

Dec 19, 2025

Version 1

Ancient DNA extraction part I: lysate preparation V.1

DOI

<https://dx.doi.org/10.17504/protocols.io.4r3l212oqg1y/v1>

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Ancient DNA Core Unit

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Protocol Citation: Sarah Nagel, Anna Schmidt, Matthias Meyer, Elena Essel 2025. Ancient DNA extraction part I: lysate preparation. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.4r3l212oqg1y/v1>

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Protocol status: Working

We use this protocol and it's working

Created: August 15, 2025

Last Modified: December 22, 2025

Protocol Integer ID: 224743

Keywords: DNA extraction, ancient DNA , lysate, ancient DNA extraction, DNA, extraction, ancient bone, extraction buffer, liquid handling, ancient DNA core unit, workflows of the ancient DNA core unit, ancient dna extraction, ancient dna extraction part, manual dna extraction, dna purification, automated dna purification, workflows of the ancient dna core unit, ancient dna core unit, ultrashort dna fragment, lysate preparation, more extraction, extraction method, ml lobind eppendorf tube, single lysate, sediment, subsamples of sediment, based extraction method, subsamples of bone, cap tube, lysate

Funders Acknowledgements:

Max Planck Society

Grant ID: -

Abstract

We here provide a protocol for the first step of ancient DNA extraction - lysate preparation - from bones, teeth and sediments, building on the silica-based extraction method described by Dabney et al. 2013 and refined by Rohland et al. 2018. The protocol includes specific adaptations to the clean room environment and workflows of the Ancient DNA Core Unit at the MPI-EVA, as well as preparatory steps for downstream automated DNA purification using the Bravo NGS (Agilent Technologies) or NGS Star (Hamilton) workstations. Sample material (drilled subsamples of bones/teeth, subsamples of sediment) should be provided in 2.0 ml LoBind Eppendorf tubes. Lysates are prepared in volumes ranging from 300 to 2000 μ l, depending on the sample weight. A standard volume of 150 μ l, aliquoted to screw-cap tubes (option A), is subsequently used for DNA purification (see separate protocol), allowing two or more extractions from a single lysate. Alternatively, aliquots with different volume (option B) may be removed for manual DNA extraction or other purposes.

References

Dabney, J., Knapp, M., Glocke, I., Gansauge, M.-T., Weihmann, A., Nickel, B., Valdiosera, C., García, N., Pääbo, S., Arsuaga, J.-L., & Meyer, M. (2013). Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proceedings of the National Academy of Sciences of the United States of America*, 110(39), 15758-15763.

Rohland N, Hofreiter M. Ancient DNA extraction from bones and teeth. *Nat Protoc*. 2007;2(7):1756-62. doi: 10.1038/nprot.2007.247. PMID: 17641642.

Materials

Materials

	Reagent	Supplier	Cat. no.	Decont. *
	Buffers/reagents			
	Extraction Buffer Stock Solution (decontaminated) [†]	self	-	UV
	Proteinase K (10 mg/ml) dissolved [†]	self	-	UV
	Consumables			
	FluidX tubes 0.8 ml external thread jacket in racks	Brooks	68-0801-11	-
	1.5 ml LoBind Tubes	neoLab Migge	VB-0285	UV
	2.0 ml LoBind Tubes	neoLab Migge	VB-0286	UV
	50 ml Falcon tube with stand	Greiner Bio-one	210-261	-
	250 ml square media bottle	VWR	391-0629	-
	Filter tip, natur, 1250 µl, low retention	Greiner Bio-one	778363	-
	Filter tip, natur, 300 µl, low retention	Greiner Bio-one	775353	-
	50 ml serological pipette	Corning BV	357550	-
	25 ml serological pipette	Corning BV	357525	-
	5 ml serological pipette	Corning BV	357543	-

* See documents in the Appendix for decontamination instructions.

† See documents in the Appendix for preparation of buffers and enzymes.

