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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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1 Works for me dx.doi.org/10.17504/protocols.io.betsjene

CARRTEL

Cecile Chardon

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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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FORK FROM

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PROTOCOL INTEGER ID

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GUIDELINES

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

MATERIALS TEXT

- 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

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Precaution phrases : 210, 260D, 264W, 280sh, 301+312, 330

BEFORE STARTING

- 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	103.6mM	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 :

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- 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

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- 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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