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Subsampling bones and teeth for ancient DNA extraction V.1

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We use this protocol and it's working

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Abstract

This protocol describes how to subsample non-human mammalian bones and teeth for ancient DNA extraction. This is an invasive/destructive protocol.

We specify this as a non-human protocol mainly due to the surface cleaning approach applied here: In human studies, the outer layer of bone/tooth is (usually) removed with the drill to get rid of any potential contaminant human DNA on the surface, whereas in this protocol we target the outer layers and thus do not remove them with the drill, but instead do a less conservative cleaning with bleach and ethanol. The advantage of working with non-human mammals is that we can tolerate some human DNA contamination from the surface of the specimen (though still not ideal), as any sequencing reads originating from contaminant human DNA can be removed bioinformatically.

In this protocol, we first discuss what to consider when subsampling bones and teeth (e.g. which bones or which areas of the tooth are best to sample for aDNA), then we describe the procedure for subsampling in an ideal setting (i.e. a dedicated aDNA clean lab), and finally we discuss some considerations when subsampling in a non-ideal setting (e.g. at a museum or in the field).

At the end of this protocol, you will have ~50 mg of bone/tooth powder in a 1.5 or 2 mL Eppendorf tube ready to be used for DNA extraction. It is assumed that the extracted DNA will then be sequenced using next-generation sequencing technology. Optional: We also address subsampling for collagen extraction to be used for radiocarbon dating and/or stable isotope analysis, in which case ~250-1000 mg of bone/tooth subsample is obtained.

See the Materials for a list of equipment and reagents required.

Key references:

- Alberti, F., et al., Optimized DNA sampling of ancient bones using Computed Tomography scans. *Molecular Ecology Resources*, 2018. **18**(6): p. 1196-1208. <https://doi.org/10.1111/1755-0998.12911>
- Damgaard, P.B., et al., Improving access to endogenous DNA in ancient bones and teeth. *Scientific Reports*, 2015. **5**(11184): p. 11184. <https://doi.org/10.1038/srep11184>
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- Hansen, H.B., et al., Comparing Ancient DNA Preservation in Petrous Bone and Tooth Cementum. *PLOS ONE*, 2017. **12**(1): p. e0170940. <https://doi.org/10.1371/journal.pone.0170940>

Materials

Equipment & consumables

- Dental drill (e.g. Proxxon Micromot, Dremel)
- Diamond-coated drill bits (e.g. Proxxon 1.8 mm diamond bur (No. 28 222), but good to have various sizes to choose from based on the specimen and target area) and cutting disks. Two of each is preferable.
- Mortar and pestle (2x)
- Hammer and protective plate
- UV cross-linker cabinet
- Extractor hood/fume hood/designated subsampling area
- (Aluminium) Foil
- Weigh boats
- Scale (to weigh the bone/tooth powder)
- Sample tubes (e.g. 1.5 or 2 mL Eppendorf tubes)
- Disposable cloth
- Tissue paper
- Protective clothing (e.g. nitrile/latex gloves, face masks to cover nose & mouth, protective eyewear, hair nets, coveralls (e.g. MICROGARD 2000 coveralls from Merck), sleeves, lab shoes/protective footwear).

Reagents

- 5% bleach (sodium hypochlorite) solution
- 70% ethanol
- DNA Away

Protocol materials

 DNA AWAY™ Surface Decontaminant Thermo Scientific Catalog #10223471

Before start

Before starting, it is a good idea to **take photos** of the specimen from multiple angles (with a ruler/scale in frame) to record its pre-subsampling condition. Also consider making a **physical or digital copy of the specimen** by making a cast, 3D scanning, or photogrammetry, if possible and feasible. In this way, a copy of the specimen exists for future study (e.g. morphometrics). This may already be a prerequisite from the museum collection curator/ethical authority that provided permission for destructive sampling. Even if it is not explicitly required, it is still good practice to do this, as future researchers and curators will thank you.

Once a copy is made, do still take care to do only the damage required to obtain the subsample for DNA extraction, thereby preserving as much of the specimen as possible.

