UNIVERSITY OF TARTU
Institute of Genomics

VERSION 1

MAR 30, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.n92ldzbpxv5b/v1

Protocol Citation: Marcel Keller, Christiana L Scheib 2023. Ancient DNA extraction (chunk samples/high volume). **protocols.io** https://dx.doi.org/10.17504/protocols.io.n92ldzbpxv5b/v1

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Protocol status: Working
We use this protocol and it's working

Created: Nov 26, 2021

Last Modified: Mar 30, 2023

PROTOCOL integer ID: 55435

Ancient DNA extraction (chunk samples/high volume) V.1

In 1 collection

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ABSTRACT

Extraction protocol for ultra-short ancient DNA molecules from skeletal chunk samples modified from Dabney et al. (2013) PNAS (doi: 10.1073/pnas.1314445110).

GUIDELINES

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

MATERIALS

Reagents:

	A	B	C	D	E	F	G
	Step	Reagents	Conc.	Unit	Manufacturer	Kit/full description	Product number
	Extraction	EDTA pH 8.0	0.5	M	Fisher Scientific	N/A	BP24821
	Extraction	Proteinase K	N/A	N/A	Roche	High Pure Viral Nucleic Acid Large Volume Kit	05114403001

Equipment and consumables:

	A	B
	Number (for 15 samples + 1 blank)	Equipment and consumables
	depending on sample weight	5 ml tubes
	depending on sample weight	15 ml tubes
	15	weighing boats (small)

Keywords: ancient DNA, aDNA, archeogenetics, archaeogenetics, paleogenetics, palaeogenetics, DNA extraction

	A	B
	1	tweezers
		Parafilm
	1	5 ml rack
		200 µl filter tips
		1000 µl filter tips
		5000 µl filter tips
	5.5 ml	Ultra-pure water (for Proteinase K if necessary)

Lab equipment:
Laminar flow hood
Scales
Nutating mixer
200 µl pipette
1000 µl pipette
5000 µl pipette

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.

EDTA

- H373 May cause damage to organs through prolonged or repeated exposure.

Proteinase K

- H315 Causes skin irritation.
- H319 Causes serious eye irritation.
- H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
- H335 May cause respiratory 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.


UV

OZON

BEFORE START INSTRUCTIONS

Previous steps:
This protocol follows the sampling of chunk samples (tooth roots, petrous cores, other bones) and decontamination. Step 2 assumes that the samples have been dried under UV light in the laminar flow hood (see decontamination). Alternatively, the samples can be transferred into tubes.

Following step:
This protocol ends with a 72 h incubation step. Proceed with the purification (chunk samples/high volume) after the incubation.

Equipment and consumables:

	A	B
	Number	Equipment and consumables
	4	disposable 100 ml beakers
	2x100 ml	NaOCl (6% v/v)
	100 ml	MilliQ water
	100 ml	70% ethanol
	2	tweezers
	depending on sample weight	5 ml tubes (LoBind)
	depending on sample weight	15 ml tubes (LoBind)
	[# of samples]	weighing boats (small)
	1	tweezers
		Parafilm
	1	5 ml rack
		200 µl filter tips
		1000 µl filter tips
		5000 µl filter tips

Prerequirements

1

Note

This protocol assumes that the samples are drying in the hood after decontamination when you start the protocol. Alternatively, if the hood needs to be cleared between decontamination and extraction, dry samples can be stored in tubes.

2

Note

This protocol is to be followed by the purification protocol after 3 days (72 h). Please plan the purification of the extracts accordingly.

Extraction

3 Set up decontamination station:
4 disposable 100 ml beakers
6% NaOCl (bleach) aliquot [2x]
MilliQ water [1x]
70% ethanol aliquot [1x]

4 To decontaminate tweezers, soak in 6% bleach for 5 minutes, rinse with MilliQ water, rinse with 70% Ethanol.

5 Clean table bench surface with DNA Exitus and wipe dry. Turn the hood on full power and open the glass.

6

*

Note

Only applicable if the samples have been dried in the hood previously after decontamination.

Move dry samples from the hood to the table bench and put down a new sheet of aluminum foil.

7 Clean hood surface with DNA Exitus and put down a new sheet of aluminum foil. Wipe down reagents with DNA Exitus.

8 Place all reagents (except proteinase K) and consumables in the hood and UV while weighing.

9

Note

Only applicable if the samples were not placed in tubes after drying and weighed before.

Tare scale with small weighing boat labeled with the sample number, place tooth/petrous core on the weighing boat with the tweezers and record weight. Change gloves when finished with weighing.

10 Make EDTA [M] 0.5 Molarity (M) pH 8.0, Proteinase K [M] 18.18 mg/mL, and tube calculations:

	A	B	C	D	E
	Sample ID	Weight (mg)	EDTA (ml)	PK (µl)	Tube
	[Sample ID]	x	2 ml per 100 mg (x/50)	50 µl per 100 mg (x/2)	5 ml < 250 mg < 15 ml
	ABC001A	100	2	50	5
	ABC001A	300	6	150	15

11 Label your tubes for the extractions:

	A	B	C
		Top	Side
	Project ID	PROJ	PROJ
	Sample ID	ABC001A	ABC001A
	Extraction #	1	1

	A	B	C
	Initials		YZ
	Date		DD/MM/YYYY
	Content	P	Pellet

12 If necessary: Make Proteinase K solution by adding 5.5 mL PCR clean water to 100 mg of powder. Shake to dissolve. Let sit before using. Store in the freezer at -20 °C.

13 Add calculated amount of EDTA to each tube. If the tubes are empty you can use the same pipette tip as long as you do not get liquid into the filter or touch any surfaces with the tip.

14 Add the calculated amount of Proteinase K to each tube and pipette-mix. Change your tips each time.

15 Add samples to their individual extraction tube.

16 Make sure that the lids are tightly sealed to avoid leakage.

17 Wipe tubes with DNA Exitus, wrap tubes with Parafilm.

18 Place on the nutating mixer for 24 rpm, Room temperature , 72:00:00 .

Note

Continue with the purification protocol after 72 h.