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We use this protocol and it's working

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Multi-Step Ancient DNA Extraction Protocol For Bone And Teeth

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ABSTRACT

The protocol described here is a multi-day extraction protocol for the recovery of fragment DNA molecules from bone or teeth powder obtained from ancient or historical remains.

The protocol is based on a silica-column method described initially in (Yang et al, 1998). Further modifications were made to this base protocol and reported (MacHugh et al, 2000), (Gamba et al, 2014), (Daly et al, 2018), and (Verdugo et al, 2019).

The instructions presented here describe the totality of these modifications and are one of the aDNA extraction methods employed by the Molecular Population Genetics group at Trinity College Dublin.

MATERIALS

Sodium Hypochlorite (14%, dilute to 0.5%)
Water (Laboratory grade)
EDTA (0.5M, pH 8)
Tris-HCl (1M, pH 7.4)
SDS (2% final)
Proteinase K (2.5 U/mg or 50U/mL)
Tween (20%)
Qiagen PB, PE, and EB Buffers
EBT buffer: 7.5 ul of 20% Tween in 15ml EB buffer

1.5 ml Eppendorf tubes
Parafilm

Thermomixer
Standard bench centrifuge

SAFETY WARNINGS

> The described protocol should be performed in dedicated ancient DNA facilities. Workers should wear full-body PPE (gloves x2, body suits, masks) to avoid contamination of material. Extra care must be made to avoid cross-contamination of low concentration material typical of ancient/historical DNA projects.

Stage 1 - Preparation (day 1)

30m

1 In advance of extraction, prepare bone powder. Measure 0.09 mg to 0.120 mg of bone powder into a 2 mL Eppendorf tube

2 Prepare the extraction buffer where n is the number of sample and control tubes plus one (for pipetting error). In the example below 15 sample tubes and one extraction control tube are used (15 + 1 + 1 = 17) 30m

When preparing the extraction buffer and **prior to the addition of proteinase K**, subject the buffer to 00:30:00 (30 minutes) of UV light

	A	B	C	D
	Reagent	x 1 (ul)	x 17 (ul)	x n (ul)
	Tris-HCl, 1M	20	340	
	SDS (2% final)	17	289	
	EDTA, 0.5	940	15,980	
	*** UV Prior To The Addition Of Proteinase K ***			
	Proteinase K (50 U/mL)	13	221	

Stage 2 - Extraction (day 1)

3 To each tube add 990 µL of extraction buffer to each tube of bone powder

4 Cover each tube in parafilm and vortex until the bone pellet is completely in solution.

5 Incubate Overnight (20-24h) using a thermomixer 700 rpm, 37°C.

Stage 3 - Preparation (day 2)

40m

6 Prepare a fresh batch of extraction buffer where *n* is the number of sample and control tubes plus one (for pipetting error). In the example below 15 sample tubes and one extraction control tube are used (15 + 1 + 1 = 17) 30m

When preparing the extraction buffer and **prior to the addition of proteinase K**, subject the buffer to 00:30:00 (30 minutes) of UV light

	A	B	C	D
	Reagent	x 1 (ul)	x 17 (ul)	x n (ul)
	Tris-HCl, 1M	20	340	
	SDS (2% final)	17	289	
	EDTA, 0.5	940	15,980	
	*** UV Prior To The Addition Of Proteinase K ***			
	Proteinase K (50 U/mL)	13	221	

7 Remove each tube from incubation, then spin each sample tube using a bench centrifuge for 00:10:00 (10min) at 10000 rpm. 10m

8 Transfer the supernatant to new labelled 1.5 mL tubes, parafilm and freeze.
Note: DNA can be purified from this supernatant. Defrost and move onto "Stage 7: Purification"

Stage 4 - Extraction (day 2)

9 Add 990 µL of extraction buffer to each sample tube containing the remaining bone pellet.

10 Cover each tube in parafilm and vortex until the bone pellet is completely in solution.

11 Incubate Overnight (20-24h) using a thermomixer 700 rpm, 37°C.

Stage 5 - Preparation (day 3)

40m

12 Remove each tube from incubation, then spin each sample tube using a bench centrifuge for 00:10:00 (10 min) at maximum speed 13300 rpm / 17000 x g. 10m

If a substantial amount of bone pellet remains, an additional extraction step can be performed, for a total of three overnight digestions.

