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Wedge sampling of pars petrosa (os temporale) for ancient DNA extraction



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We use this protocol and it's working

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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 more precise way by cutting a wedge into the bone before drilling.

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](https://doi.org/10.1371/journal.pone.0129102)).

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
 - three 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>.

Materials

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 × 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)

Round diamond saw blades (e.g. Diamond Discs Superflex, 22 mm from Kahla, SKU 806 104 358 514 220)

Tooth plier (e.g. mf-dental.de, Zahnzange, OK Prämolaren, Anatom. Griff, A127)

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

- H290 May be corrosive to metals.
- H314 Causes severe skin burns and eye damage.



Note: Both bleach solutions and DNA AWAY are used for decontamination. DNA AWAY is less corrosive than bleach and should be preferred 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





Before start

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

10m

1

10m

Safety information

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 a pair of tooth pliers or forceps on a second sheet of aluminium foil on an easily accessible clean surface outside of the hood. Alternatively, you may place them within the 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 Biopure tubes
- Weighing trays/pans
- Clean and labelled sample bags according to the amount of samples
- Small pieces of aluminium foil for wrapping the bone (optional)
- Small tape strips to fix the aluminium foil while covering the bone (optional)
- Paper towels
- Diluted commercial bleach solution (1:10)
- UV-irradiated filtered/deionised water

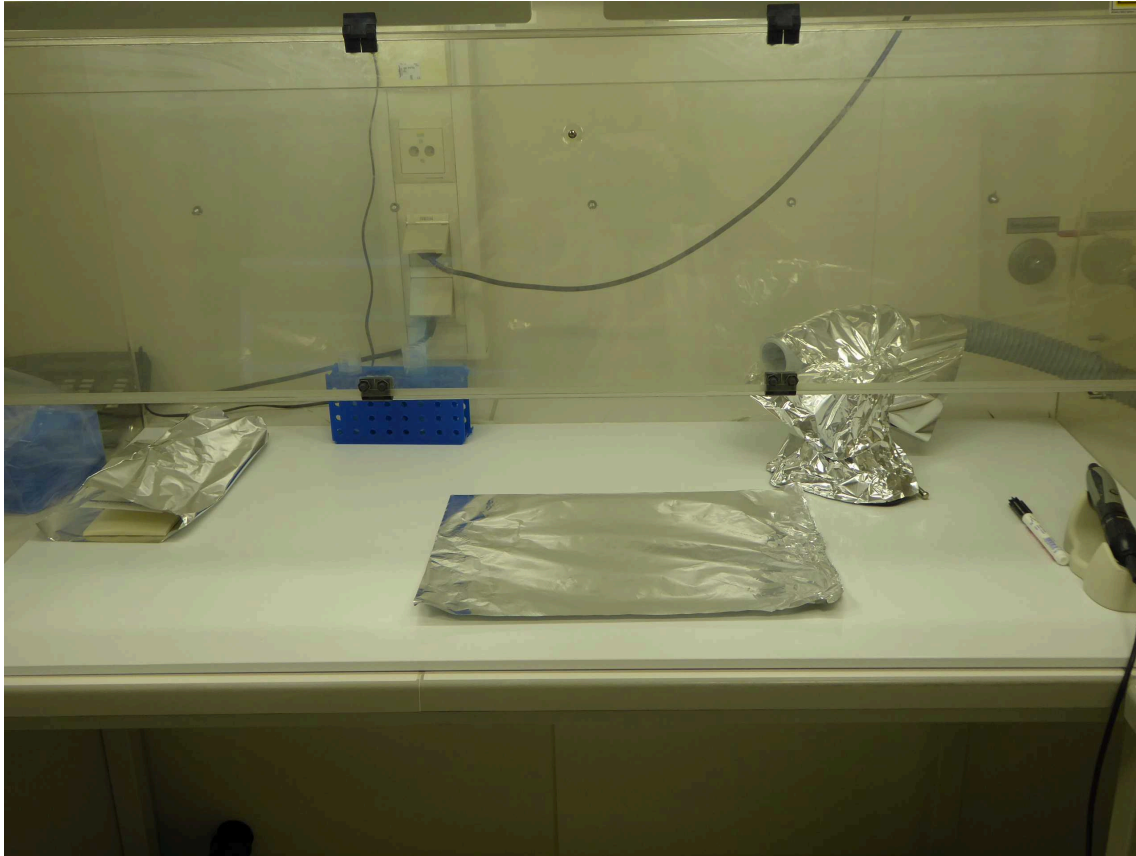


Figure 1. Possible Set up of the workstation

Preparation of sample

30m

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

30m

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.

Safety information

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



Note

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 with the sample ID on top of the lid and on the side of the tube. Tare the precision scale with the tube placed in the center.

