Forensic Science International: Genetics Supplement Series 8 (2022) 85–87

Contents lists available at ScienceDirect

Forensic Science International: Genetics Supplement Series

journal homepage: www.elsevier.com/locate/fsigss

# Recovery of DNA from bone without demineralization

Sasitaran Iyavoo [a,b], William H. Goodwin [a,*]

[a] School of Natural Sciences, University of Central Lancashire, Preston, United Kingdom
[b] School of Chemistry, College of Science, University of Lincoln, Lincolnshire, United Kingdom

ARTICLE INFO

*Keywords:*
DNA extraction
Bone
Decalcification
Demineralization
Human identification
Forensic

ABSTRACT

This study assessed the performance of five different DNA extraction methods for the recovery of DNA from bone: ChargeSwitch® gDNA Plant Kit, DNA IQ™ System Kit, DNeasy® Blood & Tissue Kit, PrepFiler® BTA Forensic DNA Extraction Kit and phenol-chloroform-isoamyl alcohol. DNA was extracted from pig rib and femur bones that was fresh, had undergone surface decomposition for three months, and had undergone surface decomposition for one year. Extracted DNA was analyzed using real-time PCR and amplification of an in-house PCR multiplex that assessed the quality and quantity of DNA and for the presence of inhibitors. The phenol-chloroform-based method consistently yielded the highest amounts of DNA and DNA IQ the lowest; however, all methods produced relatively high yields of DNA from both pig rib and femur samples that could be amplified without any detected inhibition. The data demonstrate that with reasonable quality bone samples any of the tested methods can isolate DNA that can be successfully analyzed. The effective use of internal PCR controls is also demonstrated.

## 1. Introduction

Bone samples represent one of the most challenging sample types for forensic genetics. The hard nature of bones necessitates extra steps compared to the cellular material and soft tissues, and additional challenges can present in the form of PCR inhibitors that can co-extract with DNA.

A body of work has demonstrated the advantages of demineralization when extracting DNA from bone samples that have been exposed to environmental insult, and often contain low amounts of highly degraded DNA [1]. However, the extra processes involved in demineralization add to the time and cost of processing samples, and in many cases, when working with samples that are in a relatively good condition, are unnecessary.

## 2. Materials and methods

### 2.1. Samples and preparation

Pig bone (femur and rib) were used in this study as a model for human bones. They were recovered from animals exposed to the environment for 0, 3 and 12 months at the University of Central Lancashire's TRACES facility (Taphonomic Research in Anthropology: Centre for Experimental Studies) (UK).

Where relevant soft tissue/marrow and algae/bacteria on the bone was removed using scalpels, dental burrs, and abrasive paper; the bones were then soaked in 5% sodium hypochlorite for 15 min, rinsed with water and air-dried. Portions of each bone weighing between 1 g and 2 g were prepared and pulverized under liquid nitrogen.

### 2.2. DNA extraction

Samples were extracted using ChargeSwitch® gDNA Plant Kit (Thermo Fisher Scientific), DNA IQ™ System Kit (Promega), DNeasy® Blood & Tissue Kit (Qiagen), PrepFiler® BTA Forensic DNA Extraction Kit (Thermo Fisher Scientific) and a conventional phenol-chloroform method, according to the manufacturer's instructions and published methods [2]. Approximately 250 mg of pulverized bone was used for each sample. The lysis step was carried out on 50 mg and was repeated five times for each sample. Lysed samples were pooled and concentrated using an Amicon 30 kDa filtration unit (Merck Millipore) before DNA extraction. Extractions were carried out in triplicate and the final elution volume was set at 100 µl in each extraction.

* Corresponding author.
*E-mail address:* WHGoodwin@uclan.ac.uk (W.H. Goodwin).

https://doi.org/10.1016/j.fsigss.2022.09.031
Received 18 September 2022; Accepted 28 September 2022
Available online 13 October 2022

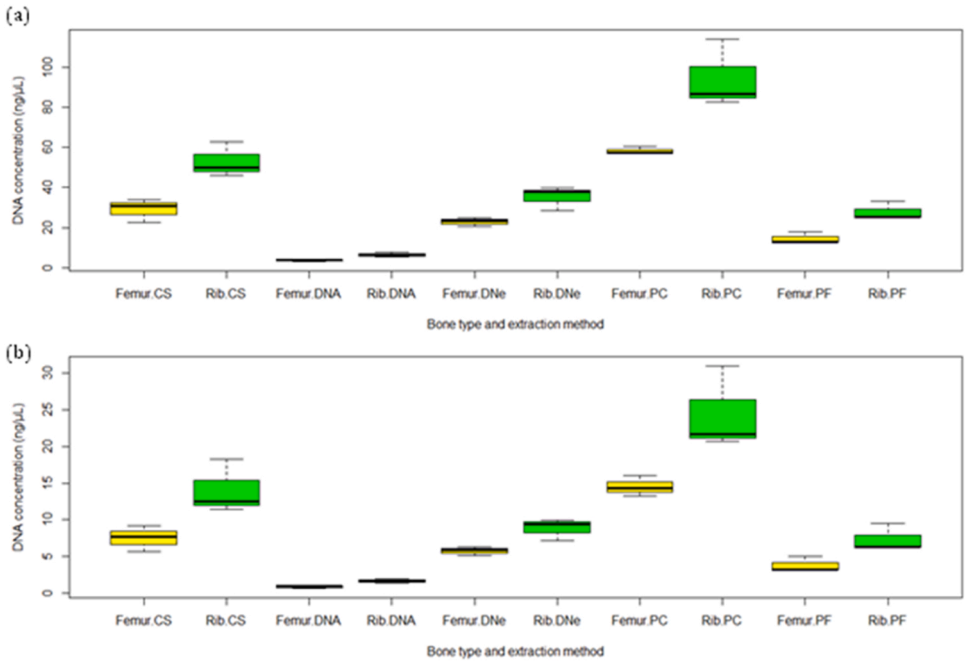


**Fig. 1.** Boxplots showing the recovery of DNA extracted from rib and femur bones recovered from a) animals that had been exposed to the environment for 3 months, and b) animals that had been exposed to the environment for 1 year. 250 mg of starting material were used. CS.: ChargeSwitch, DNA: DNA IQ, DNe: DNeasy, PC: Phenol-chloroform, PF: PrepFiler. Each sample type and method were repeated three times.

