UNIVERSITY OF TARTU
Institute of Genomics

VERSION 2

MAR 30, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.n92ldzbpxv5b/v2

Protocol Citation: Marcel Keller, Christiana L Scheib 2023. Ancient DNA extract purification (chunk samples/high volume). **protocols.io** https://dx.doi.org/10.17504/protocols.io.n92ldzbpxv5b/v2Version created by Marcel Keller

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

Created: Nov 30, 2021

Last Modified: Mar 30, 2023

PROTOCOL integer ID: 55514

Ancient DNA extract purification (chunk samples/high volume) V.2

In 1 collection

Marcel Keller[1,2], Christiana L Scheib[1,3]

[1]Institute of Genomics, University of Tartu;
[2]Institute of Forensic Medicine, University of Bern;
[3]University of Cambridge

Marcel Keller
Institute of Forensic Medicine, University of Bern, Institut...

ABSTRACT

Protocol for the purification of extracts, 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
	Purification	PB Buffer	N/A	N/A	Qiagen	MinElute PCR Purification Kit	19066
	Purification	PE Buffer	N/A	N/A	Qiagen	MinElute PCR Purification Kit	19065
	Purification	EB Buffer	N/A	N/A	Qiagen	MinElute PCR Purification Kit	28006

Equipment and consumables:

	A	B
	Number	Equipment and consumables
	1	1.5 ml tube rack
	1	5 ml tube rack
	1	50 ml Falcon rack (big)
	1	50 ml Falcon rack (small)

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

	A	B
		100 µl filter tips
		200 µl filter tips
		1000 µl filter tips
		5000 µl filter tips
	[# of samples]+1	1.5 ml tubes
	[# of samples]	MinElute columns
	[# of samples]	Roche columns (assembled with reservoir in Falcon)
	[# of samples]	Sartorius concentrators Vivaspin 15, 30kDa
	1	50 ml Falcon (waste)

Lab equipment:
Laminar flow hood
Centrifuge (50/2/1.5 ml)
Heat block
Mini table centrifuge/vortexer
100 µl pipette
200 µl pipette
1000 µl pipette
5000 µl pipette

Other consumables:
DNA ExitusPlus
Paper towels

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.

Guanidinium hydrochloride (GuHCl) (in PB buffer of Qiagen MinElute kit)
- H302 Harmful if swallowed.
- H332 Harmful if inhaled.
- H315 Causes skin irritation.
- 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 extraction protocol and is to be performed on day 3 after the extraction (after 72 h).

Following step:
Proceed with one of the library preparation protocols.

Equipment and consumables:

	A	B
	Number	Equipment and consumables
	2	1.5 ml tube rack
	1	5 ml tube rack
	2	50 ml Falcon rack (big)
	1	50 ml Falcon rack (small)
		100 µl or 200 µl filter tips
		1000 µl filter tips
		5000 µl filter tips
	[# of samples]+1	1.5 ml tubes
	[# of samples]	MinElute columns
	[# of samples]	Roche columns (assembled with reservoir in Falcon)
	[# of samples]	Sartorius concentrators Vivaspin 15, 30kDa
	1	5 ml tube
	2	50 ml tubes

[# of samples] includes the blank(s).

Prerequirements

1

Note

This protocol follows on the extraction protocol and is to be performed on day 3 after the extraction (after 72 h).

Preparation

1h 30m

2 Turn the hood on full power and open the glass.

3 Spray hood and table bench surfaces with DNA Exitus, let sit a minute and wipe down with paper towels.

4 Wipe down outside surfaces of reagents/tips with DNA Exitus and place in the hood.

5 Label the 50 ml waste tube, PB tube and PE tube as well as the 5 ml EB tube.

6 In the hood, make EB Buffer aliquot (100 µL per sample) in a 5 ml tube.

7 Prepare PE (wash) buffer by adding ethanol.

8 Aliquot PE Buffer to 50 ml tube: [# of samples]x1000 µl plus 10%.

9 Aliquot PB Buffer to 50 ml tube: [# of samples]x2.4 ml plus 10%.

10 Check that the centrifuge rotor is the bucket and add 5 ml inserts. Wipe the rotor and inserts with DNA Exitus after taking them out of the centrifuge and before putting them into the centrifuge.

Purification

1h 30m

11 Bring in samples and on the table bench, remove parafilm and wipe with a small amount of DNA Exitus before placing in the centrifuge.

12 Spin down pellets at 4000 rpm, 00:05:00 . 5m

13 Change gloves and label your concentrators on lids and sides with sample ID numbers.

14 Transfer samples to the hood without disturbing the pellet

15 Add as much supernatant to the labeled concentrators as possible without disturbing the pellet. Chunks of collagen can interfere with the concentration process.

16 Change centrifuge inserts from 5 ml to 50 ml. Wipe inserts with DNA Exitus after taking them out 1h 10m

of the centrifuge and before putting them into the centrifuge. Centrifuge at 4000 rpm for 00:25:00 to 00:45:00 or until extracts are 250 µl.

17 While tubes are spinning: Change gloves and label large volume columns (Roche) with Sample IDs on lids and sides (e.g. 12 on top and 12 on the side) and label Eppendorf 1.5 ml tubes:

	A	B	C
		Top	Side
	Project ID	PROJ	PROJ
	Sample ID	ABC001A	ABC001A
	Extraction #	1	1
	Extraction	"E"	"Extraction"
	Initials		YZ
	Date		DD/MM/YYYY

18 When samples have concentrated, add 2.5 mL or at least 10x [extract volume] PB (binding) buffer to the Roche columns using the 5 ml pipette. Do not let this sit too long as the buffer starts to leak through the membrane quickly.

19 Add concentrated extract to the PB buffer inside the Roche column and gently pipette to mix. Use 1000 µl LONG tips.

Note

Change tip for each sample.

20 Spin Roche columns at 4000 rpm, 00:01:00. 1m

21 Change gloves and place your columns back in the hood.

22 Add 1000 µL of PE (Wash/Ethanol) buffer to each Roche column.

Note

change tip each time.

23 Spin at 4000 rpm, 00:01:00. 1m

24 Change gloves and place your columns back in the hood.

25 Using a clean 1.5 ml tube rack, take a collection tube from the bag without touching the inside of the bag or the inside of the tube and place it in your 1.5 ml rack.

26 Remove the inner column from the 50 ml Roche tube and place it inside the clean collection tube. Pull the release tab on the front and twist it forward to free the column from the funnel. Discard the funnel and small plastic bits.

Note

Do not touch the funnel on the sides, but on the edge on top. Change gloves if you do touch the sides by accident.

27 Close the lid on your column/tube and label immediately with the sample ID.

28 Change gloves and change the rotor in the centrifuge to the 2 ml rotor. Wipe the rotor and inserts

with DNA Exitus after taking them out of the centrifuge and before putting them into the centrifuge.

29 Spin columns + collection tube at 13000 rpm, 00:01:00 to dry the membrane. 1m

30 Turn on the heat block 37 °C.

31 Back in the hood, take the silica column out of the collection tube and place it into the corresponding clean, labeled 1.5 ml Eppendorf lo-bind tube.

32 Change gloves and add 100 µL of EB (Elution) buffer to each sample.

Note

Change tip each time.

33 Incubate samples in the heat block at 37 °C for 00:10:00. 10m

34 Spin at 13000 rpm, 00:02:00. Be careful to arrange lids so that they will not break. 2m

35 Back in the hood, discard the silica column, close lid, wipe tubes with DNA Exitus and wrap with Parafilm if stored for more than a few days before library prep.

36 Store at -20 °C