General considerations when subsampling bones and teeth

- 1 The preservation of endogenous DNA in ancient skeletal elements (bones and teeth) is somewhat of a stochastic process. While we know that time since death (i.e. the geological age of a specimen) and the temperature of the immediate surroundings of a specimen are probably the main drivers of endogenous DNA preservation, there can be many other factors that influence this process, from the climatic down to the micrometer scale.

When subsampling a single bone or tooth, one area might yield endogenous DNA while sampling from another area a few centimeters away on the same specimen might not yield any endogenous DNA.

This does not mean that we just sample random areas of a bone or tooth and hope for the best. We can lean on scientific studies that have tested which skeletal elements (and areas of those elements) provide us with the best chance of obtaining endogenous DNA.

Where such studies are lacking for a particular species or bone, we lean on our knowledge of the anatomical and cellular structure of the element in question and/or scientific principles in general and extrapolate the findings of the other (often human-based) studies to our species of interest or skeletal element in question.

Nevertheless, it is important to realise and acknowledge (perhaps for your sanity) that even though we try to use scientific evidence and principles to give ourselves the best chance of obtaining endogenous DNA, all this does is slightly improve the usually low probability of obtaining any endogenous ancient DNA at all, due to that certain amount of randomness in the preservation of DNA in ancient specimens.

- 2 Drilling bones and teeth with a dental drill can generate a lot of heat through friction. This heat appears to result in the further degradation of any DNA that might be present ([Adler et al., 2011](#)). Therefore:
 - Use the **lowest drill speed** possible;
 - Drill in **short bursts** to prevent heat generation and allow cooling of the drill bit and the specimen between drill bursts;
 - **Target new areas** with each drill burst if possible (e.g. move along the bone or tooth root as you drill);
 - **Crush and grind** (with a mortar and pestle or pulverize with a bone mill) bone/tooth material to a fine powder whenever the opportunity presents itself. This way, drilling is either avoided altogether, or only a limited amount of drilling/cutting is done to get pieces to crush and grind.
- 3 Try to avoid damaging anatomically important areas/features, such as ligament/tendon attachment sites on bones (these are characteristic small holes/cavities in the bone), or teeth crowns, that can be useful for future researchers. Root tips generally have limited morphological research value and more cementum than the rest of the root and are thus a good target for aDNA subsampling.

Considerations when subsampling bones

- 4 **Which bones to target for aDNA extractions?**
 - The **petrous part of the temporal bone** (humans) or **petrosal bone** (a separate bone in other mammals), and in particular the **otic capsule** part of this inner-ear bone, is widely accepted as the best skeletal source of aDNA in mammals (i.e. it usually preserves the highest proportion of endogenous DNA) ([Pinhasi et al., 2015](#)). Thus, if possible, target this bone for aDNA extractions.
 - However, the petrosal bone is well-protected in the skull and can be difficult to get to in well-preserved skulls, requiring some damage to the skull to get to it. It is also not a

very common bone to find for non-human mammals at archaeological or palaeontological sites.

- Often, we are limited to fragments of **long bones**, other limb bones (e.g. **metatarsals**, **metacarpals**, **phalanges**, etc.), or cranial fragments (e.g. **mandibles**, **maxillae**, etc.).
- Depending on the size of the bone fragment, either use all of it for DNA extraction if it is very small (in which case crush & grind with a mortar and pestle, don't drill), or target the densest part of the bone to subsample via drilling (see next step).

