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We use this protocol and it's working

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Decontamination of tooth roots/petrous bone cores for ancient DNA extraction

In 1 collection

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ABSTRACT

Protocol for decontamination of tooth roots and petrous bone cores from archaeological human remains for reduction of surface contaminations and increase of endogenous DNA prior to DNA extraction.

GUIDELINES

Please read the general guidelines for working in the Ancient DNA protocol collection – University of Tartu, Institute of Genomics.

MATERIALS

Equipment and consumables:

	A	B
	Number	Equipment and consumables
	[# of samples]x2	50 ml tubes
	3	Disposable 100 ml beakers
	[# of samples]	Weighing boats (medium)
	[# of samples]	Weighing boats (small)
	2 of each	Toothbrushes, dental scaler, tweezers
	2	50 ml racks
	[# of samples]/6 (round up)	Lid of multi-rack
	[# of samples]x5 ml plus 100 ml for decontamination	NaOCl (6% v/v)
	[# of samples]x5 ml plus 100 ml for decontamination	MilliQ water
	[# of samples]x5 ml plus 100 ml for decontamination	Ethanol (70%)

Lab equipment:

Laminar flow hood

[Scales]

Other consumables:

DNA ExitusPlus

Paper towels

Aluminum foil

SAFETY WARNINGS

Reagents

NaOCl (bleach) solution (6%)
- H290 May be corrosive to metals.
- H314 Causes severe skin burns and eye damage.
- H411 Toxic to aquatic life with long lasting effects.
- EUH206 Warning! Do not use together with other products. May release dangerous gases (chlorine). Remove from surface after recommended incubation time with water-soaked tissue.

DNA ExitusPlus
H319 Causes serious eye irritation.

Ethanol
- H225 Highly flammable liquid and vapor.
- H319 Causes serious eye irritation.

Equipment

UV radiation
- UV radiation can damage eyes and can be carcinogenic in contact with skin. Do not look directly at unshielded UV radiation. Do not expose unprotected skin to UV radiation.
- UV emitters generate ozone during operation. Use only in ventilated rooms.

BEFORE START INSTRUCTIONS

Previous step:
This protocol follows the sampling protocol (tooth roots and petrous bones).

Following step:
Proceed with the extraction protocol (chunk samples/high volume).

Equipment and consumables:

	A	B
	Number	Equipment and consumables
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	[# of samples]/6 (round up)	Lid of multi-rack
	[# of samples]x5 ml plus 100 ml for decontamination	NaOCl (6% v/v)
	[# of samples]x5 ml plus 100 ml for decontamination	MilliQ water
	[# of samples]x5 ml plus 100 ml for decontamination	Ethanol (70%)

Preparation

1 Clean drill hood and table bench surfaces with DNA Exitus and rinse with water

2 Set up 100 ml beaker decontamination station:
1x NaOCl (bleach, 6% v/v)
1x MilliQ water
1x Ethanol

3 Place toothbrush and tweezers in bleach.

4 Prepare two 50 ml tubes per sample, label with sample ID and B for bleach and E for ethanol.

5 Add around 5 mL NaOCl (bleach, 6% v/v) to each B tube (sample needs to be submerged).

6 Add around 5 mL ethanol (70%) to each E tube (sample needs to be submerged).

7 Place paper towels on a drying rack (e.g. upside-down multi-rack lid) and tape in place. Label with sample IDs. Place in the hood on a piece of aluminum foil and UV the rack in the drill hood while decontaminating samples.

Decontamination

8

Note

Change gloves during the Decontamination steps whenever they get dirty or wet.

9

Note

Steps 10.1-10.3 only apply if the samples is visibly dirty.

9.1 Put out one medium weighing boat for each sample and label it in the corner.

9.2 Take sample to be used for extraction and place in weighing boat labeled with sample ID.

9.3 Remove surface dirt gently from the sample using the toothbrush soaked in NaOCl. Be careful with scrubbing to not spray bleach/dirt onto yourself or other samples.

10 Place samples in NaOCl (B tube) for 00:05:00 and shake while incubating. 5m

11 Pour excess NaOCl down the sink with normal faucet running to dilute the bleach, careful not to drop the samples.

12 Rinse with MilliQ water 3 times.

13 Shake, pour out excess water, and place sample in ethanol for 00:02:00. 2m

14 Wash down sink with normal facet to dilute/remove remaining bleach in the drain.

15 Pour out excess ethanol from tubes into the sink and place back into the rack. Rinse with normal faucet water when finished.

Drying

2h

16 In the hood, place paper towels in front of the rack for catching excess ethanol. One at a time, remove as much ethanol as possible before placing samples onto the square with the corresponding sample ID on drying rack.

17 Leave UV on while allowing the samples to dry at least for 02:00:00 or Overnight . 2h

18

Note

Either continue on the same day with the extraction protocol or stop here:

To continue, see with protocol "ancient DNA extraction (chunk samples/high volume)".

To stop, prepare weighing boats (small), one for each sample, and label with sample ID. Tare scale with small weighing boat labeled with the sample number, place tooth/petrous core on and record weight. Place sample in a respective tube (5 ml tube for <250 mg; 15 ml tube for >250 mg).