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DNA extraction protocol from frozen filtration capsules

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Protocol status: Working

We use this protocol and it's working



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Abstract

The DNA extraction protocol presented below is based on the *FISH DNA extraction protocol* developed in the Eco-AlpsWater Project (https://www.alpine-space.org/projects/eco-alpswater/deliverables-final/dt1.1.2.--8.2-fish_dna_extraction_vigidna.pdf), adapted from the NucleoSpin® Soil kit (MACHEREY-NAGEL) according to the study of Pont et al (2018).

The methodology described here is the extraction of DNA, from eDNA samples collected with filtration cartridge adapted to treat large volume of water (e.g. VigiDNA® 0.45-µm capsule). **The cartridge is stored at -20°C** until extraction (no preservation buffer is added).

Materials

DNA EXTRACTION MATERIAL LIST

- Samples (Frozen Cartridge)
- NucleoSpin® Soil kit (MACHEREY-NAGEL)
- ATL buffer from the DNeasy Blood & Tissue Extraction Kit (Qiagen) (6 mL per sample)
- Mild lysis buffer (solution 1:10 ATL buffer and autoclaved milliQ water)
- Absolute ethanol: 33 mL per sample
- Proteinase K: 20 µL per sample
- Sodium acetate solution (3 M): 1.50 ml per sample
- Flask shaker (e.g. S50 shaker (cat Ingenieurbüro™))
- A microcentrifuge for 1.5 to 2 mL tubes (relative centrifugal force needed: 11,000 to 18,000 x g)
- A refrigerant centrifuge for 50 mL tubes (relative centrifugal force needed: 15,000 x g at a temperature of 6°C)
- Freezer (-20°C)
- Vortex
- Incubator (56°C)
- Gloves
- 5/10 mL pipette + tips 5/10 mL
- 1000 µL pipette + tips 1000 µL
- 200 µL pipette + tips 200 µL
- 50 mL sterile tube: 1 per sample
- 2 mL sterile microcentrifuge tubes
- 1.5 or 2 mL sterile microcentrifuge tubes to collect DNA at the end of extraction : 1 per sample

Precautions before sampling

- 1
 - Wear gloves throughout the extraction process
 - Clean the bench with DNA off or 10% commercial bleach before and after manipulation
 - 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).**
 - If possible work in a room equipped specifically for rare/degraded DNA** (i.e. room equipped with positive air pressure, UV treatment, frequent air renewal, and accessible only with full protective clothing and mask). in any case the extraction can be done in a room where PCR products are amplified

DNA extraction procedure

- 2
 - Sample preparation**
 - Add 50 mL of the mild lysis buffer to the frozen filtration capsule and store them for 2 h at 4°C
 - Agitate the filtration capsule for 15 min on a flask shaker at 800 rpm
 - Collect 30 mL of buffer from the filtration capsule and divide it equally in two 50 mL tubes (15 mL each)
 - Add 33 mL of absolute ethanol and 1.5 mL of 3M sodium acetate to the 50 mL tubes
 - Store the tubes at least one night at -20°C
 - Centrifuge the tubes at 4,000 x g for 15 min at 6°C
 - Discard the supernatant, resuspend the pellet and merge it into one tube

The following part of the protocol is reported exactly as described in the FISH DNA extraction protocol developed in the Eco-AlpsWater Project

- 3
 - Addition of Lysis buffer**
 - Add 720 µL of ATL buffer from the DNeasy Blood & Tissue Extraction Kit (Qiagen)
 - Vortex at full speed for 5 min.
 - Transfer the mixture to a 2 mL tube containing 20 µL of Proteinase K
 - Mix by pipetting
 - Incubate 2 hours at 56°C
 - Precipitate contaminants**
 - Centrifuge 2 min at 11,000 x g at 4°C
 - Transfer the clear supernatant to a new 2 mL tube
 - Add 150 µL of buffer SL3 and vortex 5 s.
 - Incubate 5 min at 0-4°C
 - Centrifuge 1 min at 11,000 x g at 4°C