5 Which areas of bones to target for aDNA extractions?

- **Alberti et al. (2018)** showed that the **densest part of bones** usually preserve the highest proportion of endogenous DNA (Figure 1).
- Thus, **sample the densest part of the bone to obtain powder for DNA extraction**. That is to say, try to avoid the spongy (trabecular/epiphyseal) areas of the bone. It is a safe bet to apply this approach to any bones you wish to subsample, unless there is evidence to the contrary for specific bones.
- The hypothesis behind this is that the dense parts have a highly compact bone structure with few internal open spaces. This leads to improved DNA preservation due to less leaching of DNA and less exposure to external processes (e.g. microbes, water providing a substrate for chemical reactions, etc.) and contamination, as compared to spongy bone which has a much more porous structure and is thus more exposed to DNA leaching (loss of DNA to the environment) and external processes. Therefore, there is higher endogenous DNA in dense bone. See the Discussion of Alberti et al. for more details.
- **How do you identify the densest parts?** While Alberti et al. used CT-scanning to identify the densest parts of bones, you will probably not have access to a CT-scanner, or if you do then it still might not be worth the time and effort to scan every specimen before drilling. (Exceptions might be unique, high-value specimens where you only get one chance for DNA extraction.) Thus, **use your eyes** to inspect the bone and identify the densest-looking part. This becomes clearer when you have the bone in front of you and also gets easier with more experience. It can also be helpful to look up the structure of various bones, to have a general idea of which parts are dense and which parts are spongy.
- For **long bones** or long bone fragments, the **outermost layer** that surrounds the midshaft was shown to be the densest part of these types of bones. Therefore, **do not remove this outer layer** to get rid of potential contamination, **but rather target this outer layer for DNA extraction!** See the subsampling sections below for guidance on how to decontaminate before sampling the outer layer.

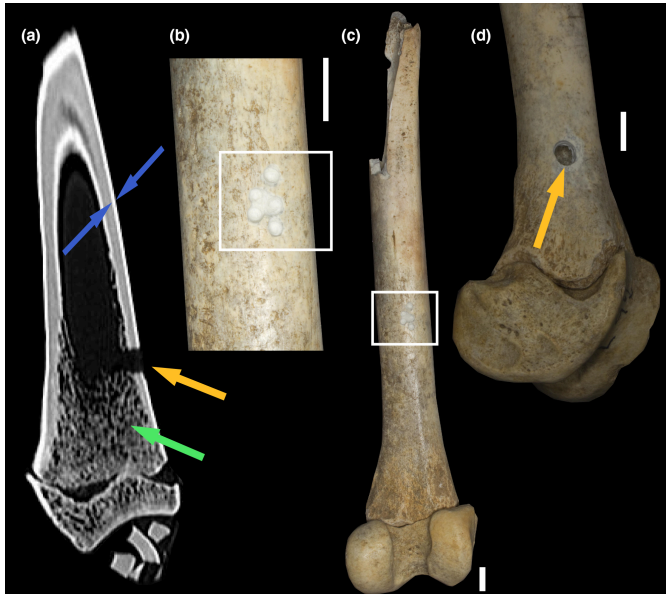


Figure 1 Long bone sampling. (a) CT scan image showing variation in bone density. The high-density outermost bone layer is clearly visible (blue arrows). The trabecular region is indicated by the green arrow. The location of conventional sampling is visible in the lower-right section of the CT scan (orange arrow). (b) Photograph showing the surface of the midshaft of the same bone, and the sampling location of the outermost layer, which was performed by drilling shallow holes into the surface of the bone (white square) according to the thickness of the outermost layer identified by the CT scan. (c) Photograph showing the complete bone. The white box indicates the sampling location of the outermost layer, which is barely visible at this magnification. (d) Conventional sampling of the same bone (orange arrow). After removal of the outermost bone surface, a small piece of the bone was removed using a core drill (orange arrow). All scale bars represent 1 cm. A movie animation of the CT slices along the coronal section of this bone is shown in Supplementary Video S1 [in Alberti et al., 2018].

Figure and caption reproduced from [Alberti et al. \(2018\)](#) (*Molecular Ecology Resources*, Volume: 18, Issue: 6, Pages: 1196-1208, First published: 07 June 2018, DOI: 10.1111/1755-0998.12911) under the [Creative Commons Attribution 4.0 International \(CC BY\) license](#).

Considerations when subsampling teeth

6 Which teeth to target for aDNA extractions?

- As far as I am aware, there have not been any studies done to determine whether any particular type of tooth (e.g. incisors, premolars, molars) preserves DNA better than others.
- Thus, if you have a choice of multiple teeth, target those which visually appear to have the best-preserved cementum on their roots (see next step).
- If you have specimens with teeth still embedded in the mandible or maxilla, target one or more of these teeth (if damaging the mandible/maxilla has been approved) by drilling from the bottom with the tooth *in situ* in the bone to target the root tip (see below) with minimal damage to the bone, or by pulling or cutting them out. The roots have been provided some protection by the bone/alveolar sockets and thus DNA might be better preserved in such specimens.