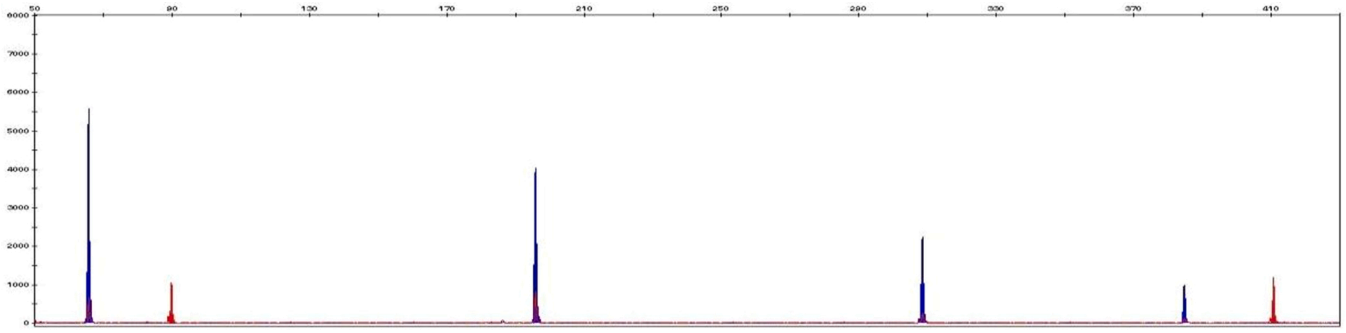


**Fig. 2.** Example of an electropherogram generated from extracted DNA of rib bone samples recovered from an animal that had been exposed to the environment for 1 year. The blue peaks correspond to fragments of 70 bp, 194 bp, 305 bp and 384 bp. In addition, two internal amplification controls (IACs) of 90 bp and 410 bp, were included and can be seen as red peaks – both IAC fragments have amplified which indicates that no PCR inhibitors are present.

### *2.3. DNA quantification and amplification*

The extracted DNA samples were quantified with GoTaq® qPCR Master Mix (Promega) amplifying a 70 bp target using Applied Biosystems® 7500 Real-Time System. Primers used were designed to amplify both human and porcine DNA: F-5′-CCTCAAAGTCATGGGCAGC-3′ and R: 5′-GACTCTCCAGGTCAGTAGG-3′. The standard DNA dilution series was made using control DNA 9947 A (Thermo Fisher Scientific).

Reactions comprised 6.25 µl 2x GoTaq® qPCR Master Mix, 4.75 µl $H_2O$, 0.50 µl primers and 1.00 µl template DNA. The thermal cycler conditions were: 95 °C for 2 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min

The DNA concentration for each sample was estimated in ng/µl. All DNA extracts were amplified using an in-house multiplex (4-plex & Internal Amplification Controls) [3].

## 3. Results and discussion

A total of 30 extractions were undertaken for each of the three timepoints, with each condition tested in triplicate. DNA could be recovered from all pig bones with all methods, however, clear differences existed between the methods in terms of the quantity of DNA recovered (Fig. 1). Phenol-chloroform-isoamyl alcohol extraction method consistently performed better than other extraction methods in terms of quantity of DNA extracted; this was followed by ChargeSwitch® gDNA Plant Kit, DNeasy® Blood & Tissue Kit, PrepFiler® BTA Forensic DNA Extraction Kit and the DNA IQ™ System Kit. The results for time zero were very similar to the 3-month time point shown below.

Despite the varying amounts of DNA extracted, amplification was successful for all DNA extracts, with fragments amplified between 70 bp and 384 bp; no inhibition was detected in any of the extracts (Fig. 2). Inhibition may present more of a problem when processing buried bone, which, depending on the characteristics of the soil, is more likely to accumulate PCR inhibitors.

## 4. Conclusions

The data presented illustrates that several methods are suitable for recovering DNA from bone that is not highly degraded. With such

samples demineralization is not necessary, allowing quicker processing of samples in comparison to methods that incorporate demineralization or total demineralization. The samples used in this study were aged for up to one year and therefore further work is required to evaluate the suitability of not incorporating a demineralization step in samples that show higher levels of degradation.

## Conflict of interest statement

The authors declare no conflict of interests.

## Acknowledgments

This work was supported by a grant from the Public Service Department of Malaysia.

## References

[1] O.M. Loreille, T.M. Diegoli, J.A. Irwin, M.D. Coble, T.J. Parsons, High efficiency DNA extraction from bone by total demineralization, Forensic Sci. Int. Genet. 1 (2) (2007) 191–195.
[2] S. Iyavoo, S. Hadi, W. Goodwin, Evaluation of five DNA extraction systems for recovery of DNA from bone, Forensic Sci. Int. Genet. Suppl. Ser. 4 (1) (2013) e174–e175.
[3] M.S. Nazir, S. Iyavoo, S. Alimat, N. Zahra, S.H. Sanqoor, J.A. Smith, C. Moffatt, W. Goodwin, Development of a multiplex system to assess DNA persistence in taphonomic studies, Electrophoresis 34 (24) (2013) 3352–3360.