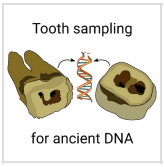

Version 1

Dec 04, 2020

Tooth Sampling from the inner pulp chamber for ancient DNA Extraction V.1

In 1 collection

Gunnar U Neumann[1], Aida Andrades Valtuena[1], James A Fellows Yates[1], Raphaela Stahl[1], Guido Brandt[1]

[1]Max Planck Institute for the Science of Human History

2 *Works for me* dx.doi.org/10.17504/protocols.io.bakqicvw

MPI-SHH Archaeogenetics

James Fellows Yates
Max Planck Institute for the Science of Human History

ABSTRACT

This protocol describes how to obtain powder from the inner pulp chamber of teeth for the extraction of ancient DNA. It is ideal for the simultaneous isolation of both host endogenous and microbial DNA (e.g. for pathogens).

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COLLECTIONS

Ancient DNA optimised protocols for Illumina Next Generation Sequencing

KEYWORDS

Tooth, ancient DNA, sampling

IMAGE ATTRIBUTION

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31088

PARENT PROTOCOLS

Part of collection
Ancient DNA optimised protocols for Illumina Next Generation Sequencing

GUIDELINES

Working in an Ancient DNA Laboratory
All steps of the protocol should take place in a clean room facility specifically designed for ancient DNA.

The researcher performing lab work should be dressed in correspondingly suitable lab-wear, such as:
- full-body suit with hood (e.g., Tyvek)
- hairnet
- face mask
- two pairs of clean gloves
- clean shoes
- protective glasses

- Sample processing should be carried out in separated work benches with integrated UV irradiation (e.g. Dead Air PCR work bench)
-Surfaces and equipement should be regularly decontaminated with e.g. bleach solution and/or Thermofisher's DNA AWAY (or similar).

Please see the following for more detailed guidance:

Llamas, B. et al., 2017. From the field to the laboratory: Controlling DNA contamination in human ancient DNA research in the high-throughput sequencing era. *STAR: Science & Technology of Archaeological Research*, 3(1), pp.1–14. Available at: https://doi.org/10.1080/20548923.2016.1258824.

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MATERIALS TEXT

MATERIALS

Safe-Lock Tubes 2 ml Biopur (preferably packed individually) **Eppendorf Catalog #0030121597**

Weighing Paper MN 226 block of 100 sheets 9 x 115 cm **MACHEREY-NAGEL GmbH & Co. KG Catalog #186002**

Weighing pans ROTILABO® blue antistatic 330 ml 140 mm 140 mm **Carl Roth Catalog # 2159.2**

DNA AWAY® 4000 ml **Carl Roth Catalog #X996.2**

Lab equipment

PCR work bench (e.g. AirClean Dead Air PCR Werkbank, 48´´)
Air extraction/vacuuming unit (optional)
UV irradiation box or shortwave cross linker (e.g. Vilber Lourmat Bio-Link BLX-254)
Drill Lab Handpiece (e.g. K-POWERgrip™ Installation Lab Handpieces from Kavo Dental Excellence; SKU: 10022916)
Round diamond saw blades (e.g. Diamond Discs Superflex, 22 mm from Kahla, SKU 806 104 358 514 220)
Rounded dental drill bit (e.g. NT1 from Kahla; SKU: H1-016 HP)
Scientific scale (e.g. Ohaus Adventerer balance AX124; SKU: OHAUS 611-3213)
Anti-static instrument (e.g. Zerostat 3 from Zerostat; SKU: SAFAZ108812) (optional)
Thin nose pliers
Aluminium foil
Paper towels
Polyethylene clear plastic bags
Bench vice (optional)
Camera

Generic Reagents

UV-irradiated and deionised tap water
Solution of household bleach (2-6% NaClO, then diluted to a working solution concentration of 0.2-0.5% NaClO)
DNA away

SAFETY WARNINGS

Reagents

Household bleach solution (2-6%) diluted to a working concentration of 0.2-0.5 % NaClO in total.
Remove from surface after recommended incubation time with water-soaked tissue.
- 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 AWAY
- H290 May be corrosive to metals.
- H314 Causes severe skin burns and eye damage.

Note: Both bleach solutions and DNA AWAY are used for decontamintation. DNA AWAY is less corrosive than bleach and should be preferred for decontamination of sensitive equipments such as surfaces of electric devices.

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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.

Usage of sharp tools
Always hold the sample with pliers while cutting and drilling to avoid injuries

ABSTRACT

This protocol describes how to obtain powder from the inner pulp chamber of teeth for the extraction of ancient DNA. It is ideal for the simultaneous isolation of both host endogenous and microbial DNA (e.g. for pathogens).

BEFORE STARTING

General considerations
Teeth are often also used for isotope and other anthropological studies. Please consider ethical use and stewardship, by sharing remaining valuable source material and coordinating with sample providers and project partners. For example, in the use case here, samples from the tooth enamel and dentine could also be used for further in isotope or radiocarbon dating studies after being sampled for DNA.

Planning
The sampling procedure of any skeletal element for DNA extraction should be performed ideally in a dedicated sampling room. This should **not** happen in a buffer preparation room.

Sampling of each tooth takes around 20 minutes, but this can vary depending on the nature of the sample.

Equipment
Make sure all necessary equipment is available (see Materials)

Documentation
This protocol is destructive, therefore for all samples consultation and permission with curators should be performed prior running this protocol.

All anatomical and morphological features of the tooth should be documented (e.g. pre-sampling photos, CT-scans, moulds) before sampling.

Dirt/soil should be removed from the sample (e.g. by scraping off with a dental scalar) to avoid contamination.

Workstation preparation

1

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Everything that comes into contact with the sample needs to be decontaminated in order to avoid cross-contamination between samples. Change gloves regularly, especially between different samples.