13 Optional additional extraction: prepare a fresh batch of extraction buffer where *n* is the number of sample and control tubes plus one (for pipetting error). In the example below 15 sample tubes and one extraction control tube are used (15 + 1 + 1 = 17) 30m

When preparing the extraction buffer and **prior to the addition of proteinase K**, subject the buffer to 00:30:00 (30 minutes) of UV light

	A	B	C	D
	Reagent	x 1 (ul)	x 17 (ul)	x n (ul)
	Tris-HCl, 1M	20	340	
	SDS (2% final)	17	289	
	EDTA, 0.5	940	15,980	
	*** UV Prior To The Addition Of Proteinase K ***			
	Proteinase K (50 U/mL)	13	221	

14 Optional additional extraction: transfer the supernatant to new labelled 1.5 mL tubes, parafilm and freeze.

Note: DNA can be purified from this supernatant. Defrost and move onto "Stage 7 - Purification"

Stage 6 (optional) - Extraction (day 3)

15 To each tube add 990 µL of extraction buffer to each tube of bone powder.

16 Cover each tube in parafilm and vortex until the bone pellet is completely in solution.

17 Incubate Overnight (20-24h) using a thermomixer 700 rpm, 37°C.

Stage 7 - Purification (final day)

18m

18 Optional: If a third overnight extraction was performed, remove each tube from incubation, then spin each sample tube using a bench centrifuge for 00:10:00 (10 min) at maximum speed 13300 rpm / 17000 x g. 10m

19 Prepare labelled Amicon filter columns, 1 per sample. Add 3 mL of TrisEDTA (x1) to each Amicon filter and add the supernatant (~ 1 mL) to the appriopriate column, **taking care not to transfer bone powder**. The remaining bone powder tubes and can frozen and re-extracted if needed.

20 Spin columns at 2500 rpm (1200 x g) in a centrifuge with a swing bucket rotor until the supernatant + TrisEDTA is at the 250 µL mark**.** This is typically 6-10 minutes, but sometimes longer.

21 Remove the columns from the centrifuge. Add an additional 3 mL of TrisEDTA (x1) to the filters.

22 Spin at 2500 rpm (1200 x g) in a centrifuge with a swing bucket rotor until the supernatant + TrisEDTA is at the 100 µL mark. Discard flow through and keep the remaining 100 µL in the filter.

23 Prepare a MinElute Qiagen for each sample with 500 µL of PB and add the remaining 100 µL from the Amicon filter. Spin the MinElute columns for 13300 rpm, 00:01:00 (17000 x g). Discard the flow-through. 1m

24 Add 750 µL of PE buffer Qiagen Catalog #19065 to each MinElute Qiagen column. Spin the MinElute columns for 13300 rpm, 00:01:00 (17000 x g). Discard the flow-through. 1m

25 Dry-spin the MinElute Qiagen columns for 13300 rpm, 00:01:00 (17000 x g). 1m

26 Place each column in a fresh 1.5 mL labeled collection Eppendorf tube. These are the final tubes and will contain the purified DNA.

27 Heat EBT (50 µL x *n*, where *n* is the number of tubes plus one) to 65 °C using a thermomixer.

28 Using a pipette, add 50 µL of EBT (see material) to the filter of each spin column. Wait 00:05:00 (5 min), and spin down columns at maximum speed 13300 rpm / 17000 x g. Collection tube lids should be angled away from the direction of spin. 5m

29 Sample tubes containing the purified DNA extract should be stored in a -20 °C freezer.