

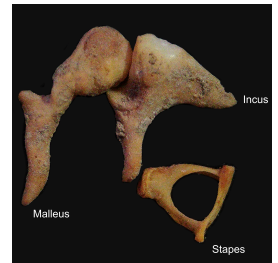
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Version 1

Auditory Ossicles Sampling Protocol for Ancient Samples V.1

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We use this protocol and it's working

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Abstract

This protocol describes how to sample the tiny auditory ossicle bones — the **stapes** (stirrup), **malleus** (hammer), and **incus** (anvil). It is particularly recommended for sampling ossicles from uncleaned whole skulls with the temporal bone present, or from isolated temporal bones with burial or depositional soil still filling the ear canal. These bones can be used to extract authentic human DNA fragments in both ancient DNA and forensic contexts.

Guidelines

All sampling procedure must be done in clean laboratory dedicated to ancient DNA experiments to avoid modern DNA contamination. Additionally, sampling particularly must be done in cleaned Class 2 Safety cabinet with air circulation on.

During sampling, always wear nitrile and powder free gloves, mask and visor to protect skin from bleach solution.



Materials

Workspace

- UV Crosslinker
- Class 2 Safety Cabinet
- Scales
- Aluminum Foil Sheets
- Notebook (Dedicated for ancient DNA laboratory use)
- Permanent Markers (Black or green ink is recommended for long-term labeling. *Blue ink may wipe off during tube cleaning with bleach solution or even due to bleach vapors.*)
- Washing bottles (for bleach solution and distilled water)
- Spatulas
- Forceps
- Cotton swaps
- Mobile lamp (for illuminating the ear canal during sampling, if needed)
- Metal support ring (use if sampling will be performed on the cranium)
- Waste Bin

Personal Protective Equipment (During Sampling)

- Full Lab Suit (must cover all exposed areas except the face)
- Disposable Shoe Covers (Also known as "*moon boots*")
- Disposable Sleeves
- Nitrile Gloves (powder-free)
- Visor
- Mask
- Hair net

Solutions

- Bleach (NaOCl) Solution (any commercial brand, unconcentrated [to be diluted to 5% as needed])
- Ethanol (EtOH, any brand with 99% purity)
- Distilled Water
- DNAway



Safety warnings

- ⚠ The bleach solution is hazardous to both skin and eyes. Avoid direct inhalation of vapors. In case of eye contact, immediately rinse the affected eye with an eyewash solution or clean saline for at least **15–20 minutes**, ensuring thorough flushing. Seek medical attention promptly.

Before start

Before starting sampling, prepare aluminum foil sheets cut to appropriate sizes for your workspace. Ensure that all utensils (spatulas and forceps) are thoroughly cleaned and UV-irradiated.

To clean the utensils, first wipe with **5% bleach (NaOCl)** solution, then with **70% ethanol (EtOH)**. After drying, expose the utensils to UV light in a **UV crosslinker for at least 30 minutes**.

Introduction

- 1 The auditory ossicles are the three tiny bones located in the middle ear: **stapes, malleus and incus**. They are the smallest bones in the human body and play a critical role in hearing by transmitting sound vibrations from the eardrum to the inner ear. Due to their small size, low weight, and delicate structure, these bones are highly susceptible to damage during handling. Careful sampling, decontamination, and handling procedures are essential to preserve their integrity for morphological or molecular analyses.

Recent studies in ancient DNA research have shown that **these tiny bones can outperform petrous bones**, which are frequently used in aDNA studies due to their high bone density and superior DNA preservation potential, in terms of the proportion of authentic human DNA recovered.

See our manuscript for details: <https://doi.org/10.1101/2025.07.17.664655>



Macro view of auditory ossicle bones. Each white and black rectangle represents 2 millimeters.

