Chapter 4

Extraction of Highly Degraded DNA from Ancient Bones and Teeth

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Abstract

We provide a DNA extraction protocol optimized for the recovery of highly fragmented molecules preserved within bones and teeth. In this method, the hard tissue matrix is degraded using an EDTA/Proteinase K lysis buffer, and the DNA is purified using spin columns with silica membranes. This method efficiently recovers molecules as short as 35 base-pairs long.

Key words Ancient DNA, DNA extraction, Degraded DNA, Silica purification, Paleogenomics

1 Introduction

Ancient DNA molecules are highly fragmented, and it has been shown that an inverse exponential relationship exists between molecule length and abundance [1]. Whereas molecules shorter than approximately 50 bp are not suitable templates for direct amplification of genomic targets by PCR, the advent of high-throughput sequencing and development of ancient DNA-specific protocols to construct genomic libraries have enabled sequencing of much shorter DNA fragments than was possible using PCR.

Here we describe a DNA extraction protocol that has been optimized for the extraction of DNA fragments as short as 35 base-pairs (bp), and that therefore recovers the full size spectrum of molecules that can be used for mapping to reference genomes. The protocol is suitable for and has been optimized for hard samples such as bones and teeth [2]. In this protocol, DNA is first released from a pulverized sample by dissolving the bone/tooth matrix in a minimal extraction buffer containing EDTA and Proteinase K. DNA is then bound to silica in the presence of guanidine hydrochloride and isopropanol, purified, and eluted in a low-salt buffer. The protocol described here has been applied successfully to a large number of Holocene and Pleistocene bones

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and teeth, including the ~430,000 years old skeletal material from the Sima de los Huesos, Spain [2, 3].

2 Materials

2.1 Chemicals and Reagents

0.5 M EDTA pH 8.0.

1 M Tris–HCl pH 8.0.

Proteinase K.

Guanidine hydrochloride.

3 M sodium acetate pH 5.2.

PE buffer (Qiagen).

Tween-20.

2.2 Consumables

1.5 mL tubes (*see* **Note 1**).

2.0 mL tubes.

15 mL tubes.

High Pure Viral Nucleic Acid Large Volume Kit (Roche)

(Optional) Bottle Top Filter, 500 mL, 45 mm neck diameter, 0.22 μm pore.

2.2.1 For an Alternative Spin-Column Assembly

MinElute PCR Purification Kit—spin columns.

Extension reservoir (e.g., from Zymo-Spin V columns, Zymo Research).

50 mL conical bottom tubes.

2.3 Equipment

Large centrifuge able to handle 50 mL conical tubes.

Benchtop centrifuge.

Incubator and rotation device (or ThermoMixer).

Scale

(Optional) Vacuum pump.

3 Methods

3.1 Prepare Buffers (See Note 2)

Extraction Buffer (0.45 M EDTA, 0.25 mg/mL Proteinase K, 0.05% Tween-20 [final concentrations]).

Example (10 mL):

1. 745 μL H_2O.
2. 9 mL EDTA (0.5 M, pH 8).

3. 250 μL Proteinase K (10 mg/mL).
4. 5 μL Tween-20.

Binding Buffer (5 M GuHCl, 40% Isopropanol [final concentrations]).

Example (50 mL) (*see* **Note 3**):

1. 23.88 g GuHCl.
2. Water to 30 mL.
3. 20 mL isopropanol.
4. 25 μL Tween-20.

(Optional) Filter the binding buffer through a Corning filter with a vacuum pump to remove residual particles from GuHCl. Prepare excess buffer as some liquid is lost during this step.

TET Buffer (1 mM EDTA, 10 mM Tris–HCl, 0.05% Tween-20 [final concentrations]).

Example (50 mL):

1. 100 μL EDTA (0.5 M, pH 8).
2. 500 μL Tris–HCl (1 M, pH 8).
3. 25 μL Tween-20.
4. Water to 50 mL.

3.2 Prepare Samples

1. Collect 10–150 mg powderized sample in a 2 mL tube (*see* **Note 4**).
2. Add 1 mL of extraction buffer. Mix well by vortexing.
3. Incubate 16–24 h, rotating at 37 °C.

   (*See* **Note 5** for setup instructions if using the alternative spin-column assembly.)

3.3 DNA Binding, Wash, and Elution

1. For each sample and control, transfer ~10 mL of binding buffer to a labeled 15 mL tube (*see* **Note 6**), and add 400 μL 3 M sodium acetate.
2. Centrifuge samples from **step 3** for 2 min at maximum speed in a benchtop centrifuge to pellet residual solid.
3. Transfer supernatant to the 15 mL tube containing binding buffer. Mix gently by shaking. The pellet can be saved for future use.
4. Pour the sample/binding buffer mixture into the reservoir of the spin-column assembly, and close the 50 mL tube with a screw cap. Centrifuge for 4 min at 400 × *g*. Rotate tubes 90° and centrifuge for another 2 min at 400 × *g*.
5. Remove the screw cap from the 50 mL tube, and transfer the spin-column assembly to a clean 2 mL collection tube. Carefully remove and discard the extension reservoir. If desired, the 50 mL tube with flow-through can be stored at −20 °C.

6. Close and label the spin-column cap.
7. Perform a dry spin for 1 min at 3000 × *g* in a benchtop centrifuge. Discard any flow-through.
8. Add 750 μL PE buffer to each column. Centrifuge for 30 s at 3000 × *g*. Discard flow-through.
9. Repeat **step 8** for a total of two washes.
10. Perform a dry spin for 1 min at maximum speed (~16,000 × *g*), turning the columns in the centrifuge 180° relative to their previous orientation.
11. Transfer the column to a clean 1.5 mL tube.
12. Add 50 μL TET buffer directly onto the silica membrane. Let sit for 5 min.
13. Centrifuge 1 min at maximum speed.
14. Repeat **steps 12** and **13** by transferring the eluate back onto the silica membrane, so that the final elution volume remains 50 μL.
15. Transfer the eluate (final DNA extract) to a clean 1.5 mL tube. Extracts can be stored at −20 °C.

4 Notes

1. We recommend Eppendorf LoBind tubes or similar that reduce the loss of DNA due to tube-wall effects.
2. Buffers can be UV irradiated before use.
3. Add salt to a 50 mL tube, and fill with water to 30 mL, using gradations on the tube. Mix to dissolve salt, and heat briefly in a microwave if necessary. Add remaining reagents.
4. Powder or pulverized material can be generated with, e.g., a drill, freezer mill, bead homogenizer, or mortar and pestle. More than 150 mg is not recommended.
5. Treat extension reservoirs with bleach and rinse well with molecular biology grade water. Let dry and UV irradiate.

   Force extension reservoir into the opening of a MinElute spin column. Remove the extension reservoir-MinElute assembly from the MinElute collection tube, and place in a 50 mL conical tube. Save the collection tube for subsequent wash steps.

   The extension reservoir-MinElute assembly may become detached during centrifugation. It is advisable here to test the assembly with a dry run in the centrifuge.
6. Using the gradations on the tube is sufficient.

References

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