Filter lysate

- Place the NucleoSpin® Inhibitor Removal Column (red ring) onto a collection tube (2 mL, lid)
- Load up to 650 µL of the clear supernatant from step 3 onto the filter and centrifuge 1 min at 11,000 x g (repeat this step as many time as there is still some supernatant from step 3 to be filtered. After each centrifugation, collect the filtered liquid in a clean tube (several collecting tubes are required, but only 1 column for all the lysate from step 3).
- Discard the NucleoSpin® Inhibitor Removal Column

Note: If a pellet is visible after the centrifugation, transfer the clear supernatant 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 (step 6 from the NucleoSpin® Soil (MACHEREY-NAGEL GmbH & Co., Düren Germany)

- Pool the lysate from step 4 in a new 2mL collection tube
- Add 250 µL of SB Buffer
- Close the lid and vortex 5s.

Bind DNA

- Place a NucleoSpin® Soil Column (green ring) in a Collection tube (2 mL)
- Load 550 µL of sample onto the column
- Centrifuge 1 min at 11,000 x g
- Discard the flow through and place the column back in a collection tube
- Load the remaining sample onto the column
- Centrifuge for 1 min at 11.000 x g
- Discard the flow through and place the column back into the collection tube

Wash and dry silica membrane

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

- 1 st wash
 - Add 500 µL of Buffer SB to the NucleoSpin® Soil Column
 - Centrifuge for 30 s at 11.000 x g
 - Discard the flow through and place the column back into the collection tube
- 2 nd Wash
 - Add 550 µL of Buffer SW1 to the NucleoSpin® Soil Column
 - Centrifuge for 30 s at 11.000 x g
 - Discard the flow through and place the column back into the collection tube
- 3 rd Wash
 - Add 650 µL of Buffer SW2 to the NucleoSpin® Soil Column
 - Close the lid and vortex for 2s.
 - Centrifuge for 30 s at 11.000 x g
 - Discard the flow through and place the column back into the collection tube
- 4 th Wash
 - Add 650 µL of Buffer SW2 to the NucleoSpin® Soil Column
 - Close the lid and vortex for 2s.

- Centrifuge for 30 s at 11.000 x g
- Discard the flow through and place the column back into the collection tube

Dry silica membrane

- Centrifuge for 2 min at 11.000 x g

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

Elute DNA

- Place the NucleoSpin® Soil Column into a new microcentrifuge tube (not provided in the kit)
- Add 50 µL of Buffer SE previously heated to 37°C to the column
- Do not close the lid and incubate for 1 min 30 s at room temperature (18-25°C)
- Close the lid and centrifuge for 30 s at 11.000 x g
- Repeat the same steps with the same column and the same collection tube to get a final extract of 100 µL: Add 50 µL of Buffer SE previously heated to 37°C to the column
- Do not close the lid and incubate for 1 min 30 s at room temperature (18-25°C)
- Close the lid and centrifuge for 30 s at 11.000 x g
- Throw the column and keep the tube containing the 100 µL of DNA
- Store DNA frozen at -20°C (or -80°C for longer storage).

DNA quantification

- 4 Different equipment can be used to quantify the total amount of DNA extracted (e.g. Qubit® Fluorometer system, Nanodrop)

References:

- 5 FISH DNA extraction: EcoALpsWater protocol applied for fish DNA extraction from VigiDNA® filtration cartridge using an adaptation of the NucleoSpin® Soil kit (MACHEREY-NAGEL)
https://www.alpine-space.org/projects/eco-alpswater/deliverables-final/dt1.1.2.-8.2-fish_dna_extraction_vigidna.pdf

NucleoSpin® Soil kit (MACHEREY-NAGEL)- User Manual

[https://www.mn-](https://www.mn-net.com/Portals/8/attachments/Redakteure_Bio/Protocols/Genomic%20DNA/UM_gDNA_Soil.pdf)

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Pont, D., M. Rocle, A. Valentini, R. Civade, P. Jean, A. Maire, N. Roset, M. Schabuss, H. Zornig, and T. Dejean. 2018. "Environmental DNA reveals quantitative patterns of fish biodiversity in large rivers despite its downstream transportation." *Scientific Reports* 8. doi: 10.1038/s41598-018-28424-8.

