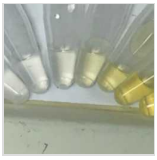

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# A method for the temperature-controlled extraction of DNA from ancient bones

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ABSTRACT

We here provide a protocol for the decontamination of ancient bones and teeth that is based on a temperature-controlled, sequential release of DNA. DNA can be extracted from all fractions generated with this method and the fraction with the highest proportion of endogenous DNA identified for further analysis. The protocol proceeds through repeated incubation of the sample powder in phosphate buffer at 37, 60 and 90 °C, followed by the complete lysis of the residual sample powder. As DNA is denatured at high temperature, subsequent DNA extraction and library preparation has to be performed using methods optimized for single-stranded DNA.

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PROTOCOL CITATION

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KEYWORDS

Ancient DNA, sequential DNA extraction, contamination removal, endogenous DNA, archaeological material



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MATERIALS TEXT

**Reagents**

Sodium phosphate solution, 0.5 M, pH 7.0 **Thermo Fisher Scientific Catalog #J60158.AP** Step 2

Water for HPLC **Sigma Aldrich Catalog #270733** In 2 steps

EDTA solution pH 8.0 (0.5 M) for molecular biology **AppliChem Catalog #A4892,1000** Step 4

Tris buffer pH 8.0 (1 M) for molecular biology **AppliChem Catalog #A4577,0500** Step 3

Proteinase K 100 mg **Sigma-aldrich Catalog #3115879001** Step 4

TWEEN® 20 **Sigma Aldrich Catalog #T2700-100ML** In 3 steps

**Consumables and equipment**

DNA LoBind Tubes 2.0 mL **Eppendorf Catalog #0030108078**

DNA LoBind Tubes 2.0 mL **Eppendorf Catalog #0030108078**

Ceramic beads 2.8 mm **VWR International Catalog #432-0292** Step 6

50 ml CELLSTAR® Polypropylene Tube 30/115 MM Conical Bottom Blue screw cap sterile skirt **greiner bio-one Catalog #210261**

Parafilm M 10 cm wid **neoLab Catalog #3-1012**

Thermomixer

| | |
|---|---|
| HLC | 52 82 00133 |

Incubator

| | |
|---|---|
| Memmert | Incubator IN55 |

Tube rotator

| | |
|---|---|
| VWR | 444-0500 |

UV cross-linker

| | |
|---|---|
| Vilber | Bio-Link BLX 254 |



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Vortex mixer

Scientific Industries SI-0236

Centrifuge

Bench centrifuge

Eppendorf 5424

DISCLAIMER:



## Buffer preparation

1

All buffers are irradiated with UV-C light at a dose of 7 kJ/cm$^2$ using a cross-linker.

2

Sodium-phosphate buffer (0.5 M sodium phosphate, pH 7.0, 0.1 % Tween 20) is prepared by combining the following reagents:

Sodium phosphate solution, 0.5 M, pH 7.0 **Thermo Fisher**

**49.5 mL** **Scientific Catalog #J60158.AP**

TWEEN® 20 **Sigma**

**50 µl** **Aldrich Catalog #T2700-100ML**

3 Tris-Tween wash buffer (10 mM Tris-HCl, pH 8.0, 0.1% Tween-20) is prepared by combining the following reagents:

Water for HPLC **Sigma**

**49.5 mL** **Aldrich Catalog #270733**

Tris buffer pH 8.0 (1 M) for molecular

**0.5 mL** biology **AppliChem Catalog #A4577,0500**

TWEEN® 20 **Sigma**

**50 µl** **Aldrich Catalog #T2700-100ML**



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4 Lysis buffer (0.45 M EDTA, pH 8.0, 0.05% Tween-20 and 0.25 mg/ml proteinase K) is prepared by combining the following reagents:

Water for HPLC **Sigma**
**3.725 mL** **Aldrich Catalog #270733**

EDTA solution pH 8.0 (0.5 M) for molecular
**45 mL** biology **AppliChem Catalog #A4892,1000**

TWEEN® 20 **Sigma**
**25 µl** **Aldrich Catalog #T2700-100ML**

**1.25 mL** 10 mg/ml proteinase K solution in water (prepared from

Proteinase K 100 mg **Sigma-**
**aldrich Catalog #3115879001** )

Proteinase K is added after UV irradiation

## Sample preparation

5 In an ancient DNA cleanroom, remove approximately **50 mg** of sample powder from each specimen using a sterile dentist drill and transfer the powder to a 2.0 ml DNA LoBind tube.

6 To facilitate resuspension of the bone powder during the subsequent incubation and wash steps, add 3-4

Ceramic beads 2.8 mm **VWR**
**International Catalog #432-0292** to the sample material.

## Temperature-controlled phosphate treatment

7 Add **0.5 mL** sodium phosphate buffer to the sample powder, completely resuspend the powder by thorough vortexing, and incubate the tube in a thermo block adjusted to the desired temperature **900 rpm, 00:15:00**

Temperature-controlled phosphate treatment steps
**37 °C** 2 times
**60 °C** 2 times
**90 °C** 2 times

At least one negative control (tube without sample material) should be included in each experiment and carried through all subsequent steps).

