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Plankton DNA extraction from Sterivex filter units

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EcoALpsWater

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ABSTRACT

The objective of this protocol is to provide a reliable and replicable method for the DNA extraction of lake micro-plankton to be used for downstream DNA analysis. This protocol is one of those proposed by the Eco-AlpsWater consortium to promote the implementation of High Throughput Sequencing (HT S) of environmental DNA (eDNA) in the biomonitoring and ecological assessment of water bodies.

The extraction is performed from samples filtered through Sterivex cartridges (Sterivex™ GP 0.22µm) and stored at -20°C, as described in the protocol dx.doi.org/10.17504/protocols.io.xn6fmhe, and with the use of the DNeasy® PowerWater Sterivex Kit (QIAGEN) with specific modifications adapted to plankton DNA extraction.

The application proposed here, in the context of EcoAlpsWater, aims at comparing DNA inventories to traditional phytoplanktonic inventories and at characterizing more broadly the micro-planktonic diversity through eDNA analysis (including bacteria).

This protocol is part of the deliverables provided by the WP1 of the Eco-AlpsWater project. All members of the EcoAlpsWater consortium (http://www.alpine-space.eu/projects/eco-alpswater/en/home) contributed to the optimization of this protocol.

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MATERIALS TEXT

- Samples (**Sterivex cartridge** stored at -20°C)

- **DNeasy® PowerWater Sterivex Kit** (QIAGEN)

- a **microcentrifuge** for 1.5 to 2 mL microtubes (relative centrifugal force needed: 4,000 to 13,000 x g)

- an horizontal **vortexer** equipped with an adapter for 5mL tubes

- **Gloves**

- **Incubator** (up to 90 ° C)

- **1000 µL pipette** + tips 1000 µL

- **200 µL pipette** + tips 200 µL

- **2 mL sterile microcentrifuge** tube to collect DNA at the end of extraction : 1 per sample

- **Fridge** (4°C storage)

- **PowerVac™ Manifold** (QIAGEN) (cat. No. 11991) or **QIAvac® 24 Plus Manifold** (QIAGEN) (cat. no. 19413) (optionnal) (If a vacuum manifold is not available, alternative methods can be used for processing a few samples at a time: see step 26 'Without vacum Manifold options' and no additional equipment is required)

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

- **Add Solution ST1A to bottle ST1B** (only required the first time you use ST1B). Mix well and store at 4°C when not in use.

- **Warm Solutions MBL and MR prior to use** (at 65°C for 5-10 minutes). Use Solutions ST2 and ST4 while still warm.

- **Shake to mix Solution PW** before use.

- **All steps have to be performed under a specific DNA-work station** (sterile area equipped with air filtration and UV systems).

1 Take the Sterivex™ filter unit (previously stored at **-20 °C** after filtration). **Remove the Inlet Cap** (Figure 1) and **add 0.9 mL of Solution ST1B** using a pipette tip.

Note: Insert the tip completely into the inlet so that the pipette tip is visible inside the unit just above the membrane.

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Note: Solution ST1A must be added to bottle ST1B prior to its first use only. Solution ST1B is a cell release solution that helps to take off the cells from the membrane, then, cells in suspension can be lysed. After Solution ST1A is added to bottle ST1B, it should be stored at 4°C.

Figure 1: Position of the two tips of the Sterivex™ filter unit

2 **Re-cap the inlet** and secure the Sterivex™ filter unit horizontally, with the inlet facing out, onto a vortex adapter. 5m
Vortex at minimum speed for **00:05:00** .

3 While still attached to the vortex adapter, **rotate the Sterivex™ filter unit** 180 degrees from the original position by marking the unit underneath with a marker and turning the unit until the mark faces up. 5m **Vortex at minimum speed for an additional** **00:05:00** .

4 Set the Sterivex™ filter unit with the inlet facing up and **remove the Inlet Cap**. **Add** **0.9 mL** **of MBL** using a pipette tip. **Re-cap the inlet**.

Note: Insert the tip completely into the inlet so that the pipette tip is visible inside the unit just above the membrane.

Note: Solution MBL must be warmed to dissolve precipitates prior to use. Solution MBL should be used while still warm. Solution MBL is a strong lysing reagent that includes a detergent to help break cell walls and will remove non-DNA organic and inorganic material.

5 **Incubate the Sterivex™ filter unit at** **90 °C** **for** **00:05:00** . 5m

Note: Place the unit so that the heat is evenly distributed around it. Do not heat at higher temperatures or for longer than 5 minutes. Heating the Sterivex™ filter units containing the bacterial release solution (ST1B) and lysis reagent (MBL) improves cell lysis of organisms that may be resistant to lysis reagents and mechanical bead beating.

6 **Cool the unit at** **Room temperature** **for** **00:02:00** . Check to make sure the caps are on tight. 2m

Note: The caps at both ends may loosen after heating. It is important to cool the unit before retightening the caps to minimize warping of the inlet.

7 Secure the Sterivex™ filter unit horizontally (with the inlet facing out) onto a vortex adapter, and **vortex at maximum speed for** **00:05:00** . 5m

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8 Set the Sterivex™ filter unit with the inlet facing up and **remove the Inlet Cap**.

