Chapter 2

Pretreatment: Removing DNA Contamination from Ancient Bones and Teeth Using Sodium Hypochlorite and Phosphate

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Abstract

DNA isolated from ancient bones and teeth comprises a mixture of microbial contamination and DNA from the organism under study. In addition, analyses of ancient human remains are often complicated by contamination with present-day human DNA, which can be introduced during excavation and subsequent handling of the specimens. In most cases, the relative abundance of contaminant DNA is much greater than that of the target organism. Here we present two techniques for reducing the proportion of contaminant DNA in bones and teeth. The first and most efficient technique uses a sodium hypochlorite (bleach) pretreatment to destroy contaminant DNA that may be bound or otherwise attached to the surface of bone/tooth powder. The second, less destructive pretreatment uses a phosphate buffer to release surface-bound DNA.

Key words Ancient DNA, DNA contamination, Pretreatment, Sodium hypochlorite (bleach), Sodium phosphate, DNA extraction

1 Introduction

Sodium hypochlorite (bleach) pretreatment of bones or bone powder [1, 2] is one of the most common methods for removing DNA contamination in forensic research and has been used occasionally to decontaminate samples for ancient DNA research [3–5]. In a recent study [6], we investigated the effect of bleach pretreatment on bone or tooth powder from 15 ancient specimens. We found that bleach pretreatment increased the proportion of endogenous sequences (sequenced DNA fragments that align to a reference genome) generated from DNA extracts by a factor of 4.6 on average (Fig. 1). We also observed a significant reduction of human contamination after bleach pretreatment in some, but not all, samples. However, this decrease of contaminant DNA came at a cost, as the complexity of endogenous DNA in pretreated libraries was reduced by 63% on average, indicating that bleach

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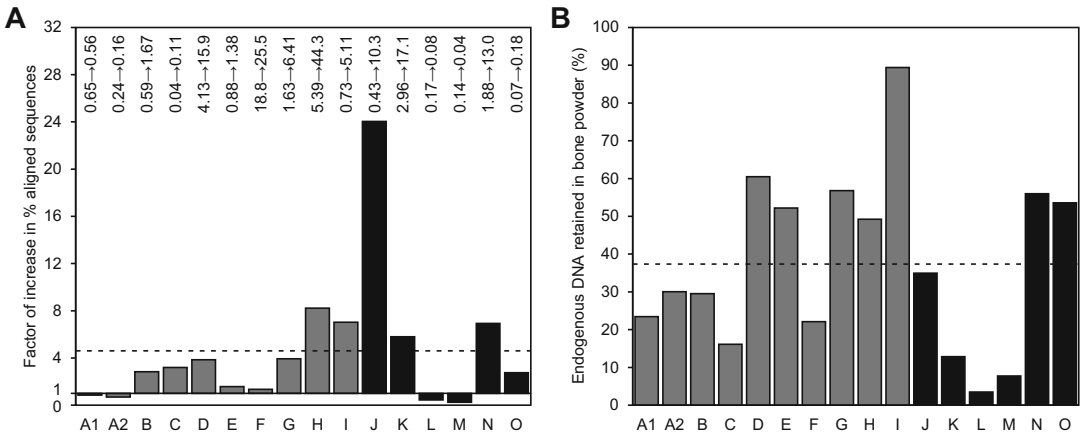

Fig. 1 Effect of bleach pretreatment on nine animal (A–I in gray) and six Neanderthal samples (J–O in black). (**a**) Increase in proportion of sequences that align to the reference genome relative to untreated control, with absolute percentages denoted above bars. (**b**) Proportion of endogenous DNA retained in the bone/tooth powder compared to the untreated control. Averages are denoted by horizontal dashed lines

pretreatment also removed a substantial fraction of endogenous DNA (Fig. 1).

Because bleach pretreatment may in some cases be overly destructive, we also explored the utility of a second decontamination approach that uses a milder phosphate buffer to release surface-bound DNA. This approach relies on the competition between free phosphate ions and phosphate groups in the DNA backbone for binding to hydroxyapatite [7, 8], the major inorganic component of the bone/tooth matrix. Occasionally, phosphate buffers have also been used as a means to recover ancient DNA during DNA extraction [9, 10]. In our study [6], pretreatment with phosphate led to a smaller but more consistent increase in the proportion of aligned sequences (2.0-fold on average) and a smaller loss of endogenous DNA (37% on average) than did pretreatment with bleach (Fig. 2). However, phosphate pretreatment proved less effective than bleach pretreatment in removing human contamination.

Since DNA is recoverable from the phosphate buffer if necessary, phosphate pretreatment can be used to reduce microbial contamination in bones and teeth even when samples are small or rare. Bleach pretreatment is more effective in removing both microbial and human contamination but may lead to a significant loss of endogenous DNA, making the procedure too risky when specimens cannot be resampled.