7 Which areas of teeth to target for a DNA extractions?

- [Adler et al. \(2011\)](#) and [Damgaard et al. \(2015\)](#) showed that the **cementum** usually preserves the highest proportion of endogenous DNA in teeth; notably higher than dentine (which has traditionally often been targeted for aDNA extraction).

- The **cementum is the outer layer of the tooth root** in humans and bovids (and most mammals) (Figure 2) and has a yellow-ish to reddish-brown colour in well-preserved teeth (Figure 3). The cementum is thickened at the tip of the root and thus targeting this area further improves the chances of obtaining endogenous DNA (Adler et al., 2011).
- Therefore, **do not remove the outer layer of the tooth root** in an attempt to get rid of contamination, as this outer layer is what will give you the best chance at obtaining endogenous DNA from your tooth!
- It is a good idea to study the tooth anatomy of your species of interest, so that you can target the cementum, wherever it may be. For example, bovids have internally secreted cementum (Figure 2) in the crown of the tooth (in addition to the root cementum) that could also be targeted for DNA extraction, as done by Adler et al. with bison teeth.
- Some advantages of targeting the root cementum are (i) that the crown remains intact and the specimen can therefore be used in future studies (e.g. morphological or stable isotopes), and (ii) the root dentine can be sampled for radiocarbon dating and/or stable isotope analysis.
- Sometimes, the cementum can be quite "flaky" and detaches in thin flakes from the dentine when trying to drill it. In such cases, collect the flakes and grind them up with a mortar and pestle.
- The root cementum is not always preserved, leaving only the dentine. In this case, either attempt to sample other cementum (e.g. internally secreted), or sample the dentine as the next best, and only other source, of endogenous aDNA in teeth.

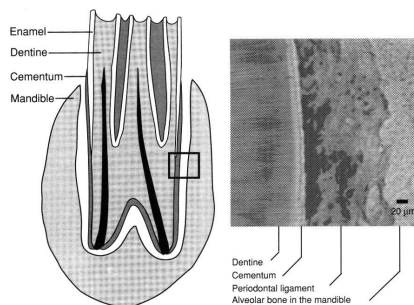


Figure 2 Diagram of generalized bovid molar in longitudinal section in the mandible (left), showing disposition of tissues. Expanded region (right) [not directly relevant for this protocol] shows the relationship between cementum, dentine and the periodontal ligament as explained in the text. Note the proliferating cells along the margin of the periodontal ligament and the cementum.

[Also shown, but not explicitly labelled, is the pulp (black) and the internally secreted cementum in the crown (same dark grey as the root cementum).]

Figure and caption reproduced with permission for use in this publication only from: LIEBERMAN, D. E., Seasonality and gazelle hunting at Hayonim Cave, *Paléorient*, 1991, vol. 17-1, pp. 47-57 © Editions du CNRS. PDF of the original publication retrieved from <https://info.persee.fr/>.



Figure 3 Visual [human] tooth preservation. Examples of poor and good tooth preservation as recorded from simple visual inspection during the sample processing. The upper (poor preservation) teeth display a root with a brittle and "chalky" appearance and the cementum layer is either fragmentary or completely gone. This in contrast to a thick, hard and compact cementum layer observed in the two lower teeth (good preservation). doi: <https://doi.org/10.1371/journal.pone.0170940.g001>.

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Subsampling in a dedicated ancient DNA clean lab

- 8
- Before going to the lab:**
 - Print a table to record information about the samples you will drill that day. See the example table below, which has been filled out with a few examples of how it could be filled-out by hand on the day. Of course, columns can be added/removed for your specific situation.

