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Sampling protocol for obtaining sediment from archaeological excavations in caves and rockshelters for the purposes of biomolecular analyses

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We use this protocol and it's working

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Abstract

The objective of this protocol is to guide systematic collection of sediment samples from archaeological excavations in caves and rockshelters in order to perform biomolecular and geochemical analyses. Although specifically designed for cave/rockshelter excavations, the protocols described here can be readily modified for archaeological excavations in other settings.

This protocol outlines the instructions for both vertical (i.e. from a profile) and horizontal (i.e. from an active spit/layer/context) sampling, as well as sampling co-occurring with the collection of a sediment block for micromorphological analysis. The sampling workflow is designed to incorporate the needs for successful biomolecular and paired geochemical/sedimentological analyses with minimal disruption to the archaeological excavation workflow. This protocol provides guidelines that can aid in a successful sampling strategy; however, sampling strategies need to be designed with initial research questions in mind and with the understanding that every site is unique. Therefore, prior to sampling, we strongly advise a thorough discussion with the site excavators and geoarchaeologists to ensure that relevant areas, layers, and features of interest are considered during sampling.

Six figures are provided in the main text. Figure 1 shows a series of photographs illustrating different stages of vertical sampling; Figure 2 shows sampling from a profile directly using a 15 ml tube; Figure 3 illustrates a vertical sampling strategy; Figure 4 shows sampling from the back of a sediment block; Figure 5 shows sampling from a scar in the profile; and Figure 6 illustrates a horizontal sampling strategy. An example of the different stages involved in preparing a geoarchaeological block sample can be found in the additional material.

Guidelines

Types and Risks of Contamination

Archaeologists may already be familiar with sampling procedures for radiometric dating, phytolith, pollen extraction and basic sedimentological analyses. Ensuring the success of biomolecular analysis requires taking contamination seriously and therefore, a more rigorous protocol is necessary. There are two main types of contamination. The first is the introduction of modern DNA that derives from the excavators and surrounding modern environment. Modern DNA can disrupt analysis, making sequencing less effective despite bioinformatic tools to help reduce the influence of such contamination. The second, and most critical type of contamination is the cross-contamination of sediments from different depths, visible sedimentary units (hereafter called layers) or contexts. Once this occurs, separating the samples back to their original sources and initial context is impossible. It may also be difficult for analysts to recognize when such contamination has occurred.

To minimize the first type of contamination, direct contact between the sediment to be sampled and the skin and bodily fluids of the excavators should be avoided at all costs. Additionally, water should not be applied to the sediment prior to sampling as this can also introduce foreign modern DNA to the sediment. To minimize the second type of contamination, it is important to isolate each context, and use clean sampling tools.
The following guidelines apply to sampling that occurs during the course of an active excavation.

Contamination Prevention

- Take the sample immediately after starting a new spit/layer as dripping water and other environmental disturbances might influence the results. If the spit is not fresh and is a few days/weeks/months old (e.g. from previous excavation seasons) rigorously remove the uppermost layer with a clean trowel.
- Assess the degree of potential contamination: if you suspect that the surface to be sampled has been walked on, touched with bare hands, or exposed to other contamination, prepare a clean, fresh surface before proceeding (as above).
- Wear sterile plastic gloves and (if possible) a face mask when taking samples. If a glove comes in contact with sediment or clothing, change to a fresh one.
- Samples should be taken with a single-use, sterile, pre-packed plastic spatula. One side of the spatula is used to remove ~1 cm of potentially contaminated sediment and the other side of the spatula is used for taking the sediment sample. The gloves should be changed in between taking samples. If the other side of the spatula comes in contact with sediment or clothing, change to a fresh spatula. Note: the plastic spatulas can also be re-used for biomolecular/geochemical sampling after cleaning with a bleach solution and rinsed with sterile water. Alternatively, they can be cleaned with alcohol and used for collecting other types of samples (i.e., phytoliths, mineral nodules, other small sediment samples for analyses such as FTIR) or as tools when sorting small finds.

