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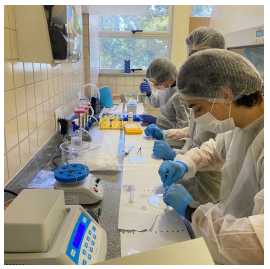
eDNA extraction from MCE filter and Sterivex cartridge - LGC PUC Minas



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We use this protocol and it's working

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Abstract

The following protocol covers the steps for extracting environmental DNA from **MCE** and **Sterivex** filters (Merck, cat. no. SVGP01050) using the **Dneasy PowerWater Sterivex kit** (Qiagen, cat. no. 14600-50-nf). This protocol has been adapted from the kit manufacturer to improve eDNA recovery.
This protocol was developed and tested, and proved to be successful for eDNA extraction. Visit our wedsite to know more about our team and our lines of research: https://sites.google.com/view/lgcpucminas

Image Attribution

Fábia Alves

Materials

Materials

- 1000 µL pipette
- 200 µL pipette
- Vortex
- Benchtop centrifuge (13,000 x g)
- Heat block
- Scapel handle (one for each sample point)
- Petri dish (one for each sample point)

Consumables

- DNeasy PowerWater Qiagen Kit
- Gloves
- Bleach
- 70% alcohol
- DNA decontamination solution
- 1000 µL tips with filter
- 200 µL tips with filter
- 2 mL tubes
- 10 mL serynge (one for each sample point)
- Scalpel blades (one for each sample point)

Reagents

- Proteinase K solution

Safety warnings

This protocol does not replace the information in the DNeasy PowerWater kit protocol produced by the manufacturer. Read it carefully before proceeding with the extraction.

Before start

- **Experimental design**

The number of samples to be extracted depends on the capacity of your centrifuge, heat block, and vortex. To prevent cross-site contamination, samples should ideally be extracted together only if they originate from the same sampling site. Check the capacity of these equipment beforehand to properly plan the following experiment.
Before starting the experiment, read this protocol from beginning to end; also read the **Dneasy PowerWater Sterivex kit** protocol and follow the manufacturer's recommendations.

- **Before proceeding with the extraction, follow these steps:**

1. Be sure not to enter any amplified DNA lab before the extraction.
2. Wear sterile gloves, mask, gown, and head cap throughout the extraction process.
3. Use pipett tips with filters to avoid cross-contaminations.
4. Properly clean all surfaces and equipment to be used, with products such as DNA decontamination solution or 5% NaClO, followed with 70% ethanol.
5. Ensure that all materials and equipment are readily available.
6. Ensure to use only filtered tips for pippeting.
7. Turn on the dry bath to 55 °C.

For Sterivex filters

1 After cleaning the bench and equipment with DNA decontamination solution, separate tweezers (one per sample) and scalpel blades for each sample. Disinfect them in [M] 25 % (v/v) bleach solution for 00:10:00. Rinse with distilled water, followed by spraying with [M] 70 % (v/v) % alcohol. Allow alcohol to evaporate completely, then flame-sterilize the instruments and place them on a sterile surface. 10m

2 Prepare a sterile 10 mL syringe for each sample site. These syringes can be used for all replicates (A, B, C, etc.) collected from the same location. Keep them on a sterile surface.

3 Use a new scalpel blade for each sample point. Partially open the blade packaging, attach the handle, and repeat for the second handle.

4 Retrive the Sterivex container from the freezer and identify the cartridges to be extracted. Keep the container closed to avoid contamination until use. Leave the frozen cartridges on the bench, separately.

5 Gather the reagents from the **DNeasy PoweraWater Qiagen Kit**, enough 2 mL tubes for all samples and replicates (one for each Sterivex filter and one for the extraction blank), and extraction bead tubes (one for each Sterivex and the blank). Organize everything on the sterilized bench.

5.1 Aliquot 1.5 mL of PW1 solution into separate 2 mL tubes for each sample/replicate (including the blank).

5.2 Preheat the tubes containing PW1 solution in a heat block at 55 °C for 00:15:00 at 1200 rpm. 15m

6 While the PW1 solution heats, label the extraction bead tubes for each sample replicate (e.g., Sample 3, replicate A: 1; Sample 3, replicate B: 2).

7 Remove the filter membranes from Sterivex cartridges while the PW1 solution is still heating. This allows for slight thawing and easier removal of the buffer liquid:

7.1 Carefully unpack the first sample's cartridge and attach the pre-filled syringe. Tighten the connection and expel the filter buffer into a designated waste container. Repeat until the inside

of the cartridge is dry.

7.2 After removing the liquid, use sharp tweezers to separate the internal cartridge from the outer plastic Sterivex casing. Locate the opening at the bottom of the casing revealing the inner cartridge edge. Use the side of the tweezers to gently pull the inner cartridge out of the casing. Avoid using the tip or dropping the filter.

7.3 Place the removed inner cartridge on a new, sterile Petri dish.

7.4 Unpack a sterile scalpel blade and carefully extract the membrane from the inner cartridge. While holding the membrane on the Petri dish, use the scalpel blade to cut it into small pieces, starting from the suture side.

7.5 Clean the scalpel handle and workbench between samples to prevent contamination.

7.6 Use sterilized tweezers to transfer the membrane fragments from each replicate into their corresponding extraction bead tubes.

7.7 Macerate the membrane fragments within the beads using the tweezers.

8 Continue from Step 13 from "For MCE Filters"

For MCE Filters

1h 23m

9 After cleaning the bench and equipment with DNA decontamination solution, separate tweezers (one per sample) and scalpel blades for each sample. Disinfect them in [M] 25 % (v/v) bleach solution for 00:10:00. Rinse with distilled water, followed by spraying with [M] 70 % (v/v) % alcohol. Allow alcohol to evaporate completely, then flame-sterilize the instruments and place them on a sterile surface.

