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RESEARCH PAPER

Modified protocol for DNA extraction from degraded and partially burnt bone samples using the EZ1 automated DNA extraction system

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ABSTRACT

Background*: Recovering deoxyribonucleic acid (DNA) from degraded skeletal remains is a key challenge in forensic science. Factors such as thermal exposure, microbial activity, and ageing reduce DNA quality, hindering DNA profiling. This study aims to optimize protocols for DNA extraction from partially burnt, exhumed, and aged bones using the EZ1 automated system.* ***Methods****: Bone and tooth samples were cleaned thoroughly and dried before processing for extraction. Tooth or a cut portion of cleaned bone was finely powdered by grinding and decalcified using 0.5 M Ethylenediaminetetraacetic acid (EDTA). Proteinase K digestion was performed, and carrier Ribonucleic Acid (RNA) was added to enhance DNA yield. DNA extraction was carried out using the EZ1 Advanced XL system. Quantification was performed using the Quantifiler Trio Kit. STR profiling was conducted with the Investigator® 24plex QS Kit and analyzed via capillary electrophoresis.* ***Results****: The modified protocol enhanced both DNA integrity and consistency. Exhumed bones exhibited the highest DNA yield (0.258 ng/µl), followed by partially burnt bones (0.072 ng/µl) and aged bones (0.013 ng/µl). All samples yielded amplifiable STR profiles. The inclusion of carrier RNA and the use of automated extraction effectively minimized DNA degradation and contamination. Conclusion: The protocol proved effective in retrieving amplifiable DNA from degraded bone samples. Its success across various bone types highlights its applicability in forensic identification. The use of automation enhances both efficiency and reliability, making this method suitable for mass disaster scenarios and the analysis of historical remains.*

Keywords: *DNA extraction; STR typing; human identification; tooth; bone; capillary electrophoresis.*

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INTRODUCTION

In anthropological research and forensic casework, skeletal remains are vital sources of deoxyribonucleic acid (DNA). However, DNA extraction is often complicated by degradation resulting from exposure to high temperatures, post-mortem changes, and environmental conditions.[1] Various extrinsic and intrinsic factors such as temperature fluctuations, microbial activity, soil pH, and burial depth affect DNA preservation.[2] Burnt bones frequently undergo irreversible heat-

induced degradation, leading to significant DNA fragmentation.[3] In exhumed bones, high levels of microbial contamination can further hinder DNA recovery, with variability influenced by soil composition and moisture content.[4] Even when stored under controlled conditions, aged bones still undergo oxidative and hydrolytic damage over time.[5]

To address these challenges, various DNA extraction techniques have been developed. While traditional phenol-chloroform methods can yield high-quality DNA, they are time-consuming and involve hazardous chemicals.[6] Automated systems such as the EZ1 Advanced XL offer reliable alternative with minimal contamination risk.[7] This present study evaluates a modified DNA extraction protocol incorporating Ethylenediaminetetraacetic acid (EDTA) -based decalcification, Proteinase K digestion, and purification with automated method to optimize DNA recovery and mitigate degradation.

MATERIALS AND METHODS

Sample collection and preparation: Bone samples were part of forensic casework for this research study to represent different degrees of degradation. Three categories of bones were included;

Partially burnt bones: These samples exhibited partial calcination or charring due to fire, depending on the extent of heat exposure. Previous research indicates that temperatures exceeding 200°C can significantly degrade DNA, with complete destruction occurring at 500°C or higher.[8,9]

Exhumed bones: These bones showed evidence of microbial activity and soil-induced degradation. DNA degradation in such samples is heavily influenced by environmental factors such as temperature, moisture, and soil composition.[10]

Aged Bones: These skeletal remains were classified as aged based on prolonged post-mortem intervals. Studies have shown that mitochondrial DNA damage tends to persist longer than nuclear DNA in aged remains.[11]

All samples were thoughouly cleaned by removing outer adhered dried tissue or other material mechanically by using surgical blades followed by 70% ethanol-based washing procedures for surface decontamination, dried, and pulverized using a sterile stainless-steel grinder. A total of 200 mg of powdered bone from each sample was taken for further processing.

DNA extraction protocol:

Decalcification: To facilitate decalcification through calcium ion chelation, 200 mg of finely ground bone powder was placed in a sterile container and incubated at 37°C for three days in 700 µL of 0.5 M EDTA. The EDTA solution promotes efficient decalcification by binding calcium, aiding in the dissolution of the bone mineral matrix.[12] Previous studies on ancient and forensic skeletal remains have shown that decalcification enhances DNA release.[13]

Proteinase K digestion: On the fourth day, 20 µL of Proteinase K was added to the sample to degrade proteins and release DNA bound to the matrix.[14] The samples were incubated at 56°C for 24 hours to ensure complete digestion. Proteinase K is widely used due to its efficiency in breaking down histone proteins and facilitating DNA release.

