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Ancient DNA Extraction from Osteological Samples

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We use this protocol and it's working

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Abstract

The Yang-urea method is used to extract ancient DNA (aDNA) from archaeological samples, e.g. bones or teeth, in a cleanroom laboratory. The bone/tooth should be cleaned mechanically and with a mild bleach solution, UV-irradiated and sub-sampled for a small piece weighing 50 - 100 mg. The sub-sample is pre-washed, demineralised and lysed, resulting in a crude extract, which is purified with MinElute Purification Kit to obtain DNA. This protocol can also be used for bone powder samples with lower concentrations of Proteinase K.

Guidelines

All work should be performed in a dedicated ancient DNA (aDNA) cleanroom facility by trained personnel and adhering to strict aDNA guidelines (Fulton & Sharpiro 2019).

The cleanroom laboratories and equipment are frequently decontaminated with sodium hypochlorite (bleach), RNase AWAY Surface Decontaminant, and/or UV irradiation. Consumables are also decontaminated, where applicable.

Personnel wear a protective coverall suit with hood, hair net, face mask with visor, shoe covers, and three layers of gloves. Entry into the cleanroom laboratories is prohibited if personnel have been in another laboratory, unless they have showered and changed into a clean set of clothes.

Materials

Sample

- Compact, dense sub-sample from a tooth or bone | 50-100 mg

Reagents

- UltraPure 0.5M EDTA **Invitrogen Catalog #**15575020
- 8M urea **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**U4883
- Proteinase K **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P6556-100mg | 10 mg/ml
- MinElute PCR Purification Kit **Qiagen Catalog #**28006

Consumables

- Filter tips 10-µl, 200-µl and 1000-µl (Thermo Scientific)
- Extended length 200-µl filter tips (Thermo Scientific #10180621)
- Amicon Ultra Centrifugal Filter, 30 kDa MWCO (Millipore #UFC803096)
- Collection tubes (Qiagen #19201)
- 1.5 ml DNA LoBind tubes (Eppendorf #0030108051)
- 2 ml DNA LoBind tubes (Eppendorf #0030108078)
- 2 ml screw cap tubes, Biosphere® plus (Sarstedt #72.694.217)

Equipment

- Pico 17 microcentrifuge (Thermo Scientific #75002410)
- Swing-bucket rotor benchtop centrifuge (Eppendorf 5804)
- Big Shot III rotary hybridisation oven (Boekel #230402-2)
- Dry bath/Block heater (Fisher Scientific #15397928)

Safety warnings

Buffer PB contains guanidine hydrochloride which form highly reactive compounds when combined with bleach. DO NOT add bleach or acidic solutions to sample waste.

Buffer PB contains isopropanol and is flammable.

Ethics statement

Obtain all permissions from owners/musuems/institutions, etc., before sampling.

Before start

- Read lab routines and risk assessment documents about working in aDNA cleanroom laboratories.
- Read product information sheets and manufacturers' instructions.
- Wipe hood and surrounding working areas with RNase AWAY Decontamination , Milli-Q water and followed by 70% ethanol before starting. Do the same after using the hood, and switch on the UV lamp. Wipe items with RNase AWAY decontamination solution before placing them in the hood.

Demineralisation and cell lysis

30m

1 Pre-treat the Compact, dense sub-sample from a tooth or bone by adding 1 mL 0.5 M EDTA to it, in a 2-ml screw cap tube.

1.1 Take a new 2-ml screw cap tube and add 1 mL EDTA . This is the extraction blank (EB) and serves as a negative control.

Note

- EB(s) should be treated as a sample and carried along with other sample(s) throughout the process.
- Prepare one EB for every 10 samples. *E.g. 25 samples should be accompanied with 3 EBs.*

1.2 Place the tubes in a rotary hybridisation oven and incubate with rotation 37 °C 00:30:00 .

30m

Note

For bone powder, incubate 37 °C for 15 min. Centrifuge the tubes 2000 rpm, 00:01:00 , carefully remove and discard the EDTA without disturb the bone powder. Then go to step #3.1

2 In the meantime, prepare Yang-urea lysis buffer (1 M urea/EDTA containing 0.4 mg/ml Proteinase K).

	Reagent	For 10 samples	Final
	0.5 M EDTA	8.35 ml	0.42 M
	8 M urea	1.25 ml	1 M

	Reagent	For 10 samples	Final
	10 mg/ml Proteinase K*	0.4 ml	0.4 mg/ml

Yang-urea lysis buffer recipe; 1 ml per sample.
*for bone powder, add Proteinase K to final conc. of 0.2 mg/ml.