8 Transfer tubes to a tabletop centrifuge and spin for 2 min at maximum speed (e.g., 16,400g/13,200 rpm).



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9 Transfer supernatant to a 1.5 mL LoBind tube and store at -20 °C until the day of DNA extraction.

Beads facilitate the resuspension of the sample powder after centrifugation steps, but make it harder to remove supernatant.
Pipette slowly and carefully.

10 Repeat steps 7-9 once at each temperature (for a total of 2 wash steps).

For the 90 °C incubation, make sure the liquid in the tube reaches 90 °C by the end of the 15 min incubation time. If necessary, set the thermo block to a higher temperature.

11 The temperature-controlled phosphate treatment is followed by a room-temperature wash step with **1 mL** Tris-Tween buffer at the end of the last temperature cycle. Completely resuspend the powder by thorough vortexing.

12 Transfer tubes to a tabletop centrifuge and spin for 2 min at maximum speed (e.g., 16,400g/13,200 rpm)

13 Transfer supernatant to a 1.5 mL LoBind tube and store at -20 °C until the day of DNA extraction.

## Final digestion of sample material

14 Add **1 mL** of lysis buffer to the sample powder, completely resuspended the powder by vortexing, and incubate overnight (8 – 16 h) with rotation at **37 °C**

Wrap the tube with parafilm to prevent leaking.

15 Transfer tubes to a tabletop centrifuge and spin for 2 min at maximum speed (commonly at 16,400 g/13,200 rpm).

16 Transfer supernatant to a 1.5 mL LoBind tube and proceed to DNA extraction or store the tube at -20 °C until the day of DNA extraction.

## DNA purification of phosphate fractions and final lysate



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17 Thaw the sodium phosphate fractions (and lysates if necessary) at 37 °C in a thermo block with gentle shaking.

Make sure the liquid is fully thawed and any crystals have completely dissolved.

If desired, DNA extraction can also be performed on the Tris-Tween buffer, but DNA yields are expected to be extremely low.

18 For the sodium phosphate fractions, purify 100 µl of the supernatant, and for the final lysate, purify 500 µl using binding buffer 'G' of the DNA extraction method described in Glocke and Meyer (2017). Final volume of all DNA extracts is 50 µl.

Glocke I, Meyer M (2017). Extending the spectrum of DNA sequences retrieved from ancient bones and teeth.. Genome research. https://doi.org/10.1101/gr.219675.116

## Library preparation, sequencing, and data processing

19 Prepare DNA libraries using 20% of the DNA extract as input, following the protocol for library preparation, quantification and indexing by Gansauge et al. (2020).

Gansauge MT, Aximu-Petri A, Nagel S, Meyer M (2020). Manual and automated preparation of single-stranded DNA libraries for the sequencing of DNA from ancient biological remains and other sources of highly degraded DNA.. Nature protocols. https://doi.org/10.1038/s41596-020-0338-0

20 Perform shallow shotgun sequencing on Illumina's MiSeq or HiSeq2500 platforms (or other Illumina platforms) using a paired-end double-index configuration (2x 76 + 2x 7 cycles).

Kircher M, Sawyer S, Meyer M (2012). Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina platform.. Nucleic acids research. https://doi.org/10.1093/nar/gkr771

## Sequence analysis

21 Trim adapters and merge overlapping paired-end reads into single-molecule sequences using leeHom.



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Renaud G, Stenzel U, Kelso J (2014). leeHom: adaptor trimming and merging for Illumina sequencing reads.. Nucleic acids research.
https://doi.org/10.1093/nar/gku699

22 Use the Burrows-Wheeler Aligner (BWA, https://github.com/mpieva/network-aware-bwa) to align merged sequences to a suitable reference genome (e.g. turTru1.75, bosTauUMD3.1, loxAfr4) using ancient parameters ("-n 0.01 –o 2 –l 16500") allowing more mismatches and indels.

Li H, Durbin R (2010). Fast and accurate long-read alignment with Burrows-Wheeler transform.. Bioinformatics (Oxford, England).
https://doi.org/10.1093/bioinformatics/btp698

Meyer M, Kircher M, Gansauge MT, Li H, Racimo F, Mallick S, Schraiber JG, Jay F, Prüfer K, de Filippo C, Sudmant PH, Alkan C, Fu Q, Do R, Rohland N, Tandon A, Siebauer M, Green RE, Bryc K, Briggs AW, Stenzel U, Dabney J, Shendure J, Kitzman J, Hammer MF, Shunkov MV, Derevianko AP, Patterson N, Andrés AM, Eichler EE, Slatkin M, Reich D, Kelso J, Pääbo S (2012). A high-coverage genome sequence from an archaic Denisovan individual.. Science (New York, N.Y.).
https://doi.org/10.1126/science.1224344

23 Restrict further analyses to sequences of length 35 bp and above to avoid spurious alignments of short sequences with random similarity to the reference genome.

24 Merge sequences with the same start- and end-coordinate into one consensus sequence using bam-rmdup (https://github.com/mpieva/biohazard-tools).

25 Generate summary statistics using samtools and choose the library with the highest proportion of endogenous DNA for further sequencing. Prepare additional libraries from remaining DNA extract if necessary.

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup. (2009). The Sequence Alignment/Map format and SAMtools.. Bioinformatics (Oxford, England).
https://doi.org/10.1093/bioinformatics/btp352



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