Benchmark

BioTechniques

A method for the temperature-controlled extraction of DNA from ancient bones

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ABSTRACT

Contamination with microbial and other exogenous DNA poses a significant challenge in the generation of genome-wide sequence data from ancient skeletal remains. Here we describe a method for separating ancient DNA into multiple fractions during DNA extraction by sequential temperature-controlled release of DNA into sodium phosphate buffer. An evaluation of the effectiveness of the method using a set of three ancient bones resulted in between 1.6- and 32-fold enrichment of endogenous DNA compared with regular DNA extraction. For two bones, the method outperformed previous methods of decontaminating ancient bones, including hypochlorite treatment, which resulted in near-complete destruction of DNA in the worst-preserved sample. This extraction method expands the spectrum of methods available for depleting contaminant DNA from ancient skeletal remains.

METHOD SUMMARY

The authors present a decontamination method for poorly preserved ancient bones that relies on a temperature-controlled sequential release of DNA in sodium phosphate buffer, followed by DNA extraction and single-stranded library preparation.

KEYWORDS:

ancient DNA ● archaeological material ● contamination removal ● endogenous DNA ● sequential DNA extraction

The analysis of ancient DNA provides direct means for assessing the genetic history of past species and populations. One of the major technical challenges associated with the retrieval of DNA from bones and teeth, which are the most commonly used sources of ancient DNA, is contamination with microbial and modern human DNA. Contaminant DNA often constitutes more than 99% of the DNA that is recovered [1], making the generation of genome-wide sequence data unaffordable in many cases, and complicating the identification of sequences from endogenous ancient molecules [2]. This problem can sometimes be alleviated by targeting parts of the skeleton that contain larger proportions of endogenous DNA, most notably the petrous bone or tooth cementum [3–5]; however, these skeletal elements are not always present in the fossil record or may not be available for sampling due to their value for morphological studies.

Alternatively, methods have been developed that aim to remove microbial and human DNA contamination prior to DNA extraction, exploiting the fact that endogenous ancient DNA tends to be preserved deeper inside the bone or tooth matrix than contaminant DNA. These methods include the predigestion of sample material with a lysis buffer containing ethylenediaminetetraacetic acid (EDTA) and proteinase K [6], and pretreatment of the sample powder with sodium phosphate buffer at room temperature [7]. Both methods promote the release of surface-exposed DNA prior to full digestion of the bone or tooth matrix, either by mild degradation of the sample matrix or by competition between free phosphate and the negatively charged DNA backbone for binding to calcium ions in hydroxyapatite, the main inorganic component of bones and teeth. A third method, pretreatment with sodium hypochlorite (bleach) solution [7–9], follows a similar principle but leads to oxidative degradation, and hence the irreversible loss of surface-exposed DNA [10], which usually includes a substantial proportion of endogenous DNA [7].

Bleach pretreatment has been particularly effective in reducing microbial and modern human contamination [7], in some instances increasing the proportion of endogenous DNA more than 160-fold [11]. However, due to its aggressive nature, bleach pretreatment is unsuitable for severely degraded bones and teeth that contain only trace amounts of endogenous DNA. These include, for example, the approximately 430,000-year-old hominin remains from Sima de los Huesos in Spain [12,13], for which only mild (and less effective) decontamination with sodium phosphate buffer was found to be suitable [13], thus limiting the amount of information that could be retrieved from this and other very poorly preserved material.