SampleID	Drill_ID	Date	Mat_group	Target_DNA	Tube_weight (mg)	Tube_powder_weight (mg)	Weight_DNA (mg)	Target_Coll	Weight_Coll (mg)	Notes
SAM-001	DRL-001	09-10-2025	Tooth	Root cementum			45	Root dentine	610	
SAM-091	DRL-002	09-10-2025	Bone	Bone (radius) outer layer			49	Bone (radius)	741	
SAM-033	DRL-003	09-10-2025	Tooth	Root material (crushed)			65	Crown dentine	255	Root was hollow, so preserved



SampleID	Drill_ID	Date	Mat_group	Target_DNA	Tube_weight (mg)	Tube_powder_weight (mg)	Weight_DNA (mg)	Target_Coll	Weight_Coll (mg)	Notes
										mabl y enric hed for ceme ntum. Root and crow n were separ ated (cut) and the root was crush ed using a ham mer.
SAM-005	DRL-004	09-10-2025	Bone	Bone (3rd phalanx) outer layer			38	Bone (3rd phalanx)	310	For DNA drillin g, the outer layer of joint was target ed (wher e the 2nd phala nx woul d join), as this looke d to be the dens est part of this bone.

Subsampling table: Record information by hand in the printed table and then copy the information to a digital version (e.g. a spreadsheet) afterwards, so that there are multiple copies of the data.

Column definitions:

- **SampleID:** The ID of the specimen being drilled. Can be a museum ID or an internal project/group/institution ID.
- **Drill_ID:** A code/ID for that specific drilling/subsampling effort ("Drill ID"). This is important, as one specimen can be subsampled several times, and each instance should have a unique ID, because different parts of the tooth/bone would be targeted for the different subsampling events, and thus one subsample might have excellent DNA and another might have none, and you must be able to keep track of this. Can also be changed to SubsampleID or similar.
- **Date:** The date when the subsampling was done.
- **Mat_group:** The broad material group to which the part of the specimen that was subsampled belongs. In this case, bone or tooth.
- **Target_DNA:** The specific part of the specimen that was targeted during subsampling for the purposes of DNA extraction. Be quite specific here. I usually also note here when I subsampled via crushing (either hammer or mortar & pestle) as opposed to drilling (the default).
- **Tube_weight (mg):** The weight of the empty tube into which the subsample (powder) will be placed.

- **Tube_powder_weight (mg):** The weight of the tube with the subsample (powder) inside it.
- **Weight_DNA (mg):** The weight in milligrams of the powder obtained for DNA extraction (=Tube_powder_weight - Tube_weight). We usually aim for ~50mg as this is what our extraction protocol requires. But if you get more, then the extra powder can be stored for future extraction attempts.
- **Target_Coll:** The specific part of the specimen that was targeted during subsampling for the purposes of collagen extraction. The collagen extract might then be used for radiocarbon dating and/or stable isotope analysis. Subsampling for both DNA and collagen at the same time saves time down the line, since everything is already set up and the specimen is then handled fewer times. Collagen is contained in bone and tooth dentine (present in the root and crown).
- **Weight_Coll (mg):** The weight in milligrams of the subsample obtained for collagen extraction. We usually aim for >250 mg, up to 1000 mg (depending on the size of the specimen).
- **Notes:** There is usually not much space for hand-written notes in this column. Thus, take notes on the back of the paper, and then transfer them to the digital copy of the table later.

Note

For collagen subsampling, I usually cut off a piece of tooth root/bone instead of drilling/crushing to get powder. In this case, I weigh the subsample for collagen in a small weigh boat and then put it in an eppie or small ziploc bag (depending on its size). If only tooth crown collagen is available, then drilling would be the way to go, as you do not necessarily want to cut off a piece of the tooth crown, so drilling into the dentine is preferable. In this case, add another Tube_weight column and a Tube_coll_weight column to the table to accurately record the powder weight.

