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Extracellular DNA extraction from sediment using phosphate buffer and NucleoSpin® Soil kit (MACHEREY NAGEL)

Forked from DNA extraction from environmental biofilm using the NucleoSpin® Soil 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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Protocol status: Working
We use this protocol and it's working

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Guidelines

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

Materials

- Samples
  - sediment ≈ 0.75g

- Reagents
  - NucleoSpin® Soil kit (MACHEREY-NAGEL)
  - Ethanol (96 - 100%), molecular grade to prepare buffer SW2
  - Saturated phosphate buffer (0.12 M Na2HPO4; 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)
  - microcentrifuge for 1.5 to 2 mL tubes (relative centrifugal force needed: 11,000 to 14,000 x g)
  - agitator for rotation with 2mL 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 : 0.0001g
  - pipettes : 1000 µL - 100 µL
  - 2 trash cans : 1 for liquid and 1 for solid

- Consumables
  - tips with filter :
    > 1000µL > 12 tips per sample
    > 100µL > 1 tip per sample
  - 2 mL sterile microcentrifuge tube > 4 per sample

(1 to transfert sediment - step 1; 1 to collect supernatant - step 3; 1 to collect solution after filter lysate - step 5; and 1 to elute DNA - step 10)

  - parafilm
  - gloves

Safety warnings

The manufacturer advise to wear gloves and goggles and to flow the safety instructions for 2 reagents :

- SB coutains Guanidinium thiocyanate 45 - 60%,
  CAS number : CAS 593-84-0
  Signal word : Irritant
  Hazard phrases : 302, 412
  Precaution phrases : 264W, 273, 301+312, 330

- SW1 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 Na2HPO4; 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	103.6 mM	14.7g

Informations on the compounds of saturated phosphate buffer

- Check buffer SW2 - 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
- Optional : Incubate the elution buffer SE at +50°C

Prepare the sample

1

- Annotate 2mL tubes, one 2mL tube per sample
- Weigh annotated tubes, use the weigh scale (0.0001g 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 ≈ 0.5g 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
- 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 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 Na2HPO4; pH ≈ 8; stored less than one week at +4°C) for one volume of sediment
e.g if you have weighed 0.5g of sediment, add 0.5mL of saturated phosphate buffer
- Close the cap and you can add parafilm to secure the closure

Desorb DNA from mineral and organic particlesAdjust lysis conditions

2

- Attach 2mL tubes (containing sediment and P buffer) to agitator for rotation
- Agitate tubes at slow speed at Room temperature for 00:15:00

Precipitate contaminants

3

- Centrifuge 10000 rpm, 00:10:00
- Transfer the supernatant (without taking the pellet) to a new 2mL tube

4

- Add 150 µL of **buffer SL3** and vortex for 00:00:05
- Incubate at 4 °C in a fridge for 00:05:00

- Centrifuge 11000 x g, 00:01:00

Filter lysate

5
- Place a NucleoSpin® Inhibitor Removal Column (red ring) in a Collection Tube (2mL, lid)
- Load up to 650 µL of clear supernatant (obtained at the step 4) onto the filter
- Centrifuge 11000 x g, 00:01:00
- Repeat the load and the centrifuge step as many time as there is still some supernatant from step 4 to be filtered

After each centrifugation, collect the filtered liquid in a clean tube : 1 single tube for all the filtration

- Discard the NucleoSpin® Inhibitor Removal Column

Note : if a pellet is visible after the centrifugation, transfer the clear supernantant to a new collection tube (not provided in the kit) to get ride of this pellet, and continue with the clear supernatant

Adjust binding conditions

6
- Add 250 µL of **buffer SB**
- Close the lid
- Vortex for 00:00:05 , make a brief centrifugation

Bind DNA

7
- Place a NucleoSpin® Soil Column (green ring) in a collection Tube (2mL)
- Load 550 µL of sample onto the column
- Centrifuge 11000 x g, 00:01:00
- Discard the flow through and place the column back into the collection tube
- Load the remaining sample onto the column
- Centrifuge 11000 x g, 00:01:00
- Discard the flow through and place the column back into the collection tube

Wash and dry silica membrane

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

8.1 1st wash :
- Add 500 µL of **buffer SB** to the NucleoSpin® Soil Column

- Centrifuge 11000 x g, 00:00:30
- Discard the flow through and place the column back into the collection tube

8.2 2nd wash :

- Add 550 µL of **buffer SW1** to the NucleoSpin® Soil Column
- Centrifuge 11000 x g, 00:00:30
- Discard the flow through and place the column back into the collection tube

8.3 3rd wash :

- Add 650 µL of **buffer SW2** to the NucleoSpin® Soil Column

Note : verify that ethanol was added to buffer SW2 during the first utilisation

- Centrifuge 11000 x g, 00:00:30
- Discard the flow through and place the column back into the collection tube

8.4 4th wash :

- Add 650 µL of **buffer SW2** to the NucleoSpin® Soil Column
- Centrifuge 11000 x g, 00:00:30
- Discard the flow through and place the column back into the collection tube

Dry silica membrane

9 Centrifuge 11000 x g, 00:02:00

Note : if for any reason, the liquid in the collection tube has touched the NucleoSpin® Soil Column after drying step, discard flow through and centrifuge again

Elute DNA

10

- Place the NucleoSpin® Soil Column into a new microcentrifuge tube (not provided in the kit)
- Add 30 µL of **buffer SE** to the column

Optional : to increase yield you can heat buffer SE at 50 °C

- Do not close the lid and incubate at Room temperature for 00:01:30
- Close the lid and centrifuge 11000 x g, 00:00:30
- Discard the NucleoSpin® Soil Column and keep the tube cointaining the DNA
- We recommend storing DNA frozen at -20°C until preparation of DNA library for HTS (or at -40°C to -80°C for longer storage)
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