Equipment



- Table centrifuge for 2.0 ml tubes (e.g. Centrifuge 5420, Eppendorf, cat. no. 5420000318)
- Table-top micro-centrifuge for 2.0 ml tubes (e.g. Carl Roth Mini-Zentrifuge ROTILABO, cat. no. T464.1)
- Serological pipette controller (e.g. battery-powered pipetting aid for glass pipettes, cat. no. TC16.1)
- Vortexer (e.g. Vortex-Genie 2, Sigma-Aldrich, cat. no. Z258415-1EA)
- Incubation oven (e.g. Big Shot II, Boekel Scientific, cat. no. 230402-2)
- Label Printer (e.g. Brady M611, Brady, cat. no. 317844)
- FluidX rack barcode reader (e.g. Brooks Life Sciences, cat. no. 20-4018)
- Tube decapper (e.g. Apero 8-Channel Semi- Automatic Screw Top tube rack decapper, Brooks Life Sciences, cat. no. 46-6502)

Appendix

Documents for buffers

Document



NAME

Extraction buffer stock solution (w/o Proteinase K)

CREATED BY

Ancient DNA Core Unit[Preview](#)

Document

NAME

Proteinase K solution for ancient DNA extraction

CREATED BY

Ancient DNA Core Unit[Preview](#)

Documents for decontamination procedures

Document

NAME

UV decontamination of reagents/buffers

CREATED BY

Elena Essel

Preview

Document

NAME

UV decontamination of materials

CREATED BY

Elena Essel

Preview

Safety warnings

 For information about potential health and safety hazards, please refer to the Material Data Safety Sheets associated with the reagents and chemicals used.

Ethics statement

The protocols.io team notes that research involving animals and humans must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Ethics Committee or Board.



Reagent preparation (days/weeks/months before the lysate preparation)

- 1 Prepare the Proteinase K solution and extraction buffer stock solution as described in MATERIALS.
- 2 Decontaminate the 2.0 ml LoBind tubes as described in MATERIALS.

Reagent preparation (day of the lysate preparation)

- 3 Check the 'Subsamples' tab of the "samples_to_screen" list located at "P:\AncientDNA\samples" to identify the subsamples requested for lysis. Calculate the required volumes of extraction buffer stock solution and Proteinase K solution based on the number of subsamples and their weight as well as the number of controls included. Always include an excess equivalent to one additional reaction in your calculations.

Note

[Note]

The required volumes for different sample weights are listed in the table below. The final concentration of Proteinase K in the extraction buffer is 0.25 mg/ml.

	Subsample (mg)	Extr. buffer (μl)	Extr. buffer stock solution (μl)	Prot. K solution (μl)
	< 10	300	292.5	7.5
	11-25	500	487.5	12.5
	26-99	1000	975	25
	100-200	2000	1950	50

[Note]

Add an extraction negative control (ENC) to every set of 20 subsamples by adding the respective amount of extraction buffer to an empty tube. Label the tube with 'ENC'.

**Note****[Documentation]**

- Create the lysate IDs for the corresponding subsamples in CoreDB.
- In the "samples_to_screen" list, move the subsamples being lysed from the 'Subsamples' tab to the 'Lysates' tab.
- Add the lysate IDs and all relevant information into the respective fields of the list.
- Save a copy of the updated list with date and initials and put the old version into the "screening_list_backup" folder.

- 4 Thaw the Proteinase K at room temperature, vortex briefly and spin down.
- 5 Combine the calculated volumes of extraction buffer stock and Proteinase K solution in a 50 ml Falcon tube or 250 ml bottle and mix by shaking to prepare the ready-to-use extraction buffer.

Note**[Note]**

For large volumes, prepare the extraction buffer in a 250 ml bottle, then transfer it to a 50 ml Falcon tube for easier pipetting.

Sample lysis

- 6 Before each experiment, create an entry in Labfolder and document the process.

Note**[Note]**

The Labfolder entry name consists of the name of the Labfolder template and the lysate IDs. To document your experiment, fill the provided data element fields in Labfolder. They serve as a template for creating the CoreDB entry. Make sure to create lysate IDs before going to the lab.

- 7 Place the 2.0 ml LoBind tubes containing the subsamples into a tube rack. If subsamples were frozen, thaw them at room temperature.
- 8 Spin the tubes in a mini centrifuge to collect the subsample material at the bottom.

**Note****[Labeling]**

Label the subsample tubes with lysate IDs, date and your initials before adding the extraction buffer. Put the new labels on top of the old subsample labels.

- 9 Add the appropriate amount of extraction buffer to each subsample as indicated above ( [go to step #3](#)). Open only one tube at a time.

Note**[Documentation]**

Note down the reagent information in the respective fields in Labfolder.

- 10 Vortex the tubes to fully resuspend the sample powder.

Note**[Note]**

Ensure the tubes are tightly closed to prevent leakage during overnight incubation.

- 11 Rotate the tubes overnight at 37 °C in the incubator.

Note**[Note]**

The minimum incubation time is 8 h.

[Documentation]

Record the incubation start time in the Labfolder entry.