The authors report a decontamination method for ancient skeletal remains [14] that builds on a gradual release of DNA from sample powder using a series of incubation steps in sodium phosphate buffer at temperatures increasing from room temperature to 90°C. The temperature gradient is followed by a room-temperature incubation of the sample powder in a digestion buffer containing EDTA and proteinase K, which releases DNA that has been trapped deep enough inside the matrix to escape exposure to sodium phosphate. Using
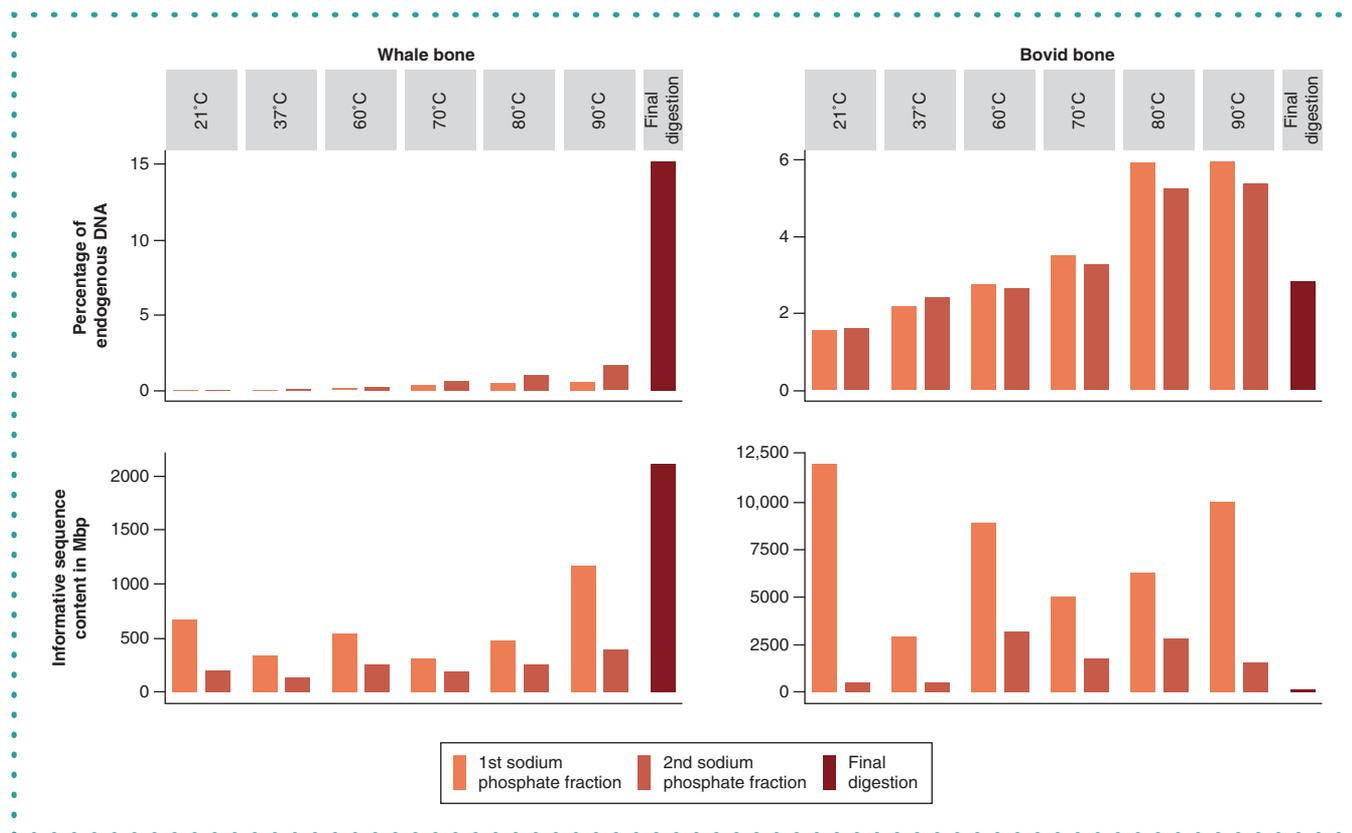
Figure 1. The relative (top) and absolute (bottom) quantities of endogenous DNA recovered in the phosphate fractions and the final lysate; absolute quantity of endogenous DNA is expressed in mega base pair (Mbp) coverage of the nuclear genome.

this method, DNA can be recovered from all sodium phosphate fractions as well as the digestion buffer using a silica-based method, which, although optimized for the recovery of extremely short double-stranded DNA fragments [15,16], also recovers single-stranded DNA arising from heat denaturation (Supplementary Figure 1). The total yield of endogenous ancient DNA (informative sequence content [17]) and its proportion in the DNA extract are then determined using single-stranded DNA library preparation [18,19] and shallow shotgun sequencing, to identify the best fraction for deeper sequencing.

In a proof-of-principle experiment, the authors applied this method to two late Pleistocene bones: a whale bone from the bottom of the North Sea and a bovid bone from Denisova Cave in southern Siberia, Russia. Samples of bone powder were obtained from the two specimens by drilling (77 and 53 mg, respectively) and subjected to serial 15-minute incubations in 0.5 ml sodium phosphate buffer (0.5 M sodium phosphate buffer; pH: 7.0; 0.1% Tween 20) at 21, 37, 60, 70, 80 and 90°C, respectively. Two incubation steps were performed at each temperature, followed by one wash in Tris-Tween buffer (10 mM Tris-HCl; pH: 8.0; 0.1% Tween 20) to reduce the carryover of DNA between fractions. Sequence data obtained from the sodium phosphate fractions show an increase in the percentage of endogenous DNA with increasing temperature for both bones (Figure 1 & Supplementary Table 1). This indicates temperature-dependent differences in the release of contaminant and endogenous DNA, with the largest amount of DNA being released in the first of the two incubation steps at each temperature. For the whale bone, the final digestion yielded the highest quantity and proportion of endogenous DNA. This contrasts with the bovid bone, where substantial quantities of endogenous DNA were released into all sodium phosphate fractions, with relatively little DNA being recovered from the bone matrix in the final digestion step. These results suggest that serial temperature-controlled sodium phosphate pretreatment enables the recovery of DNA fractions that differ substantially in their content of endogenous and contaminant DNA, and the patterns of DNA release vary greatly among samples.

