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Extracellular DNA extraction from sediment using phosphate buffer and NucleoSpin Plant II Midi kit (MACHEREY NAGEL)

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Taberlet, P., Prud'Homme, S. M., Campione, E., Roy, J., Miquel, C., Shehzad, W., ... 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. https://doi.org/10.1111/j.1365-294X.2011.05317.x
Giguet-Covex, C., Ficetola, G.F., Walsh, K. et al. New insights on lake sediment DNA from the catchment: importance of taphonomic and analytical issues on the record quality. Sci Rep 9, 14676 (2019). https://doi.org/10.1038/s41598-019-50339-1

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We use this protocol and it's working

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Abstract

This DNA extraction protocol makes it possible to extract extracellular DNA from sediment and to work with around 4g of sediment.
This protocol was adapted by INRAE - CARRTEL (Thonon les Bains, France) to work on lake sediments and perform paleolimnological analyses.
The objective of the protocol is :
* to desorb extracellular DNA from the mineral and organic particles, using a saturated phospahe buffer (Taberlet et al 2012, Giguet-Covex et al 2019). The desorption of DNA is obtained by mixing 1Volume of saturated phosphate buffer with 1Volume of sediment ; however, in some cases, when sediment contain high concentration of organic matter, it is recommand to increase the amount of phosphate buffer up to twice the weight of the soil/ sediment (Taberlet et al 2012).
* to collect DNA extracts from several grams of sediment : many DNA extraction kits (e.g. NucleoSpin soil) largely used in sedimentary DNA research do not cope with high amount of sediments (> 1 g). Though it makes sense to analyse as much sediment as possible to be representative and avoid heterogeneity of results due to non homogenous sediments. In some studies, extracellular DNA extraction was carried out from up to 24g of sediment (Giguet-Covex et al 2019), using 20mL of phosphate buffer but only a low percentage of the obtained supernatant countaining extracellular DNA was finally fixed on membranes and used for the analysis. This was due to the fact that NucleoSpin® Soil kit (Macherey-Nagel) columns used in this study, have a binding capacity of 50µg of DNA (corresponding at a maximum of 0.5g of sediment) and a maximum volume capacity of 0.650mL. For the adated protocol we tested here, we chose to use an other extraction kit, NucleoSpin® Plant II Midi (Macherey-Nagel), where columns have a binding capacity of 200µg of DNA and a maximum volume capacity of 5mL. This choice offers the opportunity to work with several g of sediment (here 4g were tested) with the possibility to keep the total volume of supernatant (4.5 - 5mL) obtained after the desorption step.

Guidelines

- Sample preparation
- Desorption of DNA from mineral and organic particles
- Contaminants elimination
- DNA fixation and washing
- DNA elution

Materials

- Samples
  - sediment ≈ 4g

- Reagents
  - NucleoSpin® Plant II Midi kit (MACHEREY-NAGEL)
  - Ethanol (96 - 100%), molecular grade to prepare buffer PW2
  - Buffer SB (MACHEREY-NAGEL)
  - Saturated phosphate buffer (0.12 M Na_2HPO_4; pH ≈ 8), stored less than one week at +4°C
  - Ethanol to sterilize spatula

- Materials
  - specific DNA-work station (sterile area equipped with air filtration and UV systems)
  - centrifuge for 15 mL tubes (relative centrifugal force needed : 4,500 x g)
  - agitator for rotation with 15mL tubes holder
  - water bath
  - spatula
  - metalic pincer
  - digital burner
  - metal or glass support
  - clean chisel wash with DNA off or ethanol
  - precision weighting scale, precision : 0.01g
  - pipettes : 10000µL - 1000 µL - 100 µL
  - 2 trash cans : 1 for liquid and 1 for solid

- Consumables
  - tips with filter :
    > 10000µL > 6 per sample
    > 1000µL > 4 per sample
    > 100µL > 2 per sample
  - 15 mL tube compatible with centrifuge > 1 per sample
  - 2 mL sterile microcentrifuge tube > 1 per sample
  - parafilm
  - gloves

Troubleshooting

Safety warnings

The manufacturer advise to wear gloves and goggles and to flow the safety instructions for 1 reagent :

- PW1 coutains Guanidine hydrochloride 36 - 50% and 2 - propanol 20 - 35%
  CAS number : CAS 50-01-1, 67-63-0
  Signal word : Irritant and Flammable
  Hazard phrases : 226, 302, 319, 336
  Precaution phrases : 210, 260D, 264W, 280sh, 301+312, 330

Before start

- The following precautions must be applied :
  - If possible, it is best to work in a room dedicated to rare and ancient DNA
  - Wear gloves throughout the extraction process
  - Clean the bench with DNA off
  - Use tips with filters to avoid contaminations
  - Include negative controls
  - **All steps have to be performed under a specific DNA-work station** (sterile area equipped with air filtration and UV systems).