Label a new sample bag with the sample ID to be used after sampling.

Safety information

To avoid contamination, tubes should only be opened inside the hood and remain closed except when the bone powder is added.

- 4 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.

Note

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

- 5 Wedge sampling of *pars petrosa*

- 5.1 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).

CITATION

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.

LINK

<https://doi.org/10.1371/journal.pone.0129102>

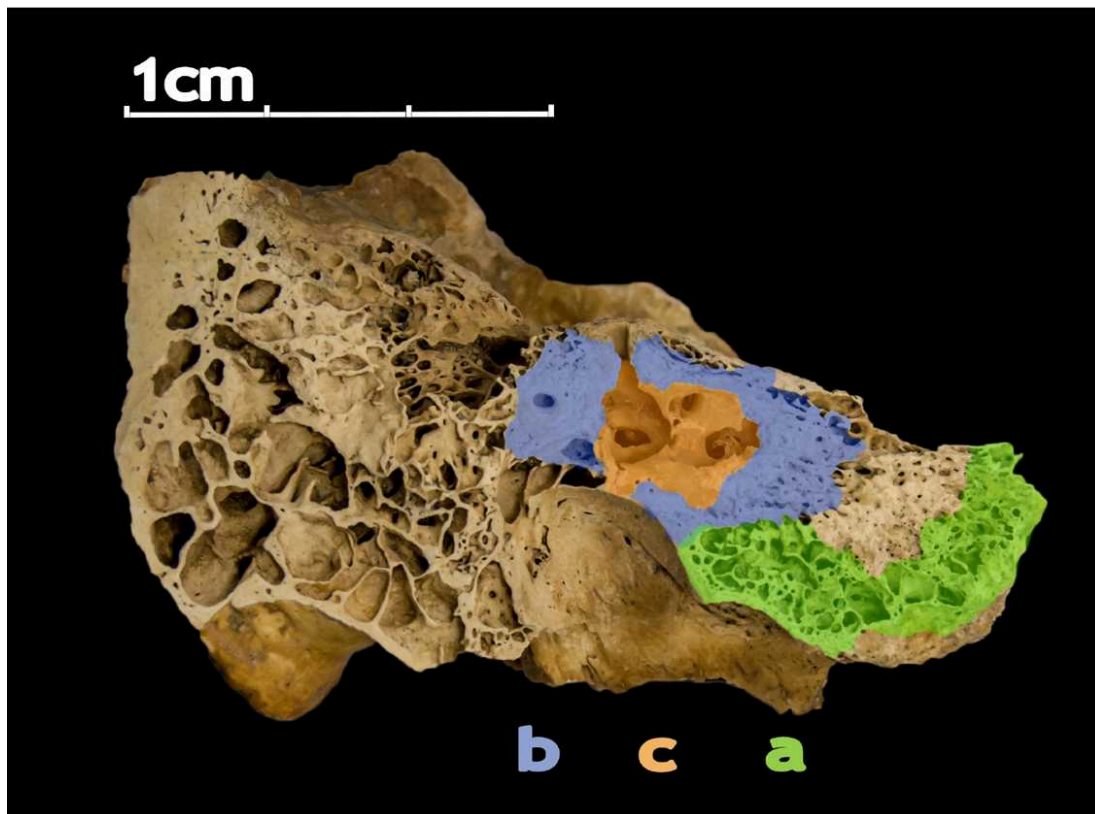


Figure 2. Dense parts around cochlea (**b & c**). Part **c** provides higher endogenous aDNA yields than part **b** and **a**. (Pinhasi et al. 2015).

To make this desired area more accessible for drilling, it is easier to turn the pars petrosa in a different angle (see Figure 2a). Locating the densest part of the bone around the

cochlea is simplified by using the wedge cutting method for sampling. Hence the petrous bone is cut in half, while holding the bone fraction with a tooth plier at the temporal bone.

