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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.bd52i88e

EcoALpsWater

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ABSTRACT

This protocol is part of the DNA workflow applied in the Eco-AlpsWater Project.
The methodological step described here is the extraction of DNA, this is a critical step for obtaining relevant results since molecular inventories might be influenced by the DNA extraction method used.
The choice of the methodology for biofilms DNA extraction is based on previous studies and in particular on the work done by Vasselon et al. (2017) where the authors have tested 5 DNA extraction methods combining various types of cell lysis and DNA purification to extract DNA (from pure diatom cultures and biofilms samples from streams and lakes).
For the Eco-AlpsWater project, after being sampled in lakes or rivers, biofilms are stored in 50 mL tubes in ethanol at 4°C, and for a maximum of 3 months before DNA extraction (the extraction should preferably be done in the month following the sampling).
The DNA extraction protocol presented below has been used in several recent studies (e.g. Vasselon et al 2017ab, 2018) focussed on the application of diatoms metabarcoding; this extraction is based on a protocol adapted from the NucleoSpin® Soil kit (MACHEREY-NAGEL) with specific modifications for biofilm DNA extraction.

EXTERNAL LINK

http:// NucleoSpin® Soil kit (MACHEREY-NAGEL) - User Manual https://www.mn-net.com/Portals/8/attachments/Redakteure_Bio/Protocols/Genomic%20DNA/UM_gDNASoil.pdf

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Vasselon V., Domaizon, I., Rimet F., Kahlert, M., & Bouchez, A. (2017a). Application of high-throughput sequencing (HTS) metabarcoding to diatom biomonitoring: Do DNA extraction methods matter? Freshwater Science, 36, 162–177
Vasselon V., A. Bouchez F. Rimet, S. Jacquet, R. Trobajo, M. Corniquel, K.Tapolczai & I. Domaizon (2017b) Avoiding quantification bias in metabarcoding: Application of a cell biovolume correction factor in diatom molecular biomonitoring. Methods in Ecology and Evolution, 9(4): 1060-1069.
Vasselon V., Rimet F., Tapolczai K., & Bouchez A. (2018). Assessing ecological status with diatoms DNA metabarcoding: Scaling-up on a WFD monitoring network (Mayotte Island, France). Ecological Indicators, 82, 1–12.

ATTACHMENTS

Genomic DNA from environmental biofilm - Protocol at a glance.pdf

GUIDELINES

- Sample preparation
- Cells lysis
- Contaminants elimination
- DNA fixation and washing
- DNA elution

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MATERIALS TEXT

- Samples
  - environmental biofilm
  - preserved in ethanol (final concentration > 70% of ethanol)
  - volume needed = 2mL of the homogeneized sample

- Reagents
  - NucleoSpin® Soil kit (MACHEREY-NAGEL)
  - Ethanol (96 - 100%), molecular grade to prepare buffer SW2

- Materials
  - specific DNA-work station (sterile area equipped with air filtration and UV systems)
  - refrigerated microcentrifuge for 1.5 to 2 mL tubes (relative centrifugal force needed: 11,000 to 18,000 x g)
  - horizontal vortexer with microtube holder
  - water bath
  - pipettes : 1000 µL - 100 µL
  - 2 trash cans : 1 for liquid and 1 for solid

- Consumables
  - tips with filter :
    > 1000µL : 12 tips per samples
    > 100µL : 1 tip per samples
  - 2 mL sterile microcentrifuge tube : 2 per sample
  (1 to collect solution after filter lysate - step 5 and 1 to elute DNA - step 10)
  - 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 STARTING

- The following precautions must be applied :
  - Wear gloves throughout the extraction process
  - Clean the bench with DNA off
  - Use tips with filters to avoid contaminations
  - **All steps have to be performed under a specific DNA-work station** (sterile area equipped with air filtration and UV systems).

- Materials preparation :

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- Clean a specific DNA - work station and apply the UV for 15min
- Turn on the centrifuge - cool at +4°C

- Solutions preparation :

- Check lysis buffer SL1 for precipitated SDS. Dissolve any precipitate by incubating the buffer at 30-40°C for 10 min and shaking the bottle every 2 min.
- 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
- Incubate the elution buffer SE at +50°C

Prepare the sample

1
- Shake the 50mL falcon tube containing the biofilm / ethanol mixture by inverting the tube in order to obtain a homogeneous solution
- Take 2 mL of this homogeneized solution by pipetting twice 1 mL into a 2 mL sterile microcentrifuge tube and close the cap

Note : between each pipetting, aspirate/discharge to resuspend the biofilm particles that may have sedimented at the bottom of the tube

- Centrifuge **18000 x g, +4°C 00:30:00 ,**
- Discard the supernantant
- Add **700 µl** of **buffer SL1** to the biofilm pellet

Note : check SL1 buffer - dissolve any precipitate by incubating the buffer at 30-40°C for 10 min and shaking the bottle every 2 min

- Resuspend the biofilm pellet
- Transfer the biofilm/SL1 mixture into the NucleoSpin® Bead Tube Type A

Adjust lysis conditions

2
- Add **150 µl** of **Enhancer SX**
- Close the cap

Sample lysis

3
- Attach the NucleoSpin® Bead Tubes **horizontally** to a vortexer

Note : using a special adapter or alternatively tape to fix the tube

- Vortex the samples at **full speed** at **Room temperature** (18 - 25°C) for **00:05:00**

Precipitate contaminants

4
- Centrifuge **11000 x g 00:02:00**
- 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**

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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

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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** previously heated **50 °C** to the column
- 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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