APR 05, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.6qpvrdr83gmk/v1

Protocol Citation: Marcel Keller, Christiana L Scheib 2023. Sampling of tooth roots for ancient DNA. **protocols.io** https://dx.doi.org/10.17504/protocols.io.6qpvrdr83gmk/v1

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

Created: Mar 25, 2021

Last Modified: Apr 05, 2023

PROTOCOL integer ID: 48558

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

Sampling of tooth roots for ancient DNA

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

Christiana L Scheib
University of Tartu, University of Cambridge

ABSTRACT

Protocol for the sampling of tooth roots of archaeological human remains for the extraction of ancient DNA.

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
	4	disposable 100 ml beakers
	2x100 ml	NaOCl (6% v/v)
	100 ml	MilliQ water
	100 ml	70% ethanol
	2 of each	drill disks/core drills, tweezers, dental scalers, tooth extractors
	1	toothbrush

Lab equipment:
Laminar flow hood
Drill

Other consumables:
DNA ExitusPlus
Paper towels
Aluminum foil

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 introduction of samples into the ancient DNA lab (documentation and changing bags).

Following step:
Proceed with the decontamination protocol.

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 of each	drill disks, tweezers, dental scalers, tweezers
	1	vice
	1	toothbrush

Preparation

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

2 Clean drill hood and table bench surfaces with DNA Exitus and wipe clean with a paper towel.

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 drill bits, drill spanner, tweezers, dental scalers and toothbrush – Soak in 6% bleach for 5 minutes, rinse with MilliQ water, rinse with 70% Ethanol. Clean the drill spanner and tooth extractors with DNA Exitus.

5 Wipe down markers and pens with DNA Exitus.

6 Label your bags and tubes:

Note

Tubes should be closed immediately after taking them from the package and opened only during sampling. This will limit contamination.

	A	B
	Bags for drilled samples	
	Sample ID	ABC001A
	Date	DD/MM/YYYY
	Initials	YZ

	A	B	C
	Tubes e.g. for collecting calculus	Top	Side
	Content		e.g. calculus/drill powder
	Project ID	PROJ	PROJ
	Sample ID	ABC001A	ABC001A
	Date	DD/MM/YYYY	DD/MM/YYYY
	Initials	YZ	YZ

7 Place down sheets of aluminum foil to cover the entire surface of the drill hood.

8 Make an L-shaped “wall” of folded foil that runs along the back of your work area and the side without the drill to keep flying dust from expanding across the hood.

9 Place down a smaller piece of foil for your working surface. Fold small pieces of foil that you can wrap around the beaks of the tooth extractor. Place a big weighing boat onto the working surface. Change this setup for each sample.

10 Gather clean, decontaminated tools for drilling and place them on the foil.

Sampling

11

Note

Aim to do all work as far into the hood as possible to avoid dust escaping and keep eyes behind the glass for protection.

12

Note

Make sure your drill bit/wheel is centered and firmly in the drill before drilling and that your foot is off the pedal when not intending to drill.

13 Turn on the drill in “FWD” mode and choose a medium speed to prevent overheating/burning of the bone or dentine.

14 Put on clean sleeves by pushing your thumbs through the plastic close to the cuffs to keep the sleeve in place, then gloves on top.

15 Place the bag with your sample and the clean sample bag and tube into the hood behind the foil wall.

16 If the tooth has calculus, use the dental scaler to remove it before sampling. Put the tooth into the mini grip bag and hold the tooth outside the bag with your fingers. Now push gently with scaler and collect all calculus inside the bag. Remove the tooth and pour the calculus on weighing paper. Use weighing paper to place calculus in a labeled 1.5 ml tube after taring.

17 Wrap foil strips around the ends of your tooth extractor (for holding the tooth).

18 Hold the tooth with the tooth extractor and drill carefully a few millimeters above the end of the root.

19 When drilling is completed, put your samples in labeled bags. Wipe these down with a small amount of DNA Exitus before adding to your sample box.

Clean-up after each sample

20 Rinse off the drill wheel with MilliQ water first then into your 6% bleach for a few minutes or while you are drilling the next tooth. Then rinse with water and finally ethanol before drying for use again.

21 Carefully remove and throw away the weighing boat, small work surface foil, dirty gloves, and sleeves. Put on new gloves.

22 For the next sample, start from step 9.

Final clean-up

23 When finished for the day, wipe down the tooth extractors and drill spanner with DNA Exitus and put away.

24 Use a clean toothbrush with a little DNA Exitus to remove sample powder from the top/inside of the drill head.

Note

Do not use bleach! This will rust the inner components.

25 Leave drill bits and scalers to soak in bleach while you throw away all foil and other consumables and wipe down all inner hood work surfaces and the outside of the drill with DNA Exitus and wipe dry with paper towels.