Centrifugation and supernatant collection: Following digestion, the lysate was centrifuged at 8,000 rpm for 10 minutes. Then 400 µL of the supernatant, containing dissolved components and DNA, was carefully transferred into a fresh microcentrifuge tube.

Carrier RNA addition: To prevent the loss of low-concentration DNA, particularly in degraded samples, 2 µL of carrier RNA (1 µg/µL) was added.[15]

DNA purification method: DNA extraction was performed using the EZ1 Advanced XL automated system. The supernatant containing carrier RNA was loaded into the EZ1 instrument, and DNA was isolated following the manufacturer's Trace DNA extraction protocol.[16] The automated process minimised contamination and improved consistency.

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DNA quantification and analysis: Extracted DNA was quantified using the Quantifiler Trio Kit, which measured total DNA concentration (ng/µL), degradation index, and human DNA content. The degradation index was used to assess the extent of DNA fragmentation.

PCR amplification and STR profiling: Polymerase chain reaction (PCR) amplification was conducted using the Investigator® 24plex QS Kit, following the manufacturer's recommended protocol. In practice, in most of the cases, it is observed that the concentration of DNA extracted by this method is usually in the range to proceed for STR profiling with a full-volume PCR reaction without dilution of DNA. Capillary electrophoresis was performed on an ABI 3500 Genetic Analyser. Resulting STR profiles were analysed using GeneMapper® ID-X software. Both positive and negative controls were included throughout the process to validate the results and ensure quality assurance.

RESULTS

The modified methodology consistently produced high-purity DNA suitable for downstream applications, including PCR, STR analysis.

Table 1 DNA yield and degradation index in different bone types (ng/µl)

Bone Sample/Casework	Quantity (ng/µl)	Degradation Index
Partial burnt bone case	0.072315	1.988205338
Exhumed bone case	0.25882	1.851606727
Aged bone case	0.013796	2.494036436

Table 1 illustrates the DNA yield (ng/µL) and corresponding degradation index across different bone types, including burnt, exhumed, and aged samples. The results indicate that exhumed bones yielded the highest DNA concentration, followed by partially burnt and aged bones. This variation suggests that burial conditions may be more conducive to DNA preservation compared to thermal exposure or extended post-mortem aging.

DNA quality and quantity were evaluated using the Quantifiler Trio Kit in combination with the QuantStudio 7 Real-Time PCR System. The addition of carrier RNA significantly improved DNA recovery, particularly in burnt and exhumed bone samples. The use of the automated EZ1 extraction system reduced the risk of contamination and enhanced overall efficiency.

Further experiments demonstrated that, the process successfully recovered small DNA fragments for PCR and sequencing from partially burned bone as shown in **Figure 2** and **Figure 3**. The methodology successfully recovered DNA from partially charred bones exposed to heat in burning cases when the body was recovered from the crime scene, though with a lower yield.

