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Ancient DNA Extraction from Bones and Teeth

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We use this protocol and it's working

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Abstract

Ancient DNA Extraction Protocol (spin column-based)

- This protocol outlines the procedure for extracting ancient DNA from bone or tooth powder using Monarch spin columns. The final DNA extract volume will be 35 µL.

Based on:

- The spin column protocol is based on Dabney et al. 2013 (DOI: 10.1073/pnas.1314445110)
- The modified binding buffer is from Allentoft et al. 2015 extraction (DOI: 10.1038/nature14507)

Protocol initially written up by Michael V. Westbury on 20-08-2018.
Annotations added by Andrea A. Cabrera and Alba Rey-Iglesia on 08-11-2019.
The procotols.io version was created by Wenxi Li and Deon de Jager.

Protocol materials

- Buffer EB **Qiagen** **Catalog #**19086
- Tween 20 **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**P1379-500ml
- 0.5M EDTA **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**03690
- Proteinase K, recombinant, PCR Grade **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**RPROTKSOL-RO
- Buffer PB **Qiagen** **Catalog #**19066
- 5M Sodium acetate **Merck MilliporeSigma (Sigma-Aldrich)**
- 5M Sodium chloride **Fisher Scientific** **Catalog #**10609823
- Sodium acetate buffer solution 3M pH 5.2 **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**S7899

Before start

- Ensure that ~50 mg of bone or tooth powder is prepared in advance (DO NOT treat with UV).
- UV-treat aliquots (PB buffer, PE buffer, EB buffer, ddH_2O) for 10 minutes before use.
- Do not UV Eppendorf Tubes/PCR TUBES
- PE buffer: Add Molecular Grade Ethanol as indicated on the bottle before use.
- Use Qiagen EB buffer directly if preferred.
- Clean all surfaces with 5% bleach solution followed by 70% ethanol before and after use.
- Clean all equipment with 70% ethanol before and after use.

Buffer Preparation

1 **Digestion Buffer**

Choose the appropriate option based on **[ProK]**.

Prepare fresh for each use.

0.5M EDTA **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**03690

Proteinase K, recombinant, PCR Grade **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**RPROTKSOL-RO

Option 1: Use 10mg/mL ProK

	Reagent	Volume per sample (µL)	Final Concentration	Master Mix (14 samples)
	0.5M EDTA	900	0.45	12,600
	10 mg/mL ProK	25	0.25	350
	ddH2O	75	N/A	1,050
	Total	**1,000**		**14,000**

Option 2: Use 19mg/mL ProK

	Reagent	Volume per sample (µL)	Final Concentration	Master Mix (14 samples)
	0.5M EDTA	900	0.45	12,600
	19 mg/mL ProK	13.15	0.25	184.1
	ddH2O	86.85	N/A	1,215.9
	Total	**1,000**		**14,000**

2 **Modified Binding Buffer**

Prepare in bulk

Option 1 (with 5M NaOAc)

- **500 mL** Buffer PB **Qiagen Catalog #**19066

- **9 mL** 5M Sodium acetate **Merck MilliporeSigma (Sigma-Aldrich)**
- **1.25 mL** 5M Sodium chloride **Fisher Scientific Catalog #**10609823
- Adjust to **pH 4-5** (~2 mL Hydrochloric acid)

Option 2 (with 3M NaOAc, pH 5.2)

- **500 mL** Buffer PB **Qiagen Catalog #**19066
- **15 mL**
  Sodium acetate buffer solution 3M pH 5.2 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S7899
- **2.5 mL** 5M Sodium chloride **Fisher Scientific Catalog #**10609823
- No pH adjustment necessary as the 3M sodium acetate is pH buffered at pH5.2.

Note

- The NaOAc and NaCl can be added directly to the 500 mL of Buffer PB. Make sure to label it as **Modified Buffer PB**.
- Make individual aliquots in 50 mL Falcon tubes.

3 **PE Buffer**
Remember to add Molecular Grade Ethanol to PE when opening a new bottle. See instructions on
the PE buffer bottle.