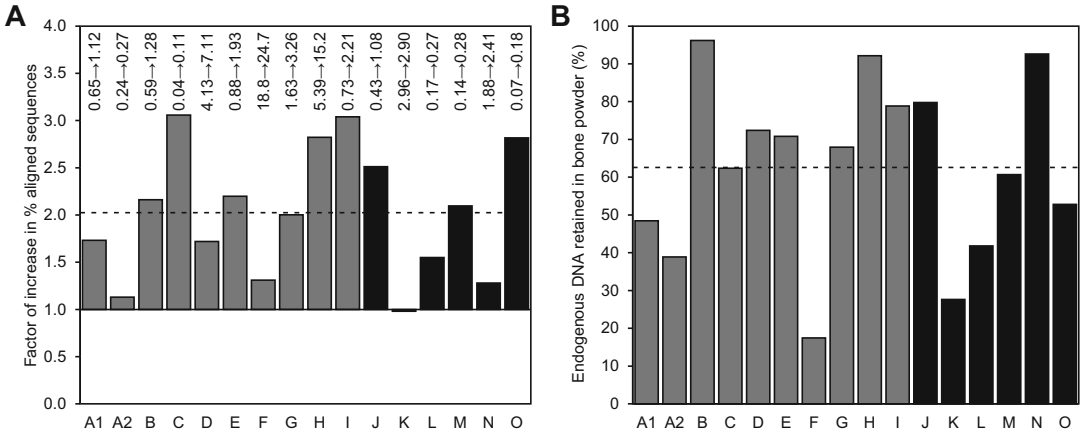

Fig. 2 Effect of phosphate pretreatment on nine animal (A–I in gray) and six Neanderthal samples (J–O in black). (**a**) Increase in proportion of sequences that align to the reference genome relative to untreated control, with absolute percentages denoted above bars. (**b**) Proportion of endogenous DNA retained in the bone/tooth powder inferred directly from the number of endogenous DNA molecules recovered from the phosphate and final extraction buffers. Averages are denoted by horizontal dashed lines

2 Materials

The starting material for both decontamination procedures is bone or tooth powder. While several methods exist to powder ancient samples, we recommend using a dentistry drill at low speed to avoid heating up the sample. All dilutions should be prepared with HPLC (high-performance liquid chromatography) grade water (*see* **Note 1**). Both procedures should be performed in 2.0 mL tubes with a round bottom (*see* **Note 2**) to which ceramic beads are added (*see* **Note 3**).

2.1 Bleach Pretreatment

1. 0.5% bleach solution (*see* **Note 4**): 0.5% sodium hypochlorite in water. Use within 1 month.

2.2 Phosphate Pretreatment

1. Phosphate buffer: 0.5 M sodium phosphate buffer (pH 7.0), 0.1% Tween 20 (*see* **Note 5**).
2. TT buffer: 10 mM Tris–HCl (pH 8.0), 0.1% Tween 20.

3 Methods

Add three ceramic beads and 30–60 mg of drilled bone or tooth powder to a 2.0 mL tube. Carry out all reactions at room temperature.

3.1 Bleach Pretreatment

1. Add 1 mL 0.5% bleach solution and suspend the bone/tooth powder by shaking or vortexing. Rotate for 15 min. Centrifuge

3 min at maximum speed in a benchtop centrifuge. Pipette off and discard the supernatant (*see* **Note 6**).

2. Add 1 mL HPLC grade water, resuspend by vortexing, and rotate for 3 min. Centrifuge 3 min at maximum speed in a benchtop centrifuge. Pipette off and discard the supernatant. Repeat this step twice (three water washes in total).
3. Continue with DNA extraction, e.g., following Chapter 3.

3.2 Phosphate Pretreatment

1. Add 1 mL phosphate buffer and suspend the bone/tooth powder by shaking or vortexing. Rotate for 15 min. Centrifuge 3 min at maximum speed in a benchtop centrifuge. Transfer supernatant to a new 2.0 mL tube and store the tube at −20 °C (*see* **Note 7**). Repeat this step twice (three phosphate washes in total).
2. Add 1 mL TT buffer and resuspend by vortexing. Centrifuge 3 min at maximum speed in a benchtop centrifuge. Transfer supernatant to a new 2.0 mL tube and store the tube at −20 °C (*see* **Note 8**).
3. Continue with DNA extraction, e.g., following Chapter 3.

4 Notes

1. Water, phosphate buffer, and TT buffer should be decontaminated in a UV cross-linker (UVC wavelengths) before usage.
2. Very fine sample powder does not resuspend well in V-shaped tubes.
3. Ceramic beads (suggested bead size ~2.8 mm) help breaking compact bone powder pellets that form in the centrifugation steps. Metal beads are not stable in bleach and should be avoided.
4. In some cases, a bleach concentration higher than 0.5% might be more effective (up to 6%). We recommend a titration series in cases where the success of decontamination is unsatisfactory with 0.5% bleach.
5. Detergent is added to facilitate penetration of the buffer into the sample powder.
6. Remove supernatant as completely as possible without removing too much sample material. Centrifuge twice if necessary. Proceed to the wash step immediately to avoid excessive exposure of the bone/tooth powder to bleach.
7. DNA can be successfully isolated from the phosphate buffer using standard DNA extraction approaches, such as that described in Chapter 3.

8. TT buffer is used to wash away leftover phosphate buffer and does not release DNA from the bone powder. Since it retains minimal amounts of DNA, it can be safely discarded after some time to save storage space.

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