Materials

- Single-use plastic tools (spatula) for sampling https://www.carlroth.com/com/en/spatulas/spatula-micro-disposable/p/hep7.1
- 5-15ml plastic conical centrifuge tubes with a screw-cap (falcon tubes) https://www.starlabgroup.com/en/product/15-ml-centrifuge-tubes-pf-sl-458011.html
- Polyethylene zippered sampling bags, with writing surface
- Permanent markers
- Colored pins
- Measuring tape
- Digital camera
- Scale bars for photography
- PPE (nitrile gloves 1x long-sleeved, 1 x short-sleeved, hairnet, face mask)

Before start

Documentation:

- Name of person collecting sample *
- Date of sample collection *
- Name of site *
- Province, Region, Country *
- Latitude, Longitude and/or GPS coordinate of the site (can be withheld at request of excavator or archaeological authority) *
- Contact Person, Affiliation, Contact Email *
- Archaeological sample ID *
- Lab ID (if different from Archaeological sample ID)
- Sample material, General description *
- Wet weight (g) of the samples within the sampling bags *
- Layer/Square/Coordinates/etc. *
- Biotic factors, such as roots or animal life, that could influence sediment characteristics
- Cultural affiliation, Age (years BP), Method of age determination, Lab ID (dating), Material used for dating (e.g., charcoal, bone, etc.)
- Hominin association (Homo sapiens, Homo neanderthalensis, etc.)
- Photographs of the sampling area before and after sampling *
- Notes, such as stratigraphy, colour, texture, and any visible inclusions (e.g., charcoal, bone fragments)
- References

Obligatory fields marked with *

1. General sampling procedure

1 Take ~5 g (for reference a sterile 5 ml falcon tube filled to the top will contain approximately 5 g of sediment) of sediment using the sterile spatula and place it into a sterile falcon tube or sterile plastic bag (Figure 1).

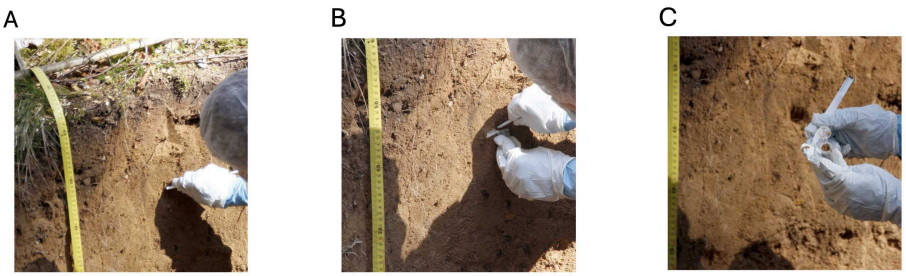

Figure 1: Stages of sampling from the profile. A: Remove 1 cm from the exposed surface with one end of sterile spatula. B: Use the other end of the spatula to scrape sediment from the desired location into a 5 ml or 15 ml falcon tube. C: Close the tube immediately after sampling.

Alternatively, if the sediment is soft, an opened 15 ml falcon tube can be directly pressed into the sediment and extracted again to collect the desired material without the use of a spatula (Figure 2).

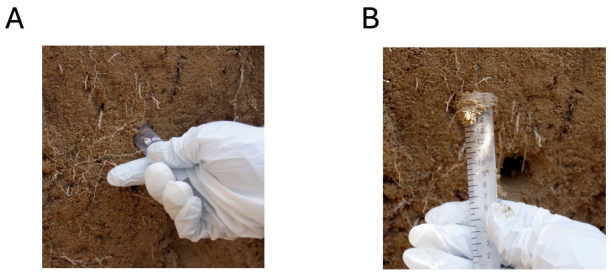

Figure 2: Sampling directly from the profile using a 15 ml tube.

2 Close the tube/plastic bag and label it with the date and lab ID. Record in the notes and site database all additional information such as the x;y;z coordinates, the site ID, layer, and the find number or Sample ID corresponding to the number assigned on the site database.

3 Place the initial sealed and labelled tube/bag inside a second bag. If the project employs a barcode system, ensure that the barcode is inside or attached to this second bag.

