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Extracellular DNA extraction from lake sediments V.2

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ABSTRACT

Over the past decade, an increasing number of studies has used environmental DNA from lake sediments to trace past lake ecosystem and landscape changes, agricultural activities or human presence and more broadly the biodiversity. In the environment, DNA can be found as intracellular and extracellular DNA (iDNA and exDNA). The contribution of each of these pools varies according to the environments, but exDNA often represents a high proportion of the total DNA (e.g. Vuillemin et al. 2017 and reference therein). Focusing the analyses on these different pools will lead to different community composition and structure of communities (e.g. Vuillemin et al. 2017). For plants, we propose to focus on the exDNA fraction to avoid the extraction of DNA from plant macro-remains, which might lead to an overrepresentation of these taxa and limit the detection of the other, “rarer” taxa.

The manipulation of ancient DNA is delicate, and the biases brought during the experiments can be multiple. Therefore, it is essential to work carefully, under strict laboratory conditions, with multiple controls and several replicates of samples or extraction or PCR (e.g. Fulton 2012).

This protocol details a sampling and extraction method of exDNA from sediments. This method was firstly developed for soils by Taberlet et al. 2012. It uses a phosphate buffer to desorb the DNA fragments from particles such as clays and then, the binding, wash and elution buffers from the NucleoSpin® Soil kit (Macherey-Nagel). Here we present a modified protocol from Taberlet et al. 2012. One modification consists in the addition of a concentration step (by using the amicon ultra centrigugal filter system) after the mixing of sediments with the saturated phosphate buffer. This step allow to increase the DNA yield. The quantity of phosphate buffer is usually based on the quantity of wet sediments (e.g. for 15g of wet sediment/soil, we add 15 ml of phosphate buffer). However, because lake sediments can have very different water content (depending on the composition and compaction) and because the exDNA is adsorbed onto particles, we now propose to base the phosphate buffer quantity on the dry weight of sediments.

Fulton T., 2012. Setting Up an Ancient DNA Laboratory. Book Chapter, Ancient DNA in Methods in Molecular Biology edited by Shapiro B. and Hofreiter M., 1-11.

Taberlet, P., Prud’Homme, S.M., Campione, E., Roy, J., Miquel, C., Shehzad, W., Gielly, L., Rioux, D., Choler, P., Clément, J.-C., Melodelima, C., Pompanon, F. and Coissac, E, 2012. Soil sampling and isolation of extracellular DNA from large amount of starting material suitable for metabarcoding studies. Molecular Ecology, 21: 1816-1820.

Vuillemin A., Horn F., Alawi M., Henny C., Wagner D., Crowe S. A., Kallmeyer J., 2017. Preservation and Significance of Extracellular DNA in Ferruginous Sediments from Lake Towuti, Indonesia. Frontiers in Microbiology 8, 1440.

PROTOCOL CITATION

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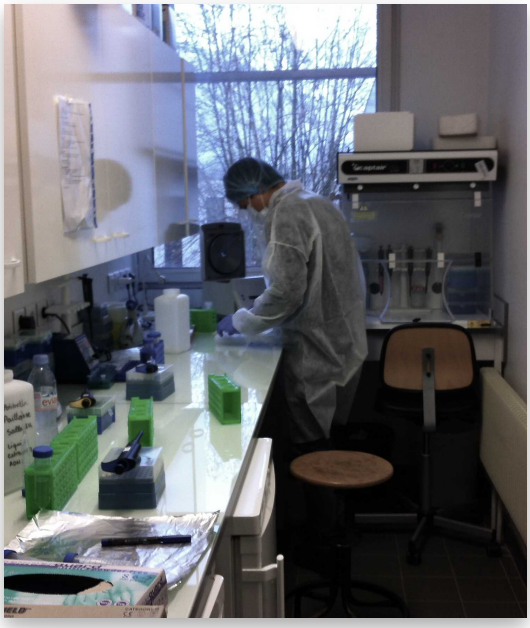
GUIDELINES