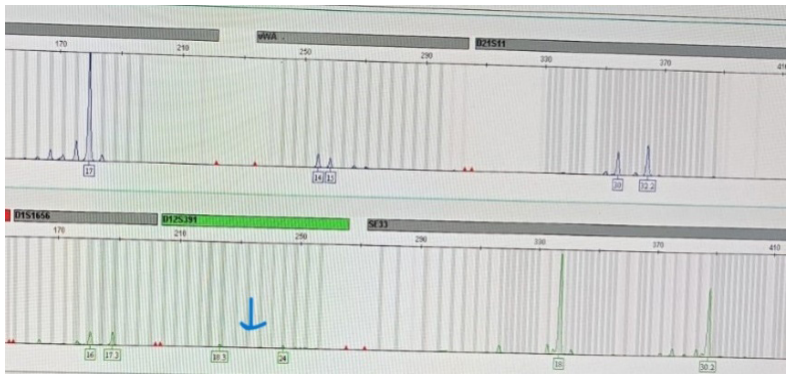

Figure 1 Result of modified DNA extraction method

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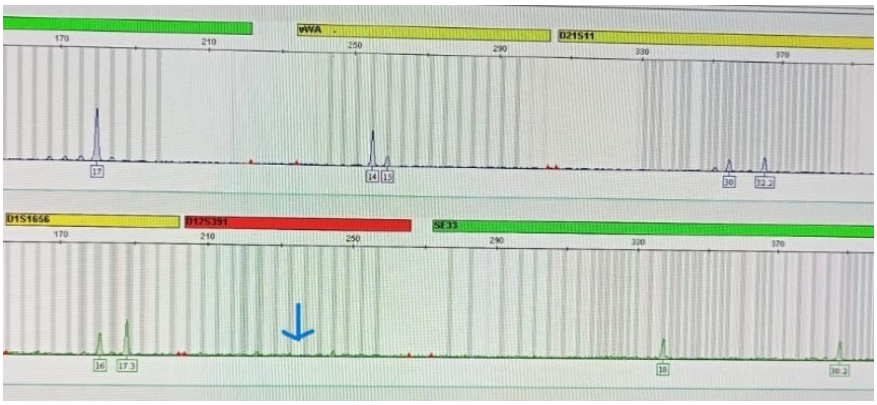

Figure 2 Result of original DNA extraction method

To validate the reliability of the results, several extractions from selected instances were repeated, resulting in consistent DNA recovery. Furthermore, negative controls were included during both the extraction and PCR amplification steps to ensure the absence of contamination, thereby reinforcing the credibility of the results.

DISCUSSION

The recovery of DNA from degraded skeletal remains presents a significant challenge in forensic identification. While genetic material may be retained in the skeletal bones, DNA in soft tissues is destroyed or denatured by severe heat insult.[3] Heat exposure in the presence of moisture causes the backbone's phosphodiester links to break, resulting in sheared DNA in bone cells.[17] Amplification of genetic markers may be challenging or impossible due to the high degree of degradation of DNA isolated from charred bone fragments. Furthermore, external DNA contamination of severely burnt bones is highly likely.[18] The credibility of ancient DNA research can be impacted by the scarcity, severe degradation, and high susceptibility to external contamination of ancient DNA isolated from bone remains.[19] DNA degradation is a powerful instrument in forensic research, notwithstanding its difficulties. For DNA degradation to be used effectively in criminal casework, it is imperative to comprehend the mechanisms and causes driving it. Degraded DNA analysis is becoming more and more reliable and useful due to continuous improvements in forensic methods and technology.[20]

This present study demonstrates that a modified extraction protocol, utilising the EZ1 automated system, enhances both the yield and quality of DNA obtained from partially burnt, exhumed, and aged bones. Among these sample types, exhumed bones produced the highest DNA concentration (0.258 ng/µL), likely due to more favourable burial conditions and the absence of thermal damage. Conversely, aged samples yielded the lowest DNA quantities and exhibited the highest degradation indices, which can be attributed to prolonged environmental exposure and progressive molecular degradation over time.

These findings are consistent with previous studies that highlighted the effectiveness of decalcification and enzymatic digestion in enhancing DNA recovery from bones.[21,22] The use of carrier RNA significantly improved DNA yield, especially in samples with limited nucleic acid preservation, corroborating the results of Dilley K et al.[15] The EZ1 system demonstrated efficiency and reduced contamination risks compared to manual extraction methods, in agreement with other automated extraction studies.[23] Although statistical analysis was not performed due to the casework-based nature of the study, reproducibility was confirmed through replicate extractions. In forensic contexts, particularly in mass disaster or cold

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case investigations, this optimised protocol offers a reliable solution for obtaining DNA profiles from compromised skeletal remains.

Microbial contamination in exhumed bone samples was effectively mitigated through ethanol-based washing steps, which enhanced the purity of extracted DNA. Ancient skeletal samples exhibited significant DNA fragmentation due to post-mortem degradation; the recovered material remained suitable for mitochondrial DNA analysis. This protocol demonstrates particular utility in forensic cases involving thermal exposure, soil contamination, and prolonged burial scenarios often associated with severely compromised skeletal remains.

Limitations and future directions: The study concentrated on certain deterioration conditions; future research should include varied environments. Further studies using a larger sample set and integrating next-generation sequencing could enhance interpretability and sensitivity. One limitation is the small number of samples in each category, which may affect generalisability. However, consistent STR profiling success across bone types validates the protocol's robustness.

CONCLUSION

The proposed procedure provides a dependable and fast way to extract DNA for STR profiling from severely damaged bone samples. It is useful in forensic, archaeological, and medicinal studies, considerably enhancing DNA recovery from difficult samples. Future research should look into its usefulness in a variety of environments and how it might be integrated with improved sequencing technology. The use of automated solutions, such as the EZ1 platform, improves reproducibility and efficiency in forensic casework.

Author declaration: There is no conflicting interest in this study.

Ethical clearance: This is based on samples received for analysis of casework, with prior consent of the donor. Modified protocols were applied to get better results. Further, this paper has analysed the data that has already been generated in the CFSL centre as per the protocol.

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