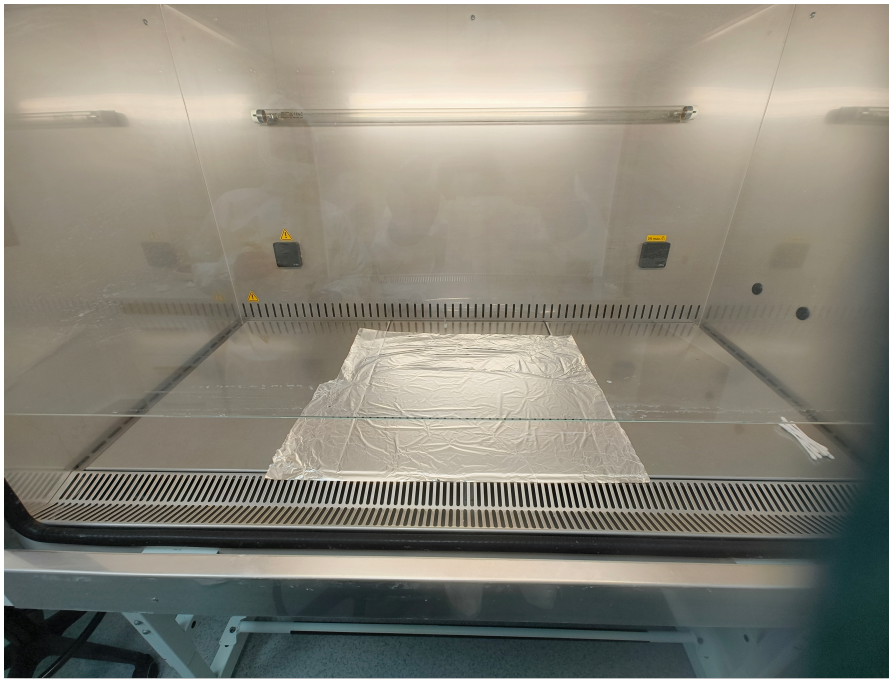
Workplace Preparation

- 2 Wear the full set of personal protective equipment, including a full-body lab suit, visor, mask, hair net, disposable sleeves, shoe covers, and **two pairs of powder-free nitrile gloves**. Ensure all protective gear covers exposed skin completely before beginning sampling.

Note

Change the outer layer of gloves between each sampling. After putting on a new glove layer, thoroughly rinse the gloves using a washing bottle containing **5% bleach (NaOCl) solution**. Ensure gloves are dry before handling any skeletal material to prevent sample damage.

- 2.1 Open the Class II safety cabinet. Turn on the lights and activate air circulation. Place an appropriately sized aluminum foil sheet inside the workspace to cover the working area, and wipe it thoroughly with a cloth rinsed in **5% bleach (NaOCl) solution**.



- 2.2 Place the skeletal material **carefully** on the workspace bench. Record its skeletal ID in the laboratory notebook dedicated to ancient DNA work. Prepare appropriately labeled screw-cap tubes for sampling auditory ossicle bones, ensuring they are clean and contamination-free.

**Note**

To decontaminate screw-cap tubes, place them into UV-Crosslinker at least **30 minutes**. Labels must contain following information:

- Skeletal ID
- Laboratory ID
- Responsible Laboratory Technician
- Date
- Bone Type (*to be written after sampling*)
- Bone Weight (*to be written after sampling*)

- 2.3 Place two smaller aluminum foil sheets. Position the first smaller sheet on top of the large aluminum foil sheet to create a clean working surface for sampling. Place the second smaller sheet directly on the working surface. Arrange sterile, UV-irradiated spatulas, forceps, and cotton swabs on this sheet to maintain contamination control.

Note

To decontaminate metal utensils (spatulas and forceps), follow steps below:

1. Immerse the utensils in **5% bleach (NaOCl) solution** for at least **5 minutes**. Do not exceed 5 minutes, as bleach may corrode metal surfaces and cause rusting.
2. Rinse the utensils in **distilled water** for at least **5 minutes**.
3. Immerse the utensils in **70% ethanol (EtOH) solution** for at least **5 minutes**.
4. Allow the utensils to dry completely.
5. Place the utensils inside a UV crosslinker and irradiate for at least **30 minutes**.

Sampling of Auditory Ossicle Bones

- 3 Place the skeletal material inside the Class II safety cabinet. Double-check its skeletal ID and open the bag carefully. Place the skeletal material on the prepared aluminum foil sheet. Place the empty bag in an available designated space within the cabinet.

Note

If sampling directly from the cranium, ensure the use of a circular metal support ring to prevent damage to the cranium.



3.1 Check the ear canal(s) for the presence of depositional soil.



3.2 Carefully scrape the depositional soil from the ear canal using sterile forceps or spatulas, transferring it onto the second layer of aluminum foil. After decomposition, the auditory ossicle bones may become dislodged into the ear canal and trapped within the soil. Gently shake the material if needed to collect the soil sample, taking care not to damage any bones.



Note

EXERCISE EXTREME CARE DURING SCRAPING. Due to their small size and low weight, these bones are highly sensitive to even minimal pressure. If they break during sampling, it will be difficult to identify them morphologically and distinguish them from other small cranial fragments.



Note

Prefer to use the pointy edge of the forceps if the soil is hardened or compact. Once the soil has softened, use a spatula to remove it.