- 12 After overnight incubation, briefly spin down tubes and move them to the freezer until further processing or directly continue with lysate aliquoting into FluidX tubes for automated DNA purification (Option A) or Eppendorf LoBind tubes for manual DNA extraction (Option B).

**Note****[Documentation]**

- Record the end time of incubation in the Labfolder entry.
- Update the storage location in CoreDB.

Option A: Lysate aliquoting into FluidX tubes for subsequent automated DNA purification

- 13 If stored at -20°C , remove the lysates from the freezer and thaw at room temperature for at least 30 minutes. Alternatively, thaw the lysates in a Thermo shaker at 37°C for approximately 10 minutes.
- 14 Prepare one or more FluidX racks by inserting empty FluidX tubes column-wise, matching the number of lysate tubes. When arranging the tubes, follow these rules:
 1. Use only odd-numbered columns (1, 3, 5, ...) and leave even-numbered columns empty to minimize electrostatic effects that can cause liquid spills.
 2. After each ENC, start filling a new column. At a later stage, this will allow for visually controlling the correct positioning of ENCs after lysate transfer.

Note**[Labelling]**

Label the FluidX rack(s) with Lysate IDs, date and initials.

- 15 Start the FluidX barcode reader software and allow the camera to initialize.
- 16 Use the FluidX barcode reader to read the bottom of all empty tubes in the rack and the rack ID in the front.
- 17 Move the FluidX document containing the FluidX tube IDs and rack ID under `"/home/public/AncientDNA/FluidX BarCodeReader/FluidX_Data/FluidX_ROOM"` to your personal folder under `"/home/public/AncientDNA/FluidX BarCodeReader/FluidX_Data/NAME"` and add the Lysate IDs from [go to step #3](#) to the corresponding FluidX IDs.
- 18 Thoroughly vortex the lysate tubes after thawing.



- 19 Spin the lysate tubes at maximum speed for 5 minutes in a tabletop centrifuge inside the sample hood.

Note**[Note]**

Handle the lysate tubes carefully to avoid disturbing the fragile pellet at the bottom.

- 20 Open the first column of empty FluidX tubes using the decapper and store the caps on an empty and clean cap rack. The caps will be used afterwards when closing the FluidX tubes.
- 21 Transfer 150 µl of supernatant from each lysate tube into a FluidX tube. Carefully pipette the lysate supernatant to avoid transferring any sample material. If the sample pellet begins to dissolve, repeat centrifugation before transferring the supernatant (i.e., [⇒ go to step #19](#)).

Note**[Note]**

Use the previously created list ([⇒ go to step #17](#)) to verify that each lysate tube ID matches the corresponding FluidX tube barcode during liquid transfer. If the lysate volume is less than 150 µl, transfer the entire remaining volume to the FluidX tube, then fill up to 150 µl with extraction buffer stock solution. In this case, also update the lysate input column in the "samples_to_screen" list and mark the lysate as 'used up' in CoreDB.

FluidX tubes should not be removed from the rack during liquid transfer. If it becomes necessary to move or remove FluidX tubes, scan the FluidX rack again and update the tube barcodes accordingly.

- 22 Close the current column of FluidX tubes using the decapper. Then proceed to the next full or partial column ([⇒ go to step #20](#)) and continue until all lysate supernatants have been successfully transferred.
- 23 Store the FluidX lysate rack in the freezer until further processing and return the lysate tubes to the freezer.
- 24 Update the "samples_to_screen" list located at "P:\AncientDNA\samples".

**Note****[Documentation]**

- Add the FluidX rack ID and the FluidX tube IDs to the corresponding fields.
- Save a copy of the updated list with date and initials and put the old version in the "screening_list_backup" folder.

Option B: Lysate aliquoting for manual DNA extraction or other purposes

- 25 If stored at -20°C , remove the lysates from the freezer and thaw at room temperature for at least 30 minutes. Alternatively, thaw the lysates in a Thermo shaker at 37°C for approximately 10 minutes.
- 26 Thoroughly vortex the lysate tubes after thawing.
- 27 Spin the lysate tubes at maximum speed for 5 minutes in a tabletop centrifuge inside the sample hood.

Note**[Note]**

Handle the lysate tubes carefully to avoid disturbing the fragile pellet at the bottom.

- 28 Transfer the volume of lysate supernatant into a new 1.5 or 2.0 ml LoBind tube. Carefully pipette the lysate supernatant to avoid transferring any sample powder. If the sample pellet begins to dissolve, repeat centrifugation before transferring the supernatant ([⇒ go to step #27](#)).
- 29 Store the tube containing the lysate aliquot in the freezer until further processing and return the lysate tubes to the freezer.