VERSION 1

APR 07, 2020

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.bdws7ee

Protocol Citation: Charline Giguët-Covex, Pierre Taberlet, Francesco Gentile Ficotola 2020. Extracellular DNA extraction. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bdws7ee>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's working

Created: Mar 19, 2020

Last Modified: Apr 07, 2020

PROTOCOL integer ID:
34482

Extracellular DNA extraction V.1

Pierre

Charline Giguët-Covex¹, Taberlet²,
Francesco Gentile
Ficotola^{3,4}

¹[Laboratoire Environnements, DYnamiques et TErritoires de la Montagne (EDYTEM), Université Savoie Mont Blanc, F-73376 Le Bourget du Lac, France];

²[Laboratoire d'Ecologie Alpine (LECA), Université Grenoble Alpes, F-38000 Grenoble, France];

³[Dipartimento di Scienze e Politiche Ambientali, Università degli Studi di Milano, via Celoria 26, I- 20133 Milano, Italy];

⁴Laboratoire d'Ecologie Alpine (LECA), Université Grenoble Alpes, F-38000 Grenoble, France]

Charline Giguët-Covex

Keywords: environmental DNA, ancient DNA, Lake sediment DNA, extracellular DNA extraction

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 centrifugal 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.

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

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 insert 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, NaH_2PO_4 powder and Na_2HPO_4 powder for preparing the phosphate buffer
- Amicons ultra-15, centrifugal 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 START INSTRUCTIONS

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 NaH_2PO_4 and Na_2HPO_4 in the DNA-free water (To prepare 1 L of phosphate buffer: add 1970 mg of NaH_2PO_4 powder and 14700 mg of Na_2HPO_4 powder)
3. Mix until the total dissolution of the phosphate powder. Please be sure that the phosphate powder is completely dissolved (i.e. no powder remains in the bottle)
4. If you have, put in the UV box for 00:15:00

3 Addition of phosphate buffer to samples:

30m

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)

4 Centrifugations: 4400 rpm

30m

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)

5 DNA binding:

10m

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

6.1 1st wash:

7m

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

6.2 2nd wash:

7m

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

6.3 3rd wash:

7m

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.

6.4 4th wash:

7m

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

7 Dry Silica Membrane:

3m

Centrifuge 11000 rpm, 00:02:00

8 DNA elution:

15m

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