluation

*Presenting Author