4 Store the sample(s) in a cool and dark place until transport to the laboratory, preferably in a cooling box, if available.

5 If samples are not analysed immediately, store sediment samples in a freezer at -20 °C until analysis. If freezer space is limited/not available, or if the transport to the lab takes some time, the samples can be refrigerated to hinder microbial growth. If it can be ensured that the samples are dry, they can also be kept at room temperature, away from sources of light.

2. Vertical sampling strategy (sampling for aDNA from a profile, scar, baulk, etc.)

6 The exposed profile should be completely scraped and cleaned with a clean trowel prior to sampling, starting from the top of the profile and working downwards. Do not spray the profile with water prior to or after cleaning.

7 Sample profiles from bottom to top, to minimise the risk of vertical cross-contamination from material falling from upper layers. Use one side of the sterile spatula to remove ~1 cm of sediment at the sampling location to avoid potential contamination from tools used to clean the profile.

8 From each layer, at least one sample should be collected. If a layer is 20 cm or more in thickness, take three samples, one from the bottom, one from the middle and one from the top. Aim for the samples to be taken at equally spaced intervals. When the layer is less than 20 cm aim to take one sample, ideally from the middle of the layer.

9 Special features and contexts (pits, burials, occupation horizons) present within the profiles can also be targeted for sampling in addition to the samples following the present protocol, in consultation with the excavators as part of an overall sampling strategy.

10 When vertical sampling is conducted in conjunction with collection of a micromorphology block sample from a profile, the same procedures above apply, with the following modifications and guidelines for three specific sampling strategies:

2.1 Sampling from next to a sediment block

11 Samples should be collected from as close to the location of sampling for the micromorphology block as possible. Each (micro-)layer should be sampled if possible, with sample spacing ideally no greater than 5 cm. If a finer resolution is warranted, the resonated blocks can be processed into thin sections and sampled for sedaDNA (Massilani et al. 2022).

12 In addition to documenting the x,y,z coordinates of each sample collected, an overview photograph documenting the spatial relationship of the loose samples with the block

should be taken. Colored pins can be used to indicate the location of the loose sample locations. This should be done once all loose samples are collected and prior to the removal of the micromorphology block. Care should be taken to ensure that the photograph is of high quality, as it may prove useful for documenting sample location and context in subsequent publications.

13 Select and document, including photography, the location of the micromorphology block prior to biomolecular and/or geochemical sampling. If the block is to be plastered prior to removal, do this before collecting loose samples, so that the position of the block can be used as guidance for subsequent sampling (Figure 3).

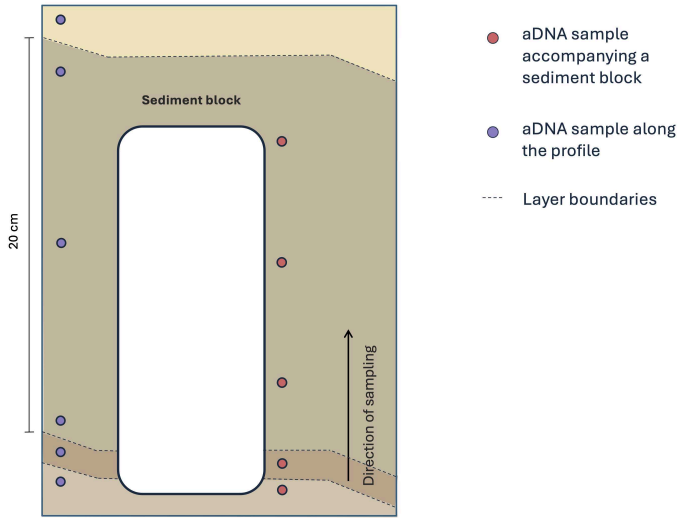

Figure 3: Vertical sampling strategy with oriented block. To minimise contamination, take samples from the bottom of the cleaned profile upwards. **Purple**: Take a sample from every visible layer. If the layer is >= 20 cm thick, take a sample at the end, center and beginning of the layer. If the layer is < 20 cm thick take one sample at the center of the layer. **Red**: All visible layers should be sampled. For thicker layers, collect multiple samples in a column at ~ 5 cm intervals. The block size should not exceed the size of a milk carton (15-20 cm long and 5-10 cm in width and depth).

