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A high-throughput ancient DNA extraction method for large-scale sample screening

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We use this protocol and it's working

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Abstract

Large-scale DNA screening of paleontological and archeological collections remains a limiting and costly factor for ancient DNA studies. Several DNA extraction protocols are routinely used in ancient DNA laboratories and have even been automated on robotic platforms. Robots offer a solution for high-throughput screening but the costs, as well as necessity for trained technicians and engineers, can be prohibitive for some laboratories. Here we present a high-throughput alternative to robot-based ancient DNA extraction using a 96-column plate. When compared to routine single MinElute columns, we retrieved highly similar endogenous DNA contents, an important metric in ancient DNA screening. Mitogenomes with a coverage depth greater than 0.1X could be generated and allowed for taxonomic assignment. However, average fragment lengths, DNA damage and library complexities significantly differed between methods but these differences became non-significant after modification of our library purification protocol. Our high-throughput extraction method allows generation of 96 extracts within approximately four hours of laboratory work while bringing the cost down by ~39% compared to using single columns. Additionally, we formally demonstrate that the addition of Tween-20 during the elution step results in higher complexity libraries, thereby enabling higher genome coverage for the same sequencing effort.



Materials

Equipment

- QIAquick 96 PCR Purification Kit (QIAGEN, cat. no. 28181). Contains 4x QIAquick 96 plates, collection racks, lids and buffers (PM, PE, EB).
- QIAvac Connecting System (QIAGEN, cat. no. 19419).
- Vacuum pump (230 V - 50 Hz) (QIAGEN, cat. no. 84020).
- QIAvac 96 (QIAGEN, cat. no. 19504). Contains QIAvac top plate, base and waste tray.

Note

QIAvac Connecting System is adapted for connection to QIAvac 24 Plus. In order to make the vacuum pump and 96 vacuum manifold compatible, we advise cutting the tubing from the vacuum valve (remove the adapter to QIAvac 24 Plus). Immerse tubing in hot water to make it more flexible and connect tubing to the QIAvac base connector, then tighten connection using a clamp (not included).

- DNA LoBind 1.5 mL tubes (Eppendorf).
- Adhesive PCR film seals, sterile (VWR, cat. no. 391-1255).
- Large centrifuge (Eppendorf).
- Microcentrifuge.
- Precision balance.
- Pulse vortexer.
- Barrier pipette tips (20, 200 and 1000 μ L).
- Finnpiptette multichannel (8) F2 10-100 μ L (Thermo Fisher Scientific, cat. no. 4662020).
- Adjustable multichannel (8) 5-100 μ L (Eppendorf Research plus Move It, cat. no. 3125000133) and custom pipette tips.
- Adjustable multichannel (8) 120-1200 μ L (Eppendorf Research plus Move It, cat. no. 3125000206).
- Incubator.
- 2 mL screw cap tubes.

Reagents

- Guanidine hydrochloride.
-  UltraPure DNase/RNase-Free Distilled Water Thermo Fisher Scientific Catalog #10977023
- Isopropanol.
- Tween-20.
- Sodium Acetate (3 M, pH 5.2).
- TET buffer (10 mM Tris-HCl, 1 mM EDTA, 0.05% Tween-20).
- EDTA (0.5 M, pH 8).
- Proteinase K (10 μ g/ μ L).

Lysis

- 1 Add ~50 mg of bone fragments or powder to a 2 mL screw cap tube. Prepare the required amount of lysis buffer per reaction as follows (from Dabney et al. 2019). Add 1000.5 μL of lysis buffer to each screw cap tube containing bone fragments/powder and mix by pipetting.

Reagent	Amount	Final concentration
EDTA (0.5 M, pH 8)	900 μL	0.45M
Ultrapure water	75 μL	
Tween-20 (10%)	0.5 μL	0.05%
Proteinase K (10 $\mu\text{g}/\mu\text{L}$)	25 μL	0.25 $\mu\text{g}/\mu\text{L}$

Incubate at 37°C under rotation overnight for powder. Bone fragments can take several days to fully dissolve and we recommend adding an additional 20 μL of Proteinase K after 48h incubation for such samples.