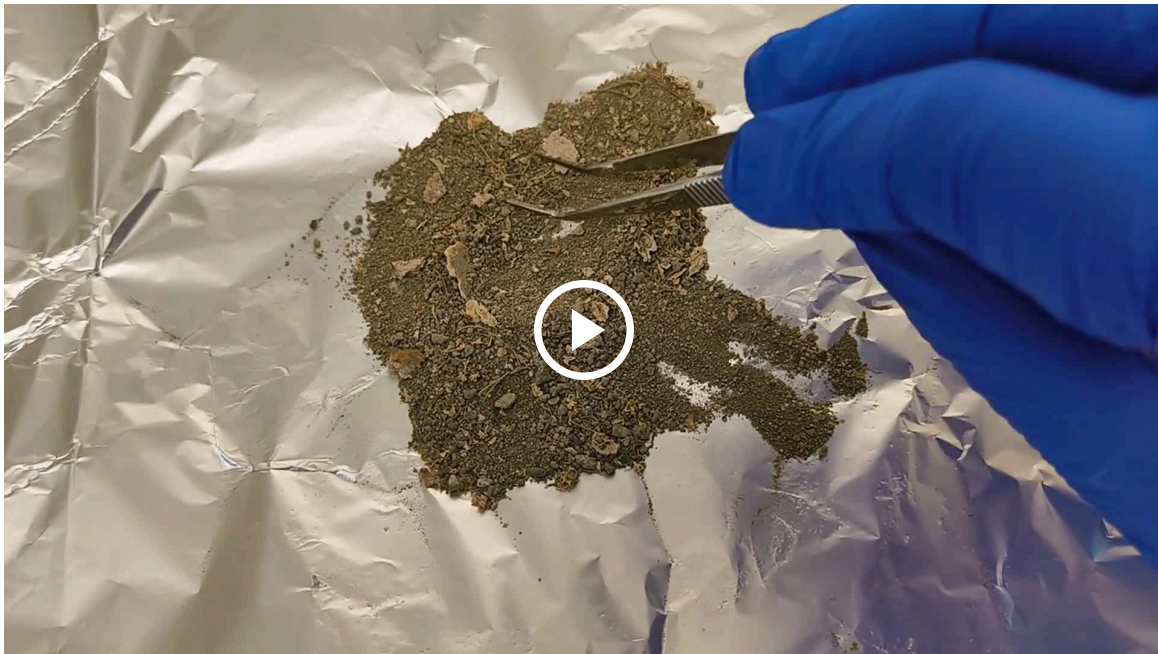


- 3.3 Observe ear canal presence of auditory ossicle bones. Some ossicles may fall out during scraping. However, due to small cavity of ear canal, some ossicle may still be trapped inside. Use mobile lamp to illuminate inside of ear canal if needed. To improve visibility, **if needed**, gently clean the ear canal using cotton swabs soaked in **5% bleach (NaOCl) solution**.

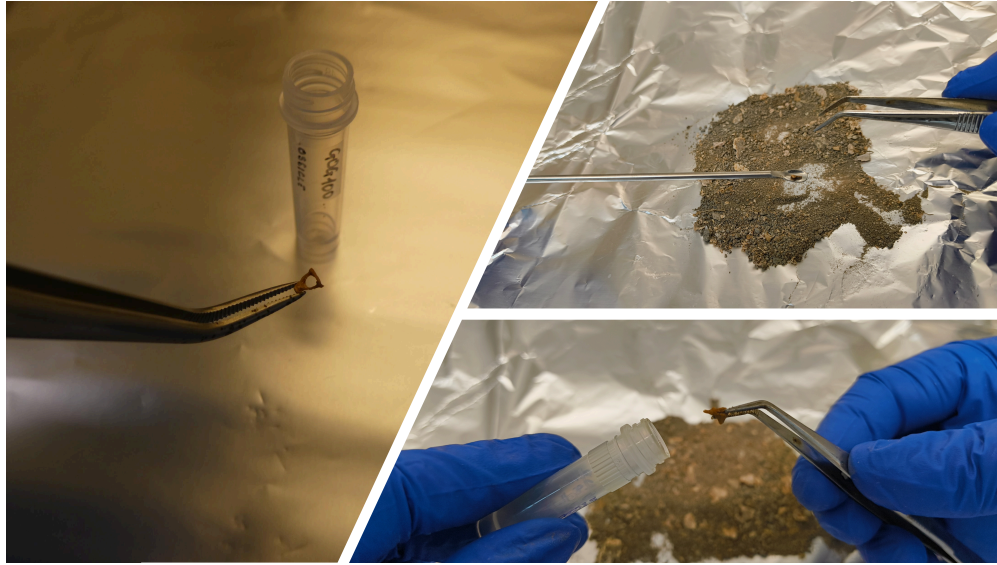


Example: Detection of an auditory ossicle bone (in this case, the **malleus**) inside the ear canal.

- 3.4 Examine the removed soil for the presence of ossicle bones. Gently move the soil using metal utensils, and use a magnifying glass if needed to identify small bone pieces.



- 3.5 Transfer the ossicle bones onto small aluminum foil sheets. Place the sheets on a scale to weigh the ossicle bones. After weighing, transfer the bones into labeled screw-cap tubes. Record the weight and bone type on both the tube and the laboratory notebook.



Clean-up and Decontamination

- 4 Fold the second layer of aluminum foil containing the removed soil from the skeletal material. Place the skeletal material and the folded foil into the original sample bag. Move the sample bag to the working bench. Discard the first layer of aluminum foil from the safety cabinet. Finally, clean the surface of the safety cabinet using a cloth rinsed in **5% bleach (NaOCl) solution**.
- 4.1 Decontaminate the metal utensils used to remove soil from the ear canal by following the guidelines described in *Step 1.3*.
- 4.2 Remove the outer layer of gloves and replace them with a new pair. Briefly clean the new gloves using a washing bottle containing **5% bleach (NaOCl) solution** before handling the next skeletal sample.