2.2 Vertical sampling from the back of a sediment block

14 In order to reduce the number of destructive scars left in a profile after sampling, it is also possible to collect loose samples for geochemical/biomolecular analysis from the exposed back of block samples after removal from the profile and prior to completely encasing the block in plaster bandages (Figure 4). In this case, sampling procedures follow those provided above, with the following modifications and considerations:

15 Expose a fresh surface for sampling on the back of the block as it might have been handled or come in contact with non-sterile equipment.

16 Once all loose samples have been collected, locations of the loose samples should be indicated with colored pins and photographed. This is particularly important, since the x,y,z of the samples cannot be directly recorded using this method, but can only be reconstructed by tying into the x,y,z values of the block sample.

17 In this case, since the available material for sampling is limited to the micromorphology block, less material can be collected in the loose sample, e.g., 2 g (ca. 2 ml). This can still be collected within the 5/15 ml falcon tubes or sterile plastic bags.

Figure 4: Sampling from the back of a sediment block sample.

2.3 Vertical sampling from scar left in profile after removing sediment block

18 If one wishes to reduce the damage to a profile and relate loose samples to a block sample, but also retain greater spatial control and x,y,z-recording of the loose samples, it is also possible to collect samples from the scar left behind after removal of the sediment block (Figure 5). In this case, the same procedures above are followed with the subsequent considerations:

19 As before, after sampling mark sample locations with colored pins and ensure good photographic documentation of sample locations, in addition to x,y,z coordinates.

Figure 5: Sampling from a scar in the profile left by a sediment block sample.

3. Horizontal sampling strategy – Excavating in arbitrary units (e.g. spits)

20 Take a sediment sample at the beginning of each new spit when the exposed sediment is fresh. If a spit is completed at the end of the day, collect the samples from the new spit surface before leaving. If multiple samples will be taken from the same area over the course of days or weeks, aim to take samples from the same location within the excavation square (Figure 6).

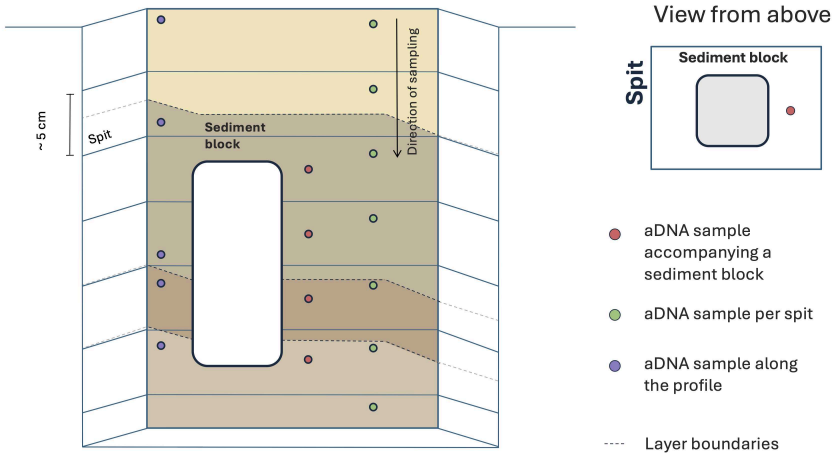

Figure 6: Horizontal sampling strategy with oriented block. **Green**: Take a sediment sample at the beginning of each new spit. **Purple**: Take a sample from every new visible layer. After the first sample, take a sample every 10 cm until the start of the next layer. **Red**: aDNA samples should be distributed evenly along the block depth, starting from the top. Aim for the samples to be in about 5 cm intervals. The block size should not exceed the size of a milk carton (~ 15-20 cm long and 5-10 cm in width and depth).