- Materials preparation :
  - Clean a specific DNA - work station and apply the UV for 15min
  - Sterilize spatulas, one spatula per sample. To limit risk of burns, during this step, you must wear a cotton blouse and do not use gloves
    * put a clean spatula in an ethanol solution
    * with metal pincer, take the spatula
    * pass the spatula through the flame, warning of the risk of burns
    * place sterilize spatula in a clean metal or glass support

- Samples preparation :
  - thaw sediment used for extraction, between 30min - 1h at room temperature or between 1h - 2h at +4°C (the time depends of the quantity of sediment and freezing temperature)

- Solutions preparation :
  - Prepare saturated phosphate buffer (0.12 M Na_2HPO_4; pH ≈ 8)(according to Taberlet et al 2012)
    * weigh the 2 compounds - see table below
    * transfer these compounds in a becher
    * dissolve them in ultra pure water
    * check pH, it will be ≈8
    * put solution into 1L graduated flask, make up to the mark with ultra pure water and mix
    * filter this solution on 0.2µM filter
    * store this buffer at +4°C, maximum one week

	Name	Linear Formula	Molecular Weigh	Final concentration	Weight for 1L of buffer
	Sodium phosphate monobasic	NaH2PO4	119.98 g/mol	16.4mM	1.97g
	Sodium phosphate dibasic	Na2HPO4	141.96 g/mol	103.6mM	14.7g

Informations on the compounds of saturated phosphate buffer

- Check buffer PW2 - before the first utilisation, you need to add the indicate volume of ethanol (96 - 100%) to buffer SW2 concentrate and mark the label of the bottle to indicate that ethanol was added. This solution is stable at room temperature (18 - 25°C) for at least one year
- Incubate the elution buffer PE at +65°C

Prepare the sample

1

- Annotate 15mL tubes, one 15mL tube per sample
- Weigh annotated tubes, use the weigh scale (0.01g of precision)
  → *On your notebook, note the name of tubes and their weights*
- With a sterilized spatula, homogenize a sediment and check that it is completely thawed (if not, wait for total defrost)
- Transfer ≈ 4g of sediment in the associated tube, 2 methods :
  - if the sediment is "compact", use a sterilized spatula to transfer the sediment
  - if the sediment countains a lot of water, use a 1000µL tip cuted with clean chisel

and transfer sediment by pipetting few times.
→ *On your notebook, note the used method and your comments about the sediment particularity*

- Weigh the tube containing sediment
  → *On your notebook, note the weight*
- If you can, transfer remaining sediment to a tube with adequate capacity (annotate it with the initial information).

On the storage tube, make a cross to show that this tube countains tawed sediment thaw once : this is highly recommended to do not subject sediment to freeze-thaw cycles in particular if the aim is to perform downstream analyses on sed-DNA extracts.
Keep this sediment at -40°C or -80°C for long term storage.
→ *On your notebook, note this tranfer and where the sediments are stored*

- Repeat this process for all the sediments that will be extracted during this session
- Add one volume of saturated phosphate buffer (0.12 M Na_2HPO_4; pH ≈ 8; stored less than one week at +4°C) for one volume of sediment

e.g if you have weighed 3.95 g of sediment, add 3.95 mL of saturated phosphate buffer

- Close the cap and you can add parafilm to secure the closure

Desorb DNA from mineral and organic particles

2

- Attach 15 mL tubes (containing sediment and P buffer) to agitator for rotation
- Agitate tubes at slow speed at Room temperature for 00:15:00

Eliminate contaminants

3

- Centrifuge 10000 rpm, Room temperature, 00:10:00
- Transfer the supernatant (without taking the pellet) to a NucleoSpin® Filter Midi
- Cenrifuge 4500 x g, 00:10:00

- Collect the clear flowthrough and discard the NucleoSpin® Filter Midi

Notes :
- *If not all liquid has passed the filter, repeat the centrifugation step*
- *If a pellet is visible after the centrifugation, transfer the clear supernantant to a new 15 mL tube (not provided in the kit)*
- *Estimate the volume of clear flowthrough, you need have around 4.5 mL - 5mL*

Adjust binding conditions

4
- Add 1.25 mL of **buffer SB**

Note : 0.25V of buffer SB for 1V of clear flowthrough
- Vortex 00:00:30

Bind DNA

5
- Place a NucleoSpin® Plant II Midi Column in a collection tube (15 mL)
- Load 4 mL of sample onto the column
- Centrifuge 4500 x g, 00:02:00
- Discardthe flowthrough and place the column back into the collection tube
- Load the remaining sample onto the column
- Centrifuge 4500 x g, 00:02:00
- Discard the flowthrough and place the column back into the collection tube

Wash and dry silica membrane

6 *Note : the same collection tube is used throughout the entire washing procedure to reduce plastic waste*

6.1 1st wash :
- Add 1 mL of **buffer SB** to the NucleoSpin® Plant II Midi Column
- Centrifuge 4500 x g, 00:02:00
- Discard the flowthrough and place the column back into the collection tube

6.2 2nd wash :
- Add 1 mL of **buffer PW1** to the NucleoSpin® Plant II Midi Column
- Centrifuge 4500 x g, 00:02:00
- Discard the flowthrough and place the column back into the collection tube

Note : although washing with buffer PW1 increase purity, it can in some case slightly reduce the final yield

6.3 3rd wash :

- Add 3 mL of **buffer PW2** to the NucleoSpin® Plant II Midi Column
- Centrifuge 4500 x g, 00:02:00
- Discard the flow through and place the column back into the collection tube

Note : verify that ethanol was added to buffer PW2 during the first utilisation

6.4 4th wash :

- Add 1 mL of **buffer PW2** to the NucleoSpin® Plant II Midi Column
- Centrifuge 4500 x g, 00:10:00 in order to remove wash buffer and dry the silica membrane
- Discard the flow through and place the column back into the collection tube

Elute DNA

7

- Place the NucleoSpin® Plant II Midi Column into a new collection tube (15mL)
- Add 100 µL of **buffer PE** previously heated 65 °C to the membrane
- Incubate at Room temperature for 00:05:00
- Centrifuge 4500 x g, 00:02:00
- Repeat this step with another 100 µL of **buffer PE** previously heated 65 °C and etlute into the same tube
- Discard the NucleoSpin® Plant II Midi Column
- Transfer the total eluted DNA volume in a 2mL tube
- We recommend storing DNA frozen at -20°C until future analyzes (or at -40°C to -80°C for longer storage)