3 Remove the tubes from the oven and quick spin them to spin down the liquids from the tube lids and walls. Aspirate and discard the EDTA.

3.1 Add 1 mL Yang-urea lysis buffer to each sample, including EB. Incubate the tubes with rotation 55 °C for 3 - 6 h .

Note

- Proteinase K has an optimal activity at 50 - 60 °C, in the presence of a chaotrophic agent such as urea.
- The digested sample, i.e. crude extract, contains biomolecules, debris and other contaminants.
- Alternatively, digest the samples at 37 °C overnight (16 - 24 h) . Continue go to step #3.2 to add Proteinase K , but incubate the samples at 55 °C for 3 - 6 h . The crude extracts can be stored at -20°C until ready to be purified.

3.2 Quick-spin the tubes and add 40 µL 10 mg/ml Proteinase K to each sample.
Incubate the tubes with rotation 37 °C Overnight 16 - 24 h .
⊗ The crude extracts and EB can be stored at -20 °C until ready to be purified.

3.3 Optional: if the sample is partially digested, quick spin the tube and collect the crude extract into a new 2-ml DNA LoBind tube and store at -20 °C . Add fresh 1 mL Yang-urea lysis buffer to the sample and incubate 55 °C for 2 - 6 h or until complete digestion. The second crude extract can be stored at -20 °C until ready to be purified. Continue with both crude extracts, go to step #4 centrifuge , collect and combine their supernatants (total volume ~2 ml).

DNA purification of crude extract

45m

4 Place the tubes into the microcentrifuge and centrifuge 12000 rpm, 00:05:00 . 5m

Note

If crude extracts were frozen, make sure that they are thoroughly thawed before centrifuging.

5 Without disturbing the pellet/debris, transfer the supernatant to a Amicon Ultra Centrifugal Filter and centrifuge in a tabletop swing-bucket centrifuge 2000 x g, 00:20:00 , Eppendorf 5804 until the retentate in the inner compartment of the Amicon Filter is reduced to 100 - 150 µl. 20m

Note

Avoid any carry-over of undissolved particles. Repeat centrifugation in step 4, if needed. The remaining bone pellet/debris can be stored at -20°C and reserved for a second round of DNA extraction. However, it may not be time-efficient as the DNA recovery rate is often 0 - 5% compared to the first round.

6 Use a 200-µl extended length tip to pipette the retentate into a new 2-ml DNA LoBind tube. Add 10x volume of Buffer PB to the retentate. Mix well by pipetting up and down. Incubate Room temperature 00:05:00 . 5m

7 Transfer the retentate-PB mix (~600 µl) into a MinElute spin column and centrifuge in a microcentrifuge 12000 rpm, 00:01:00 . Place the MinElute column to a new collection tube and discard the old one with the flow-through. Repeat go to step #7 until all the mix has passed through the MinElute column. 1m

8 Place the MinElute column to a new collection tube and discard the old one with the flow-through. Wash the MinElute column with 720 µL Buffer PE and centriuge 12000 rpm, 00:01:00 . 1m

8.1 Transfer the MinElute column to a new collection tube and discard the old one with the flow-through and centrifuge 12000 rpm, 00:01:00 to remove residual ethanol. 1m

9 Place the MinElute column in a clean 1.5 ml DNA LoBind tube without lid. Elute the DNA by slowly adding 55 µL Buffer EB drop-wise to the middle of the column, without touching the silica membrane. Incubate 37 °C 00:05:00 . Centrifuge the column/tube at 12000 rpm, 00:01:00 . 6m

9.1 Repeat by adding another 55 µL Buffer EB drop-wise to the middle of the same column/tube and incubate 37 °C 00:05:00 . Centrifuge the column/tube at 12000 rpm, 00:01:00 . 6m

10 Remove the MinElute column and transfer the eluted DNA extract into a new 1.5 ml DNA LoBind tube (total volume ~110 µl). Label the tube with "sample ID, DNA extract and date". Store the DNA extract at -20 °C .

Protocol references

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