3.1 Excavating according to sedimentary characteristics (e.g. in layers, baskets, contexts, etc.)

21 Take a sample from every new visible sedimentary unit. After the first sample at the beginning of the layer, take samples every 10 cm until the next layer becomes visible (Figure 6).

3.2 Horizontal sampling with oriented sediment block

22 If a block sample for micromorphological analysis is to be taken from within a unit during excavation, and paired analyses are warranted, try and determine the number of corresponding loose samples that are to be taken beforehand. The maximum size of the block sample should be determined in collaboration with the laboratory or analyst who will process the block sample. As a general guide, take the loose samples at an interval of ~ 5 cm, as close to the area from where the sediment block sample is being collected (Figure 6). If a higher resolution is needed, the resonated blocks can be made into thin sections and then sampled for sedaDNA (Massilani et al. 2022). Generally, and if possible, a loose sample should be collected anytime that a new layer, feature, or context is encountered, regardless of the spacing of previous samples.

4. Additional Material

23

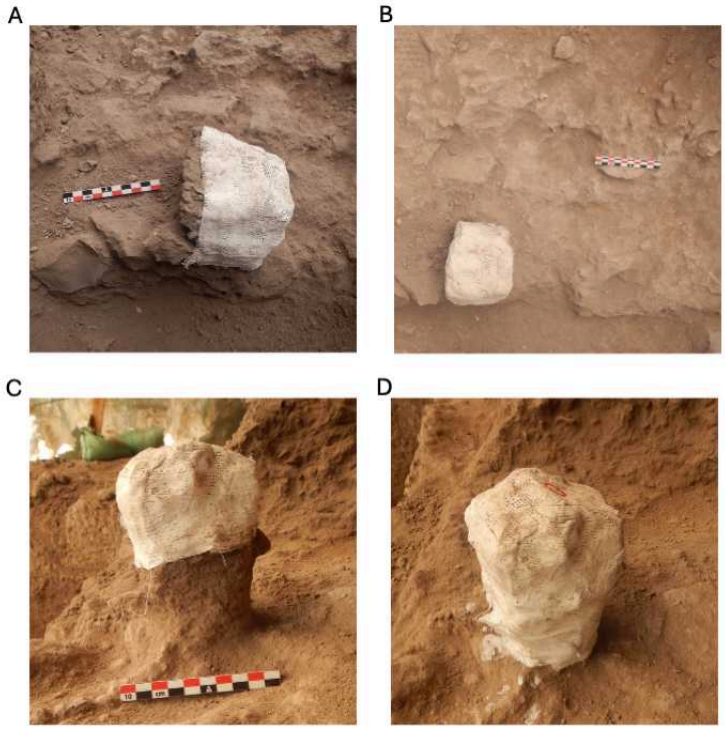

Figure 7: Procedure for collecting a micromorphology block from within an active excavation unit. A) Excavate as usual, but leave a block of sediment pedestaled. The block size should not exceed the size of a milk carton (~ 15-20 cm long and 5-10 cm in width and depth. When the block reaches a height of 3-4 cm, stabilize it with a few pieces of gypsum plaster bandage. B) Image of the same block fully encased in plaster. C) Continue to excavate downwards, maintaining the same block dimensions. Add a ring of plaster around the newly exposed sides as needed. D) When the final depth is reached, fully encase the sides with plaster and allow to dry. Then remove the block.

Protocol references

D. Massilani, M.W. Morley, S.M. Mentzer, V. Aldeias, B. Vernot, C. Miller, M. Stahlschmidt, M.B. Kozlikin, M.V. Shunkov, A.P. Derevianko, N.J. Conard, S. Wurz, C.S. Henshilwood, J. Vasquez, E. Essel, S. Nagel, J. Richter, B. Nickel, R.G. Roberts, S. Pääbo, V. Slon, P. Goldberg, & M. Meyer, Microstratigraphic preservation of ancient faunal and hominin DNA in Pleistocene cave sediments, Proc. Natl. Acad. Sci. U.S.A. 119 (1) e2113666118, https://doi.org/10.1073/pnas.2113666118 (2022).

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