Lysates can be stored at -20°C until extraction.

Extract purification (hands-on time for steps 2. to 6. is ~4 hours)

2 Buffer preparation

Prepare binding buffer, as follows for 35 mL (~35 extractions):

Reagent	Amount
Guanidine hydrochloride	16.72 g
Ultrapure water	up to 21 mL
Isopropanol	up to 35 mL
Tween-20 (10%)	17.5 μL

Aliquot 1 mL of binding buffer into 1.5 mL LoBind tubes, and add 40 μL of sodium acetate per aliquot.

**Note**

we advise UV decontamination of ultrapure water prior binding buffer preparation as well as of PE and elution buffer aliquots prior starting extraction.

Note

sodium acetate could be added to the original binding buffer mix. We recommend not storing binding buffer mixed with sodium acetate.

3 Check pressure in empty plate

Seal a new QIAquick 96 plate. Place it on the QIAvac top plate and above the waste tray.

Note

Throughout the whole procedure, keep the plate on the top plate and make sure either the waste tray or the collection rack is underneath prior pipetting on the columns.

Place this set-up in the QIAvac base.

Make sure to close the valve between the vacuum pump/connecting system and the QIAvac manifold.

Switch on the vacuum pump and let the needle stabilize. If needed, downplay the control to below -800 mbar.

Open the valve and let the needle stabilize again, to below -600 mbar.

If there is no pressure, check connections of the system. Otherwise proceed with the extraction.

4 Mix lysate and binding buffer (in tubes)

Let lysate equilibrate to room temperature and vortex.

Centrifuge lysate at 6,000 g for 3 minutes.

Mix a 100 µL aliquot of the lysate to the binding buffer aliquot (with sodium acetate).

Note

we advise using an adjustable multichannel pipette to parallelise this step for multiple samples.

Vortex vigorously.

5 Silica column binding (on the plate)

Remove the seal from the new QIAquick 96 plate.

Pipette half of the sample and binding buffer mixture onto the QIAquick 96 plate columns.

Note

we advise using an adjustable multichannel pipette to parallelise this step for multiple samples.

Note

Make sure to record the layout of samples on the plate.

Adapt the plate + top plate + waste tray to the base, and turn on the vacuum by following the procedure in step 2. although there is no need to seal the plate anymore unless you kept empty wells.

Add the remaining volume of lysate-binding buffer mix to the QIAquick 96 plate (done twice due to limitations with the maximum volume per column).

Empty waste tray following waste management for guanidine hydrochloride-containing solutions.

6 Wash

Apply 900 μ L of PE buffer to each column and turn on the vacuum. Repeat the wash a second time.

Note

we advise using a multichannel pipette.

Make sure to eliminate all wash buffer prior to elution: apply maximum vacuum to the plate for 10 minutes. Then lift the plate and top plate together, ventilate slowly and tap on top of absorbent paper until nozzles are dry.

7 Elution

Transfer the plate and top plate from the waste tray to a collection rack.



Apply 60 μ L of elution buffer (we recommend TET) to each column.

Note

we advise using a multichannel pipette.

Incubate at room temperature for 5 minutes, then turn on the vacuum for 5 minutes.

Note

we advise annotating collection tubes according to the plate map.

Library preparation

- 8 Prepare DNA library from extract according to lab-specific protocol.

Library purification (deviations from the extraction)

9 **Buffer preparation**

No binding buffer + sodium acetate preparation. [⇒ go to step #2](#)

10 **Check pressure in empty plate**

No deviation. [⇒ go to step #3](#)

11 **Mix libraries to binding buffer**

Mix 125 μ L PB buffer to 25 μ L of library. [⇒ go to step #4](#)

12 **Binding**

The whole mixture can be loaded at once. [⇒ go to step #5](#)

13 **Wash**

No deviation. [⇒ go to step #6](#)

14 **Elution**



Apply 45 μ L of elution buffer. [↗ go to step #7](#)

Protocol references

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