All steps must be carried out in a sterile laboratory with sterile equipment as well. Experimenters must equip themselves accordingly (disposable blouse, gloves, mask, disposable hair cap) in order to reduce all possible ways of contamination.

core sub-sampling for DNA analyses



exDNA extraction in the ancient DNA laboratory

MATERIALS TEXT

Equipment for sampling:

- Disposable blouse
- Gloves
- Mask
- Disposable hair cap
- Bleach (3 to 10%)
- Ethanol (70%) and a container to soak the metal plates and other tools
- Paper towel
- Indelible marker
- Metal plates to instert into the half cores
- Stainless steel forceps (to take the metal plates and then burn them)
- Balance
- Aluminium foil
- Sample carrier
- Free-DNA falcons 50ml
- Normal plastic vials with caps (for the edges of the sediment slices)

For extractions:

- Disposable blouse
- Gloves
- Mask
- Disposable hair cap
- Bleach (3 to 10%), DNA-away, Ethanol (70%) for cleaning
- Orbital shaker
- Centrifuge for the 50 ml falcons and centrifuge for eppendorfs (or QIAvac 24 plus system with the connectors and a vacuum pump)
- Clean container for preparing the phosphate buffer
- Free DNA water, NaH2PO4 powder and Na2HPO4 powder for preparing the phosphate buffer
- Amicons ultra-15, centrigugal filters Ultracel - 10K (not certified as DNA-free so put under UV before to use it)
- Tubes 1,5 ml
- Mechanical pipettes and filter tips (p1000 and p100)
- UV box (optional)
- Block heater to warm the elution buffer
- Freezer at - 20° (to keep the extracts)
- NucleoSpin® Soil kit (Macherey-Nagel) (Buffers SE, SB, SW1 and SW2, green column) and DNA-free ethanol

SAFETY WARNINGS

Take care with fire when you are sterilizing tools with the alcohol and burner (lighter).

BEFORE STARTING

Core sub-sampling: required material and working conditions

-Be in a clean room, ideally positively pressurized

-Wear gloves, disposable blouse and disposable hair cap
-Clean the work space with bleach (3 to 10%) and then with ethanol (70%)
-see Fulton 2012 for more detailed information on working conditions for ancient DNA (aDNA)

Core sub-sampling: protocol
Put the metal plates in alcohol to burn them and decontaminate them.
Insert the metal plates into the core to cut the slice. You can remove the sediment slice keeping the pressure on the slice.
Remove the edges (around 3/5 mm of thickness), which were in contact with air and the coring tube.
Cut the sample in two parts
Put the 2 samples from the heart of the sediment slice in 2 DNA-free falcons of 50 ml (we can start to extract one sub-sample and keep the second as an archive in case we need it later)
Keep open a DNA-free falcon on the laboratory working bench. You will use it as sampling control.
Extract directly after sampling or put in a freezer at - 18°C to extract later.

Keep the edges of the slices and weight wet and then dry to estimate the water content and then the dry weight of the samples for the DNA analyses. The estimation of the dry weight of the samples used for the DNA analyses will be used to determine the quantity of saturated phosphate buffer to add.

exDNA extraction 2h 30m

1 Preparation of the SW2 buffer within the kit (NucleoSpin® Soil kit from Macherey-Nagel):

This step is only FOR the FIRST USE of this BUFFER

Add correct volume of ethanol to SW2 (e.g. for 250 prep kit, add **400 mL** of ethanol to **100 mL** SW2 concentrate).

2 Phosphate buffer preparation: 15m
The phosphate buffer must be prepared the same day, before starting the extraction protocol.