10m

10 Gather the reagents from the DNeasy PoweraWater Qiagen Kit, enough 2 mL tubes for all samples and replicates (one for each filter and one for the extraction blank), and extraction bead tubes (one for each Sterivex and the blank). Organize everything on the sterilized bench.

10.1 Aliquot 1.5 mL of PW1 solution into separate 2 mL tubes for each sample/replicate (including the blank).

10.2 Preheat the tubes containing PW1 solution in a heat block at 55 °C for 00:15:00 at 1200 rpm . 15m

11 While the PW1 solution heats, label the extraction bead tubes for each sample replicate (e.g., Sample 1, replicate A: 1; Sample 2, replicate B: 2).

12 While the aliquotted PW1 remains in the heat block, remove the MCE filters from their storage tubes with tweezers and carefully place one filter into each tube containing the extraction beads, previously identified. Use a separate pair of tweezers for each replicate. Gently macerate the filters with the tweezers to increase the contact area with the reagents.

13 Retrive the Proteinase K (PK) solution from the freezer to thaw.

14 Once the filters are macerated and the PK aliquot has thawed (approximately 00:15:00 of PW1 solution heating), invert each tube containing PW1 solution into its corresponding bead tube with macerated filter membranes. 15m

14.1 Add 20 µL of PK to each bead tube containing the PW1 solution.

15 Vortex each bead tube containing the fragmented membranes for 00:02:00 . 2m

16 Incubate the tubes in the heat block at 55 °C for 00:15:00 at 1200 rpm . 15m

16.1 While the tubes are incubating, retrive 6 tubes + 1 filter tube for each replicate (and the blank) from the kit box.

16.2 Label each tube with the replicate number (1, 2, or 3) and the following step.

17 After the 15-minute incubation in the heat block, vortex each tube again for 2 minutes. Return the tubes to the heat block for another 00:15:00 at 55 °C . Repeat this cycle four times. 1h

18 Once the heat block and vortex cycles are complete, transfer the supernatant liquid from the bead tubes to the first of the labeled 2 mL tubes. Do this for each replicate. Be careful not to transfer the beads, only the supernatant.

19 Centrifuge the 2 mL tubes containing the supernatant at 13000 rpm for 00:01:00 . 1m

20 Carefully remove the tubes from the centrifuge and transfer the supernatant to a new 2 mL tube. Avoid disturbing the pellet at the bottom. If the pellet is disturbed, centrifuge again at 13000 rpm for 00:01:00 . 1m

21 Add 200 µL of the IRS solution from the kit to each new tube containing the supernatant.

22 Vortex the tubes to mix the supernatant and IRS solution thoroughly. Place the tubes in the freezer for 00:07:00 . 7m

23 Remove the tubes from the freezer and centrifuge them again at 13.000 rpm for 00:01:00 . 1m

24 Transfer the supernatant to a new 2 mL tube, avoiding the pellet.

25 Add 650 µL of PW3 solution to each tube and vortex to mix.

26 Remove 650 µL of the supernatant and transfer it to the filter tube. The filter tube cannot hold all the liquid at once, so add only 650 µL . Do not discard the remaining liquid in the tube.

27 Centrifuge the filter tubes at 13000 rpm rpm for 60 seconds.

28 Discard the filtered liquid and place the filter back at the bottom of the tube. Add another 650 µL of the remaining liquid from the 2 mL tube and centrifuge again at 13.000 rpm for 00:01:00 . 1m

29 While the tubes are centrifuging, cut the caps off of the remaining 2 mL tubes in the rack (there should be two for each replicate/filter).

30 Discard the filtered liquid. If there is any remaining unfiltered liquid, add it to the filter tubes and centrifuge again at 3.000 rpm for 00:01:00 . 1m

31 Discard the filtered liquid and replace the bottom of the filter tube with a 2 mL tube. To do this, discard the cap from the cut tube and place the filter container in the capless tube.

32 Add 650 µL of PW4 (shake before use) to the tubes with the filters inserted into the capless tubes.

33 Centrifuge all tubes with the new bottom at 13000 rpm for 00:01:00 1m

34 While the tubes are centrifuging, aliquot the EB solution from the kit into a 2 mL tube and place it in a heat block. To calculate the total amount of EB, multiply 100 µL by the total number of samples + 1. For example, if extracting 6 samples, aliquot 700 µL of the EB solution.

35 After centrifugation, discard the liquid and add 650 µL of ethanol to each filter tube. Centrifuge again at 13000 rpm for 60 seconds.

36 Discard the filtered liquid and place the filter tube back in the centrifuge, but this time for 2 minutes to ensure that all of the liquid has been removed.

36.1 While the filter tubes are centrifuging, prepare new 2 mL tubes for each replicate/filter and write the following information:

1. Collection site, abbreviated
2. Collection medium
3. Sample ID
4. Replicate type (A, B, or C)
5. Campaign date
6. Extraction date

37 After centrifugation, discard the bottom with the liquid and transfer the filter to the new bottom. Next, add only 50 µL of EB to each tube, carefully dispensing it close to the inner filter. Centrifuge at 13000 rpm for 00:01:00 . 1m

38 After the last centrifugation, do not discard the filtered liquid. It contains the extracted DNA. Add the remaining 50 µL of EB to each replicate and centrifuge again at 13000 rpm for 00:01:00 . 1m

39 Discard the filter and transfer the filtrate to the new tube that has already been labeled with the appropriate information.

40 Store the tubes containing the extracted DNA at -20 °C .

Protocol references

DNeasy PowerWater Kit Handbook: https://www.qiagen.com/jp/resources/download.aspx?id=75765ef9-2a6f-4f5d-a36b-dbd9beb43079&lang=en