To test whether the gradual DNA extraction method provides advantages over regular DNA extraction and previously described de-contamination methods (i.e., predigestion [6], room-temperature phosphate treatment and bleach treatment [7]), the authors applied all methods to a set of three samples. These included the whale and bovid bones used in the previous experiment, as well as a bone fragment from a 230,000-year-old straight-tusked elephant from Weimar-Ehringsdorf [20], which previously yielded a complete mitochondrial genome sequence, but no useful nuclear genomic data due to its poor state of preservation [21]. Approximately 550 mg of bone powder was obtained from each specimen by drilling, thoroughly mixing, and dividing into 15 subsamples of roughly equal weight (~50 mg) to allow triplicate measurements for each method. To reduce the workload associated with gradual DNA extraction, serial phosphate buffer
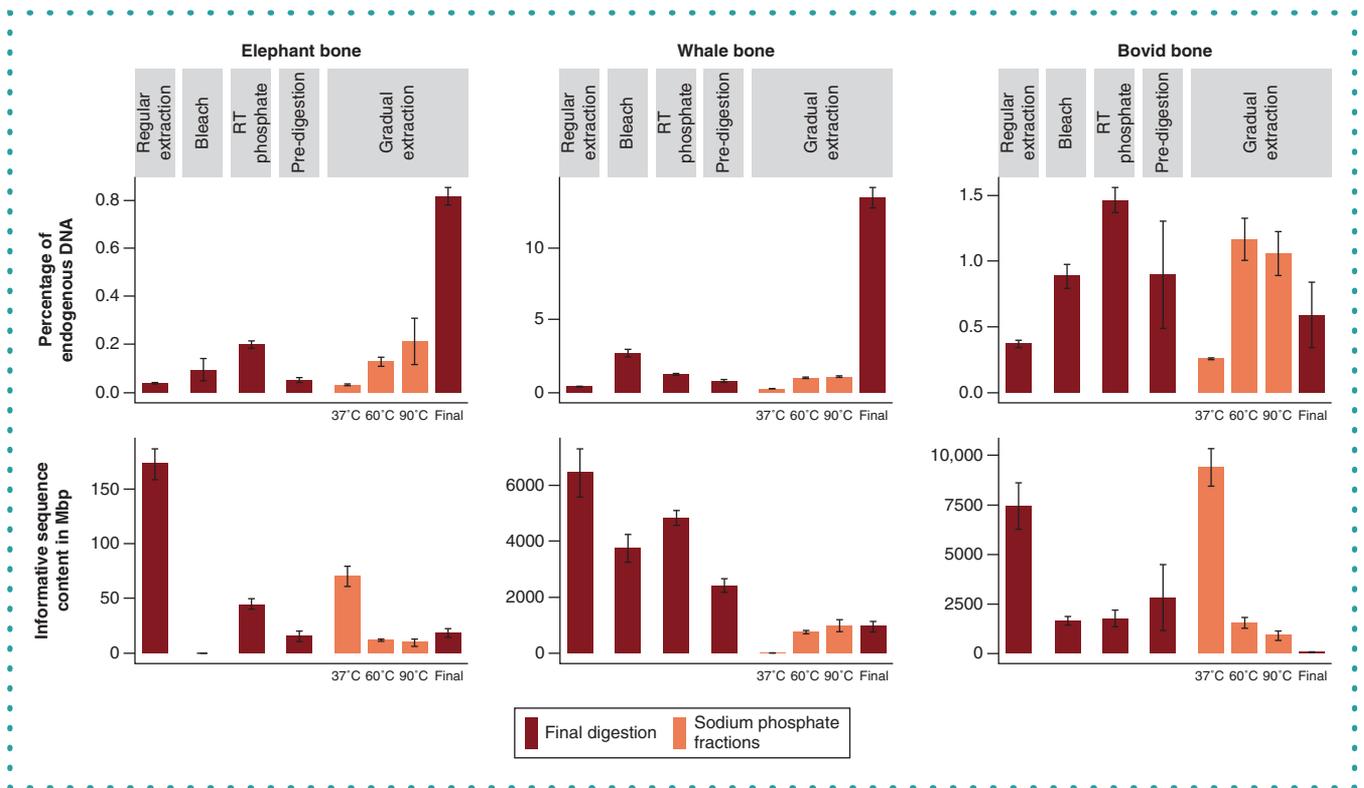
Figure 2. Comparison of gradual DNA extraction with other decontamination methods and regular DNA extraction using three ancient bone samples. Shown are the relative (top) and absolute (bottom) quantities of endogenous DNA recovered. Absolute quantity of endogenous DNA is expressed in mega base pair (Mbp) coverage of the nuclear genome. Error bars denote one standard deviation.