1. Calculate the quantity of phosphate buffer required for the extractions. Prepare a little more especially for the extraction control. You will add the same volume of phosphate buffer as 2 or 3 times the dry weight of sample depending on the quantity of sediment sampled and the water content. You will apply the same ratio for all samples of a same study. It is important to take care to not sample too much sediments to be able to add the phosphate buffer in the 50 ml falcon and avoid overflowing.
2. Add the right quantities of NaH2PO4 and NA2HPO4 in the DNA-free water (To prepare **1 L** of phosphate buffer: add **1970 mg** of NaH2PO4 powder and **14700 mg** of NA2HPO4 powder)

3. Mix until the total dissolution of the phosphate powder. Please be sure that the phosphate powder is completely dissoluted (i.e. no powder emains in the bottle)
4. If you have, put in the UV box for **00:15:00**

30m

3 Addition of phosphate buffer to samples:

1. Add the same volume of phosphate buffer to each 50 ml falcon. Each falcon should receive 2 or 3 times the dry weight of sediment (e.g. **15 mL** for **5 g** of dry sediment).
2. Mix with an orbital shaker **00:15:00**
3. During the shaking time, label the final collection tubes, Amicons and green columns and prepare the tubes for SB (**200 µL**)

30m

4 Centrifugations: **4400 rpm**

1. Centrifuge the **50 mL** falcons to separate the sediment **00:10:00** .
2. Transfer of the supernatant to the amicons and centrifuge for **00:10:00** intervals until concentrated down to about **400 µL** in filter (the time for concentration depends on the sediment type). You can decide to centrifuge nearly all the supernatant (e.g. **12 mL**) or only a part. If the concentration is fast we suggest to centrifuge all the supernatant. On an aluminium foil (avoid notebooks in a clean lab) note the quantity of supernatant added and the quantity obtained after the centrifuge to know the concentration rate (it is generally difficult to obtain exactly the 400 µl required for the following steps. We usually oscillate between around 450 and 700 µl)

Heat X ml (for X samples) of SE to **80 °C** (in 1.5 or 2 ml tubes)

10m

5 DNA binding:

1. Add the **400 µL** of the concentrated extract in the tube previously filled with **200 µL** of SB buffer, resuspend and transfer the **600 µL** of supernatant to the green column (from NucleoSpin® Soil kit (Macherey-Nagel))
2. centrifuge at **11000 rpm, 00:01:00** . Discard flow-through. (You can also use the Quiavac system to save time)
3. Prepare the control with **400 µL** of phosphate buffer + **200 µL** of SB buffer

6 Wash silica membrane

7m

6.1 1st wash:

1. Add **500 µL** SB on each column.
2. Centrifuge at **11000 rpm, 00:00:30** .. Discard flow-through.

7m

6.2 2nd wash:

1. Add **550 µL** SW1 on each column.
2. Centrifuge at **11000 rpm, 00:00:30** . Discard flow-through.

7m

6.3 3rd wash:

1. Add correct volume of ethanol to SW2 - for 250 prep kit, add **400 mL** of ethanol to **100 mL** SW2 concentrate. (only FOR the FIRST USE of this BUFFER)
2. Add **700 µL** SW2 on each column.
3. Centrifuge at **11000 rpm, 00:00:30** . Discard flow-through.

7m

6.4 4th wash:

1. Repeat previous step: add additional 700 µl SW2 on each column.
2. Centrifuge at **11000 rpm, 00:00:30** .. Discard flow-through.

3m

7 Dry Silica Membrane:

Centrifuge **11000 rpm, 00:02:00**

15m

8 DNA elution:

1. Place each column on a new **1.5 mL** collection tube (with the sample label).
2. Add **50 µL** SE to each column.
3. Incubate **00:01:00** (**Room temperature**), lid open, followed by additional **00:04:00** with lid closed.
4. Centrifuge at **11000 rpm, 00:00:30** .
5. Repeat with additional **50 µL** SE for final elution volume of **100 µL** .
6. Store at **-20 °C**

DNA is ready for quantification (we use the Qubit system or the Quantifluor® ONE dsDNA system from Promega) and PCR