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Minimally-invasive sampling of *pars petrosa* (*os temporale*) for ancient DNA extraction V.1

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1 *Works for me* dx.doi.org/10.17504/protocols.io.bdyvi7w6

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ABSTRACT

This protocol describes how to obtain bone powder from the *pars petrosa* of disarticulated *ossis temporalis*, specifically from the dense parts around the cochlea, in a minimally-invasive way by drilling from the outside.

The *pars petrosa* has been shown to consistently yield high amounts of aDNA (Pinhasi *et al.* 2015 *PLoS One,* doi: 10.1371/journal.pone.0129102).

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KEYWORDS

ancient DNA, petrous pyramid, sampling, non-invasive method, archaeogenetics, archaeology

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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 wear 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 equipment should be regularly decontaminated with e.g. bleach solution or Thermofisher's DNA AWAY (or similar) and irradiated with UV.

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

Lab materials

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

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

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

☒ Precision forceps 18/10 steel extra sharp bent points without ridges L=105mm **VWR Scientific Catalog #232-0008**

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

Lab equipment

PCR work bench (e.g. AirClean Dead Air PCR Werkbank, 48´´)
UV irradiation box or cross linker (e.g. Vilber Lourmat Bio-Link BLX-254)
Air extraction/vacuuming unit
Drill Lab Handpiece (e.g. K-POWERgrip™ Installation Lab Handpieces from Kavo Dental Excellence; SKU: 10022916)
Rounded dental drill bit (e.g. NT1 from Kahla; SKU: H1-016 HP)
Balance (e.g. Ohaus Adventurer balance AX1502)
Anti-static instrument (e.g. Zerostat 3 from Zerostat; SKU: SAFAZ108812)
Aluminium foil (lab grade or sterile preferred)
Paper towels
Polyethylene clear plastic bags
Marking or masking tape
Camera

General Reagents

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

SAFETY WARNINGS

Reagents

Household bleach solution (2-6%) diluted to a working concentration of 0.2-0.5 % NaClO in total.
- 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
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Note: Both bleach solutions and DNA AWAY are used for decontamination. DNA AWAY is less corrosive than bleach and should be prefered for decontamination of sensitive equipments such as surfaces of electric devices.

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

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This protocol describes how to obtain bone powder from the *pars petrosa* of disarticulated *ossis temporalis*, specifically from the dense parts around the cochlea, in a minimally-invasive way by drilling from the outside.

The *pars petrosa* has been shown to consistently yield high amounts of aDNA (Pinhasi *et al.* 2015 *PLoS One,* doi: 10.1371/journal.pone.0129102).

BEFORE STARTING

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 the buffer preparation room.

Sampling of each pars petrosa takes around 20-40 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 *pars petrosa* should be documented (e.g. pre-sampling photos, CT-scans) before sampling.

If petrous bones from both sides are available, sample only from one side, in order not to cause alterations to the external morphology of both.

Workstation preparation

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

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
- Small pieces of aluminium foil for wrapping the bone
- Small tape strips to fix the aluminium foil while covering the bone
- Paper towels
- Diluted commercial bleach solution (1:10)
- UV-irradiated filtered/deionised water

P1080849.JPG

Figure 1. Set up of the workstation

Preparation of sample 15m

2 Place a sheet of aluminium foil in the UV chamber. 15m

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

UV- irradiate the bone for **00:15:00** on each side.

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

Tip: It is possible to UV irradiate multiple bones at once in the same UV chamber. Ensure each bone 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.

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

4 Remove the bone from the UV chamber by using a paper towel with a small amount of household bleach solution.

Wipe the surface of the bone region you want to sample with a bleach-dampened paper towel and then carefully clean the bone with a water-dampened paper towel to remove excess bleach. Use another sheet to wipe the bone dry.

If the bone was not washed after excavation this can be a tedious step. Make sure you brush/shake off as much of the residual soil as possible before starting.

5 Take a weighing tray with a new pair of gloves.

Prepare a sheet of weighing paper by creasing it mid-way and place it under the area where you want to sample.

Bone powder can be statically charged and difficult to transfer to tubes. By slightly folding the weighing paper, it makes it easier to move the bone powder from the weighing paper into the tube.

Sampling procedure

6 Minimally-invasive sampling of *pars petrosa*

6.1 Familiarize yourself with the anatomy of the petrous bone (e.g., Pinhasi et al. 2015). Locate the dense parts around the cochlea (b & c) as shown below in Figure 2.

Ideally you will target area c (orange) shown in Figure 2, which is located roughly halfway between the jugular fossa (bottom) and the petrous crest (top). However, since this protocol does not cut the petrous bone in half, it will be easier to orient the bone so that your are looking at the posterior part, which contains the internal acoustic opening (*meatus acusticus internus*). To target the cochlear region you will have to position the drill a few millimetres laterally (the outer side where the temporale is/would be attached) and choose an angle so that the drill hole will not penetrate the auditory canal (Figure 3). Of note, the auditory canal also leads laterally/dorsally outwards, so it is advisable to drill in a parallel line.

Ron Pinhasi, Daniel Fernandes, Kendra Sirak, Mario Novak, Sarah Connell, Songül Alpaslan-Roodenberg, Fokke Gerritsen, Vyacheslav Moiseyev, Andrey Gromov, Pál Raczky, Alexandra Anders, Michael Pietrusewsky, Gary Rollefson, Marija Jovanovic, Hiep Trinhhoang, Guy Bar-Oz, Marc Oxenham, Hirofumi Matsumura, Michael Hofreiter (2015). Optimal Ancient DNA Yields from the Inner Ear Part of the Human Petrous Bone. PLoS ONE. https://doi.org/10.1371/journal.pone.0129102

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journal.pone.0129102.g001.PNG

Figure 2. Dense parts around cochlea (**b & c**). Part **c** provides higher endogenous aDNA yields than part **b** and **a**. (Pinhasi et al. 2015).

Screenshot 2020-03-26 at 11.55.54.png

Figure 3. Before (top) and after (bottom) sampling of pars petrosa.

6.2 To prevent residual dust/dirt from the outer surface and crevices to contaminate your freshly drilled bone powder, you can wrap the sample with aluminium foil and/or tape. Leave the part that you want to drill unwrapped. For ease of orientation, mark the position of the internal auditory meatus with a permanent marker (Figure 4).

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Figure 4. Wrapping the pars petrosa with aluminium foil and marking of the auditory canal.

6.3 Use the drill to clean (abrade) the surface of sample window at low speed.

Discard or set aside the powder from this cleaning step and place a new weighing tray and/or weighing paper under the sample.

6.4 Drill at low speed (and high torque) from the outside towards the cochlear region in parallel to the auditory canal.

Collect *clean* bone powder on the weighing paper. Of note, it is very likely that you will drill into internal canals and porosities that can be filled with soil. If so, stop and try to reorient your drilling angle.

Empty the drilling hole by carefully tipping it out onto the collection paper.

Pro-Tip: If residual dirt/soil is mixed in with your collected clean bone 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 or set aside the dirt.

Be careful when unwrapping the bone, the vibration of the drilling will loosen residual dirt/soil and bone powder, which will accumulate in the wrapping.

Weighing of bone powder

7 Transfer the clean powder bone into a labelled 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.

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

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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 back-up material 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.

8 Take photos after sampling for documentation.
Put bone back into a new, UV irradiated, labelled bag.

Decontamination

9

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:

9.1
- Throw away disposable material such as aluminium foil and 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 to prevent corrosion; air dry.

9.2 Drill bits can be reused after careful cleaning:
- 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 if necessary.

10 **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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