incubations were carried out at 37°C, 60°C and 90°C, thereby eliminating half of the temperature steps performed in the first experiment (see Supplementary Information for details). In addition, we increased the number of incubation steps at each temperature to three, but extracted DNA only from the first fraction, which was found to be the richest fraction in the first experiment.

All three previously described decontamination methods as well as the gradual extraction method yielded DNA extracts with a higher percentage of endogenous DNA compared with regular DNA extraction (Figure 2 & Supplementary Table 2). For the whale and elephant bone, gradual DNA extraction achieved the greatest enrichment of endogenous DNA, by a factor of 32 (0.42–13.43%) and 20 (0.04–0.82%), respectively. For both samples, the highest percentage of endogenous DNA was obtained in the final digestion of the bone material. For the bovid bone, decontamination was less effective with all methods, leading to enrichment factors between 1.6 and 4.0. The highest percentage of endogenous DNA was obtained with the room-temperature sodium phosphate decontamination method, and the second highest with gradual DNA extraction in the 60°C fraction.

All methods led to a reduction in the total yield of endogenous DNA compared with regular extraction, by between 41.5 and 99.9% with bleach pretreatment, 25.3 and 76.0% with room-temperature sodium phosphate pretreatment, 61.8 and 90.8% with predigestion, and 79.2 and 89.0% with the gradual extraction method, when considering the fraction yielding the highest percentage of endogenous DNA. Although this fraction contains only a comparatively small fraction of the endogenous DNA released from the sample material, gradual DNA extraction prevents the near-complete loss of molecules that is occasionally observed with bleach pretreatment, such as in the elephant bone. The latter observation highlights the incompatibility of bleach pretreatment with decontamination of very poorly preserved material.

In summary, though the experiments reported here include a small number of samples, data showed that gradual DNA extraction can, in principle, outperform bleach pretreatment and other decontamination methods, including when applied to severely degraded material. Comparisons across larger sample sets are needed to determine whether this improvement is consistently observed and large enough to make the method a primary choice for decontaminating ancient bones and teeth; or, whether the additional experimental efforts involved in making multiple DNA extracts and libraries from each sample (as well as the strict requirement for single-stranded library preparation) are only warranted for very poorly preserved material or when other decontamination methods have failed. However, given the sample-dependent variation in results observed with this and previous methods, it is unlikely that a single decontamination method will ultimately prove to be the best option in all cases and multiple approaches may often have to be evaluated. A particular advantage of gradual DNA extraction over bleach treatment is that it does not lead to the destruction of endogenous DNA. The method

may therefore be suitable for combining DNA extraction and decontamination for material that is too precious for repeated sampling. Finally, given that at neutral pH sodium phosphate buffer releases DNA by competing for binding sites rather than degrading the mineral or organic components of the bone matrix, it is conceivable that the method presented here may also be suitable for nondestructive DNA extraction from skeletal remains and other archaeological material if the final digestion step is omitted.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.future-science.com/doi/suppl/10.2144/btn-2021-0025

Author contributions

E Essel, P Korlević and M Meyer planned the study. E Essel and M Meyer designed the experiments. E Essel performed the experiments. E Essel and M Meyer analyzed the data and wrote the manuscript.

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Financial & competing interests disclosure

Ethical conduct of research

This study did not involve ethically sensitive material or analyses. All samples used in this study were recovered from abundant sources and, due to their limited value for other research, could be devoted to technical experiments. The bovid bone fragment was recovered during excavation at Denisova Cave, the dolphin and straight-tusked elephant bone fragments were obtained as fishery bycatch and during mining, respectively.

Data sharing statement

All numbers underlying the analyses presented are available in Supplementary Tables 1 & 2. Raw sequence data are available upon request.

Open access

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