9 Prepare work areas and equipment:



- 9.1 Follow **all contamination-prevention and personal protection procedures** of the clean lab.
- 9.2 Make sure you are **double-gloved** (two gloves on each hand), as you will be changing gloves often at between drilling different samples.
- 9.3 **Turn on extractor/fume hood** (if available) where drilling will take place.
- 9.4 Fill a small-medium sized plastic container (e.g. 250 mL) with **5% bleach solution**. Soak a piece of disposable cloth in the bleach solution. This cloth is what you will use for cleaning surfaces and specimens.
- 9.5 **Clean all work surfaces** with 5% bleach solution followed by 70% ethanol.
- 9.6 If you brought any materials, e.g. papers or sample bags, with you into the lab, **wipe them down with 5% bleach**.
- 9.7 Remove drill, drill bits, and cutting blades from storage box. It helps to have at least two of each item, as then you can use one set while another set is in the UV cross-linker (see below). This speeds up subsampling substantially.
- 9.8 **Soak drill bits and cutting blades in DNA Away** ( DNA AWAY™ Surface Decontaminant Thermo Scientific Catalog #10223471) in a small weigh boat for **~10 min**. Use 5% bleach solution if DNA Away is not available, but decrease the time to ~2 min.
- 9.9 While soaking the drill accessories, **wipe the drill down with 70% ethanol**.



- 9.10 **Clean any other tools/equipment you might use** (e.g. mortar, pestle and hammer) with 5% bleach followed by 70% ethanol.
- 9.11 **Label 1.5 or 2mL Eppendorf tubes** with your Drill_IDs and organise your specimens in the order you plan to subsample them.
- 9.12 Place a large rectangular piece of **foil into the UV cross-linker**.
Take the drill bits out of the DNA Away/bleach solution, dry with tissue paper, and place on top of the foil in the cross-linker.
Place the mortar and pestle in the cross-linker.
Switch the cross-linker on, making sure the timer is set to **10 min**.
- 10 **Subsampling:** 
- 10.1 While waiting for the UV cross-linker, prepare the drilling area by **placing a large rectangular piece of foil down on the surface** where subsampling will take place.
Wipe the foil with 5% bleach - it will stick the foil to the surface. This is your sterile working area.
- 10.2 **Weigh the Eppendorf tube** into which the tooth/bone powder will be placed. Record the weight in your printed table as the tube weight.
- 10.3 When the UV cross-linker is done, remove everything and place on a sterile (bleach-cleaned) surface. Only take the equipment you will use into the drilling area (hood).
- 10.4 Before you start drilling, place a new piece of foil into the UV cross-linker, together with any pieces of equipment that need to be sterilised again, and switch it on (for 10 min).
This way, the equipment is being sterilised while you are processing a sample, and will be done by the time you have finished subsampling. This saves time.
- 10.5 **Fold the UV-sterilised foil** to make a tray or "boat" with vertical sides - this is where you will place the specimen while drilling. It will collect the powder and the vertical sides will (hopefully) prevent most of the powder from being blown away if there is airflow from the hood or if you accidentally bump the foil.
- 10.6 Remove the specimen from its packaging and identify the area/s you want to target for subsampling, following the relevant guidelines in steps 1-8 above. **Clean the target area/s by wiping with the bleach-soaked cloth, removing any soil or other exogenous material that might be on the specimen.** Follow the bleach cleaning with 70% ethanol cleaning using tissue paper. Make sure to rinse the cloth in the bleach solution in the plastic container after cleaning the specimen.
- 10.7 Subsample the specimen by drilling and/or crush pieces with a mortar and pestle to a fine powder. 
- If using the latter, make sure to use your one hand to cover the mortar, as pieces of bone/tooth tend to ricochet out during crushing & grinding.
 - Because we are attempting the densest portion of the skeletal element, mortar and pestle may not be effective. If available, use a hammer and protection plate, both decontaminated with 5% bleach followed by 70% ethanol, on your working area.
Place the specimen on another UV-sterilized foil and fold the foil multiple times to minimize the risk of fragment dispersal during crushing.
- 10.8 "Pour" the powder into the eppendorf tube and weigh the tube.
Record the weight in your printed table.
Calculate the weight of powder to make sure you have enough (at least 50 mg). If not, subsample to obtain more powder, if possible.
- 10.9 Once you have enough powder for DNA extraction, subsample for collagen (if needed).
- 10.10 Once all subsampling is complete, return the specimen to its container.
- 10.11 **Discard both pieces of foil.**