Place a sheet of aluminium foil under the hood.

Place weighing paper and drill bits on a sheet of aluminum foil on an easily accessible clean surface outside of the hood. Alternatively, you may place drill bits and weighing paper within hood but be sure to cover them with aluminum foil or a paper towel to protect them from dust produced during sampling.

Install the air extraction unit, if present, to reduce airborne dust during sample cutting.

You will also need:

- 2 ml Safe-Lock Biopur tubes
- Weighing trays/pans (optional)
- Clean and labelled sample bags according to the amount of samples
- Paper towels
- Diluted commercial bleach solution (1:10)
- UV-irradiated filtered/deionised water

Figure 1. Set up of the workstation

Preparation of sample

2 Place a sheet of aluminium foil in the UV chamber.

Place the tooth on the aluminium foil, and place the labeled sample bag next to it *(to avoid mixing up samples in case you work on more than one)*.

UV- irradiate the tooth for 15 minutes on each side.

If possible, avoid touching the tooth directly. Use precision forceps or similar to handle each tooth individually.

Tip: it is possible to UV irradiate multiple teeth at once in the same UV chamber. Ensure each tooth is on a separate bit of foil, and keep the corresponding labelled sample bag next to it to prevent sample mix up.

3 Take a new 2 ml tube and label it accordingly. If this is the beginning of the sampling session, tare the precision scale with the tube placed in the center.

Label a new sample bag with the sample ID.

To avoid contamination, tubes should remain closed except when the tooth powder is added.

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4 Remove the tooth from the UV chamber and tranfer it to the aluminium foil in the hood. Check the tooth for calculus. If present, sample the deposit by following this protocol (ensure thorough clean up of work space before continuing).

Sampling procedure

5 On a weighing tray, hold the crown of the tooth with the pliers and use the electric drill with a diamond saw blade to cut the tooth at the cementum-enamel junction (CEJ) to separate the roots from the crown.

Alternatively, you can use a manual jeweler's jigsaw for cutting. In this case, fix the tooth in a foil-covered bench vice.

6 Remove any sawing powder that may be trapped inside the pulp chamber and root canals by gently tapping onto the weighing tray. Hold the samples with pliers.

Powder from the sawing procedure should not be used for extraction as it make contain modern surface contamination and should be set aside (clearly labeled) or discarded.

7 Put the weighing tray/pan to the side.

Take a fresh piece of weighing paper, slightly fold it mid-way and place it under the area where you want to sample in the inner pulp chambers.

Bone powder can be statically charged and difficult to transfer to tubes. By creasing the weighing paper in advance, it makes it easier to transfer the bone powder from the weighing paper into the tube when pouring.

8 Replace the diamond circular saw blade of the electric drill with a diamond ball bit.

9 Hold the crown with the pliers above the weighing paper. With the drill at low speed (and high torque), drill from inside the pulp chamber and collect the powder on the weighing paper.

In addition, you can also sample from inside the roots, if needed.

Teeth are often used as material to screen for blood-borne pathogens. For this, it is better to sample the surfaces of the pulp chamber (instead of drilling deep into the dentine) because this is where the highly vascularized pulp tissue existed during life. Deeper sampling will result in the collection of more acellular/anuclear, non-blood associated dentine tissue and will reduce possible pathogen DNA signals as well as the amount of endogenous human DNA.

Pro-Tip: If residual dirt/soil is mixed in with your collected *clean* powder, you can physically separate them by carefully tipping the edges of the weighing paper repeatedly. In doing so, the different weights and particle sizes will easily separate. Discard the dirt particles.

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cut_tooth_drawing_smooth_diagram.png

Figure 2: Sampled tooth. Crown and roots are seperated and drilling was done from inside the pulp chamber.

Weighing of tooth powder

10 Transfer the *clean* powder bone into a labeled 2 ml Safe-Lock Biopur tube. The folded weighing paper will help guide the powder into the tube.

Close the tube, wipe it with a damp paper towel and weigh the tube.

Tooth powder can become statically charged. In such case, consider using an anti-static gun and apply to the weighing paper and tube.

Ideally you wish to collect around **30 mg** to **50 mg** of powder for extraction (if using our protocol for ancient DNA Extraction). If you have more than **50 mg** then store it as a back-up in a separate tube.

Write down the weight in mg on the cap and on the side of the tube.

Store the bone powder at **-20 °C** until further processing.

11 Take photos after sampling for documentation.

Put tooth parts back into a new, UV irradiated, labeled bag.

The remaining material should be stored adequately so they can be passed on to other colleagues for additional analyses (e.g. isotope studies), to minimise unnecessary additional sampling of valuable ancient specimens.

Decontamination

12

Everything that came in contact with the sample needs to be cleaned/decontaminated in order to avoid cross-contamination between samples.

Carefully clean the workspace and the equipment before sampling the next sample:

12.1
- Throw away disposable material such as aluminium foil and plastic weighing tray/pan.
- Clean surfaces and tools such as pliers with bleach solution
- Use less aggressive DNA decontamination reagents (e.g. DNA away) for sensitive material such as electronic devices (e.g. handpiece of the drill, precision balance).
- Wipe off bleach with water-wet paper towels afterwards.

Saw blades and drill bits can be reused after careful cleaning:

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12.2
- incubate them in bleach solution (1:10 dilution) for at least 2 minutes.
  You can use a UV irradiated and bleached toothbrush for brushing the saw blade.
- Clean with UV irradiated water to remove all bleach and let it dry.

Check conditions of drill bits after ~5 uses and replace with new bits if there is excessive wear.

13 **go to step #2** (or in case of UV irradiation of multiple samples at once, **go to step #3**) and repeat the sampling procedure with the next sample.

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