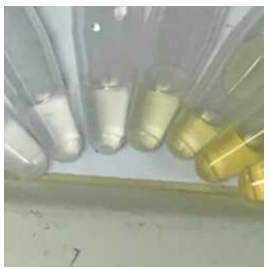
Sep 07, 2023 Version 2

A method for the temperature-controlled extraction of DNA from ancient bones V.2



Version 1 is forked from A method for the temperature-controlled extraction of DNA from ancient bones

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MPI EVA Ancient DNA C...

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Protocol status: Working
We use this protocol and it's working

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Keywords: Ancient DNA, sequential DNA extraction, contamination removal, endogenous DNA, archaeological material, dna extraction, extraction of dna, subsequent dna extraction, sequential release of dna, stranded dna, dna, ancient bone, endogenous dna, protocol for the decontamination, incubation of the sample powder, controlled extraction, extraction, decontamination, temperature, sample powder, complete lysis of the residual sample powder, phosphate buffer, high temperature

Disclaimer

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.

Materials

Reagents

- Sodium phosphate, 0.5M buffer soln., pH 7.0 **Thermo Scientific** **Catalog #**AAJ63791AP
- Water for HPLC **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**270733
- EDTA solution pH 8.0 (0.5 M) for molecular biology **AppliChem** **Catalog #**A4892,1000
- Tris buffer pH 8.0 (1 M) for molecular biology **AppliChem** **Catalog #**A4577,0500
- Proteinase K 100 mg **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**3115879001
- TWEEN® 20 **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**T2700-100ML

Consumables and equipment

- DNA LoBind Tubes 2.0 mL **Eppendorf** **Catalog #**0030108078
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- Ceramic beads 2.8 mm **VWR International (Avantor)** **Catalog #**432-0292
- 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

Equipment	
Thermomixer	NAME
HLC	BRAND
52 82 00133	SKU

Equipment

NAME	BRAND	SKU
Incubator	Memmert	Incubator IN55

Equipment

NAME	BRAND	SKU
Tube rotator	VWR	444-0500

Equipment

NAME	BRAND	SKU
UV cross-linker	Vilber	Bio-Link BLX 254

Equipment

Vortex mixer

NAME

Scientific Industries

BRAND

SI-0236

SKU

Equipment

Centrifuge

NAME

Bench centrifuge

TYPE

Eppendorf

BRAND

5424

SKU

Protocol materials

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Troubleshooting

Buffer preparation

1

Note

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:

49.5 mL

Sodium phosphate, 0.5M buffer soln., pH 7.0 **Thermo Scientific Catalog #**AAJ63791AP

50 µL

TWEEN® 20 **Merck MilliporeSigma (Sigma-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:

49.5 mL

Water for HPLC **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**270733

0.5 mL

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

50 µL

TWEEN® 20 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T2700-100ML

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:

3.725 mL

Water for HPLC **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**270733

45 mL

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

25 µL
TWEEN® 20 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T2700-100ML
1.25 mL 10 mg/ml proteinase K solution in water (prepared from
Proteinase K 100 mg **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**3115879001
)

Note

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 (Avantor) 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

Note

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

Note

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).

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

Note

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).

Note

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

Note

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

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

Note

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

Note

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.

Citation

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 LINK

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).

Citation

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 LINK

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

Citation

Kircher M, Sawyer S, Meyer M (2011)
. Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina platform..
Nucleic acids research.

https://doi.org/10.1093/nar/gkr771 LINK

Sequence analysis

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

Citation

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 LINK

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.

Citation

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 LINK

Citation

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 LINK

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.

Citation

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 LINK

Citations

Step 18

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

Step 19

Gansauge MT, Aximu-Petri A, Nagel S, Meyer M. 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.
https://doi.org/10.1038/s41596-020-0338-0

Step 20

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

Step 21

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

Step 22

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. A high-coverage genome sequence from an archaic Denisovan individual.
https://doi.org/10.1126/science.1224344

Step 22

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

Step 25

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