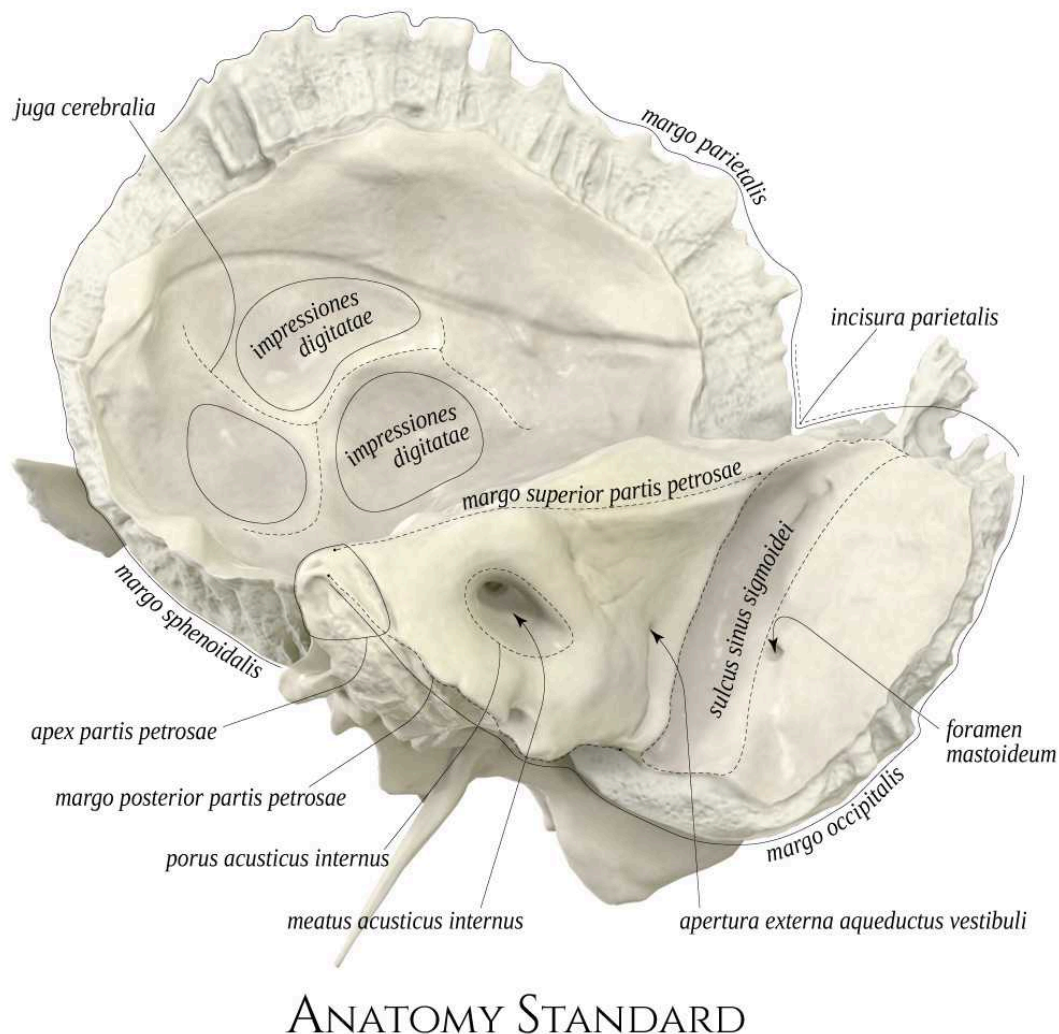


Figure 2a. Outside perspective of pars petrosa with temporal bone.
(<https://www.anatomystandard.com/Cranium/Neurocranium/Temporal.html>).

Therefore a round diamond saw blade is used to cut starting from the *apex partis petrosae*, following the *margo superior partis petrosae* and will stop doing so shortly after the internal acoustic opening (*meatus acusticus internus*). (Figure 2a)

See below a picture after cutting out this wedge. (Figure 3)



Figure 3. Pars petrosa with attached temporal bone after wedge cut.

- 5.2 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. (Figure 4).



Figure 4. Wrapping the pars petrosa with aluminium foil.

- 5.3 Drill at low speed (and high torque) within the cochlear region in parallel to the auditory canal.

Collect *clean* bone powder on the weighing paper. Of note, it is possible that you will drill into internal canals and porosities that can be filled with soil, despite your bone being cut open for better visibility. If so, follow our Pro-Tip below.

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

Note

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.

Safety information

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

- 6 Transfer the clean powder bone into a labelled 2 ml Safe-Lock Biopure tube. The folded weighing paper will help guiding the powder into the tube.




Close the tube, wipe it with a damp paper towel and weigh the bone powder. Make sure that this exact tube was used before to tare the scale.

Safety information


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



Note

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.

Note down the weight in mg on the side of the tube. Please refrain from labeling the lid with this information, it'll be too hard to read afterwards. Of course labeling the lid with the sample ID is recommended.

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

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

Decontamination

8

Safety information

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:

- 8.1
 - 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.
- 8.2 Drill bits and saw blades 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.

Note

Check conditions of drill bits after ~5 uses and replace by new ones if necessary.



9  (or in case of UV irradiation of multiple samples at once, ) and repeat the sampling procedure with the next sample.

Citations

Step 5.1

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. Optimal Ancient DNA Yields from the Inner Ear Part of the Human Petrous Bone
<https://doi.org/10.1371/journal.pone.0129102>