4 **EB Buffer**
Appropriate for when using LoBind Eppendorf tubes.

- Buffer EB **Qiagen Catalog #**19086 . Use as provided.

4.1 **EBT Buffer *optional** *

Appropriate for when NOT using LoBind Eppendorf tubes.

- Buffer EB **Qiagen Catalog #**19086
- Tween 20 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P1379-500ml at a final concentration of 0.05% (v/v)

Day 1 - Sample digestion 19h 2m

5 **Optional: Pre-digestion** 30m *

5.1 In a 2 mL LoBind Eppendorf tube:
- Add 50 mg Bone Powder to 1 mL **extraction buffer**.
- Resuspend the bone powder by **vortexing**.

5.2 Incubate at 50 °C with **constant rotation** for 00:30:00 (use Parafilm to seal the lids). 30m

6 **Digestion**

Note
- The microcentrifuge tubes are the LoBind tubes.
- Use a rotation machine to keep the samples well mixed during digestion.

6.1 Remove Parafilm and **centrifuge** for 00:02:00 at **maximum speed** to pellet the bone powder. 2m

6.2 Remove the supernatant and add 1 mL **fresh extraction buffer**.

6.3 Resuspend the bone powder by **vortexing**.

6.4 Incubate at 37 °C with **constant rotation** for ~ 18:00:00 or overnight (use Parafilm to seal the lids). 18h

Day 2 - DNA purification 41m

7 **Concentration of DNA with Amicon vivaspin 30kD column**

Note
- Amicon® vivaspin column are also called **centrifugal filter units**.
- When centrifuging the Amicon columns, do not close the tubes too tight.
- MinElute spin columns can be used instead of Monarch columns, though the latter are preferable for smaller volumes, like that used in the elution step.

7.1 Remove Parafilm from eppendorf and **centrifuge** for 00:02:00 min at **maximum speed** to pellet the bone powder. 2m

7.2 Transfer the **supernatant** (~1 mL) to an **Amicon® vivaspin 30kD column**.

7.3 **Centrifuge** at ~4,000g **until ~70 µL remains**. Start with a 00:10:00 min centrifugation, then adjust in 00:05:00 min intervals as needed. 15m

Note

- If the volume does not decrease to ~70 uL after several rounds of centrifuging, then repeat steps 7.4-7.5 until all the remaining sample is used.

7.4 **Mix** the **supernatant** with **10x** its volume of **modified binding buffer** in a **Monarch column**.
- Example: For 70 µL supernatant, add 700 µL of binding buffer.
- **Mix** by pipetting or inverting.

7.5 **Centrifuge** for 00:01:00 min at **8,000 RPM** and **discard the flowthrough**. 1m
- Tap the collection tube on a paper towel to get excess flowtrhough out before reassembling.

7.6 Add 650 µL **PE buffer** to the spin column.

7.7 **Centrifuge** at **5,000 RPM** for 00:01:00 min and **discard the flowthrough**. 1m

7.8 Repeat steps 7.6-7.7.

7.9 Centrifuge at **maximum speed** for 00:01:00 min to dry the spin column. 1m

7.10 Place the column in a clean **1.5 mL LoBind** Eppendorf tube.

8 **DNA Elution** 10m 30s

8.1 Add 17.5 µL **EB buffer** (or EBT buffer if not using LoBind tubes) to the silica membrane and **incubate** for 00:10:00 min at room temperature. 10m

8.2 **Centrifuge** at **maximum speed** for 00:00:30 seconds. 30s

8.3 **Repeat** steps 8.1-8.2 for a total of **35 µL DNA extract.** Store DNA at -20ºC.

9 **Qubit Quantification**

- If desired, measure the DNA concentration using a Qubit. See Qubit protocol for details.

Protocol references

Allentoft ME, Sikora M, Sjögren KG, et al. Population genomics of Bronze Age Eurasia. *Nature*. 2015;522(7555):167-172. doi:10.1038/nature14507

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