- 10.12 **Soaking drill bits and cutting blades in DNA Away.**
- 10.13 **Clean mortar and pestle** with 5% bleach solution, followed by 70% ethanol. Make sure there are no powder left in the mortar, as it cause the contamination across samples.
- 10.14 **Clean the drill** with 70% ethanol - focus particularly on the end of the drill where the drill bits are attached, as this tends to get coated in powder.
- 10.15 **Clean the drilling area** with 5% bleach followed by 70% ethanol.
- 10.16 **Change your outer layer of gloves.**
- 10.17 Place a **new rectangular piece of foil in the drilling area and wipe with 5% bleach** (as in the first step).
- 10.18 Remove the equipment and foil from the UV cross-linker that you placed there before starting the subsampling (if 10 min have passed, otherwise wait for the 10 min to be over). These are now sterile.
- 10.19 Place a **new piece of foil into the UV cross-linker.**
- 10.20 Remove the used drill bits and cutting blades from the DNA Away, wipe dry with tissue paper and make sure there is no visible bone/tooth powder. If there is, wipe down with DNA Away again, or 5% bleach followed by 70% ethanol. Once you are satisfied that they are clean with no powder visible, put them in the UV cross-linker, together with the mortar and pestle (if used) and switch the UV cross-linker on for 10 min.
- 10.21 Start processing the **next specimen** and repeat the above until all specimens have been subsampled.
- [⇒ go to step #10.5](#)
- 10.22 **Once done, clean all surfaces and equipment with 5% bleach followed by 70% ethanol** and/or follow the required protocols of the clean lab for post-work clean-up.
- 10.23 Make sure to treat the last batch of drill bits, cutting blades, and mortar and pestle with the UV cross-linker before storing these away.
- 10.24 Either proceed directly to DNA extraction, or **store subsamples at -20°C** for later processing.

Protocol

NAME

Ancient DNA Extraction from Bones and Teeth

CREATED BY

Vanssy Li

Preview

- 10.25 Discard the leftover, used bleach solution appropriately according to your institute's guidelines.

Subsampling at a museum or in the field

- 11 **Before going to the museum/collection:**



- Prepare all the equipment and reagents you need, including printing the subsampling table from Step 8 (➞ [go to step #8](#)).
- It is preferable to have **another drill and set of drill bits for use outside the clean lab**. Otherwise, the drill and drill bits have to be thoroughly cleaned with 5% bleach and 70% ethanol before they re-enter the clean lab.

12 Prepare work areas and equipment:

- Subsampling specimens for aDNA outside a clean lab is not ideal, because it is a non-sterile environment where there is a continual threat from many sources of potential contamination with exogenous DNA.
- Thus, we take as many precautions as reasonably possible to limit this contamination.
- This can range from setting up a complete field "clean lab", as in [Arrieta-Donato et al. \(2025\)](#) (see their Supplementary Materials), to simply ensuring the area you are working in is clean and you prevent contamination with your own DNA via bleach, gloves, a mask, and a hairnet.
- **At a minimum:** wear two pairs of gloves, a mask, and a hairnet, making sure to change the outer layer of gloves between specimens (to prevent cross-contamination), and clean the work area with 5% bleach solution and 70% ethanol before and after each specimen is subsampled.
- If possible, also wear disposable sleeves.
- Follow the same procedures as in Step 9 (➞ [go to step #9](#)), excluding the UV cross-linker steps (unless you have one available).

13 Subsampling:

- Follow the same procedures as in Step 10 (➞ [go to step #10](#)), excluding the UV cross-linker steps (unless you have one available).
- Since the foil "boat" that will collect all the drilled powder likely will not be sterilised in a UV cross-linker, make sure to clean it by wiping with 5% bleach solution and waiting for it to air-dry before drilling. In this case, you do not need to follow it up with 70% ethanol.
- If only "pre-subsampling" is to be done at the museum or in the field (e.g. a piece of bone or a tooth root is cut off for later subsampling in a clean lab), it is still advised to follow the **minimum** precautions as detailed in Step 12.

Protocol references

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