Pull back the plunger of a 3 ml Syringe to fill the barrel with 1 ml of air and attach it to the inlet of Sterivex™ filter unit. Hold the 3 ml Syringe and Sterivex™ filter unit vertically with the syringe at the bottom to allow as much of the lysate as possible to be near the inlet.

Push the air into the unit until there is resistance and release the plunger. Back pressure withdraws the lysate and fills the syringe. Continue to pull back on the plunger to **remove as much of the lysate as possible. Detach the syringe from the Sterivex**™ filter unit.

Note: Do not force the air from the syringe into the Sterivex™ filter unit.

9 **Add the lysate to the 5 ml PowerWater® Sterivex™ glass Bead Tube**. 5m

Secure the PowerWater® Sterivex™ glass Bead Tube horizontally to a vortex adapter

Vortex at maximum speed for 00:05:00 .

10 **Centrifuge the tube at 4.000 x g for 00:01:00 at Room temperature** . 1m

11 **Transfer all the supernatant** to a clean 2.2 ml Collection Tube.

Note: Placing the pipette tip down into the beads and against the bottom of the tube is required. Pipette more than once to ensure removal of all supernatant. Any carryover of beads will not affect subsequent steps. It is expected to recover ~1.5 ml of supernatant.

12 **Add 300 µl of Solution IRS** and **vortex briefly** to mix. **Incubate at 4 °C for 00:05:00** . 5m

Note: Solution I is a second reagent to remove additional non-DNA organic and inorganic material including humic acids, cell debris, and proteins. It is important to remove those contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.

13 **Centrifuge the tube at 13.000 x g for 00:01:00** . 1m

14 Avoiding the pellet, **transfer the supernatant to a clean 5 ml Collection Tube**.

Note: The pellet at this point contains additional non-DNA organic and inorganic material. For best DNA yield and purity, avoid transferring any of the pellet.

With vacuum Manifold option

15 **Place a tube extender firmly into an MB Spin Column**.

Note: A 20 ml Syringe can be used as a binding column extender.

16 **Attach the tube extender/MB spin Column unit to a VacConnector and VacValve on the Qiavac 24 Plus Manifold.**

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17 **Add 3 mL of Solution MR to the 5 ml Collection** Tube containing supernatant and **vortex to mix.**

Note: Solution MR must be warmed to dissolve precipitates prior to use. Solution MR must be used while still warm. If solution MRis not used warm then the Binding Column may clog. Solution MR is a high concentration salt solution. DNA binds tightly to silica at high salt concentrations. Solution MR adjusts the salt concentration to selectively allow binding of the DNA to the silica filter membrane, while non-DNA organic and inorganic material that may still be present at low levels is prevented from binding.

18 **Load the entire 4.5 mL of supernatant into the tube extender/MB Spin Column**.

19 **Turn on the vacuum source and open the stopcock of the port**. Allow the lysate to pass through the binding column. This should take 5 to 10 minutes.

Note: If the Binding Columns become clogged, see the Clogging of Binding Columns section under the protocol.

20 After the lysate has passed through the column completely, **close the one-way Luer-Lok™ stopcock of that port**.

Note: Close the ports to samples that have completed filtering to increase the vacuum pressure to the other columns.

21 While keeping the binding column attached to the Luer-Lok™ stopcock, **remove the tube extender** and discard.

22 **Add 0.8 mL of ethanol into the Binding Column**. **Open the stopcock** while holding the column steady. Allow the ethanol to pass through the column completely. **Close the stopcock.**

Note: Ethanol is a prewash to help remove residual contaminants which will result in higher DNA purity and yield.

23 Shake to mix Solution PW. **Add 0.8 mL of Solution PW to the Binding Column**. **Open the LuerLok™ stopcock** and apply a vacuum until Solution PW has passed through the Binding Column completely. Continue to pull a vacuum for another minute to dry the membrane. **Close the port.**

Note: Solution PW is an alcohol based wash solution used to further clean the DNA that is bound to the silica filter membrane in the binding column. This wash solution removes residual salt and other contaminants while allowing the DNA to stay bound to the silica filter membrane.

24 **Add 0.8 mL of Ethanol to the MB Spin Column**. **Open the Luer-Lok™ stopcock** and apply a vacuum until Solution ethanol has passed through the Binding Column completely. Continue to pull a vacuum for another minute to dry the membrane. **Close the port**.

Note: Ethanol ensures complete removal of Solution PW which will result in higher DNA purity and yield.

25 **Turn off the vacuum source** and open an unused port to vent the manifold. If all 20 ports are in use, break the vacuum at the source. Make certain that all vacuum pressure is released before performing the next step. It is important to turn off the vacuum at the source to prevent backflow into the columns. **Continue with Step 27**.

Without vacuum Manifold options

26 The vacuum manifold is highly recommended for this protocol. If a vacuum manifold is not available **two alternative methods** can be used for processing a few samples at a time :

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26.1 The 20 mL syringe plunger from step 15 can be reinserted and used to **manually push the lysate through the column**. Once all the lysate has been pushed through, the column can be **added to a 2.2 ml tube** (provided for Step 27). **Wash with 0.7 mL of Solution PW** and **centrifuge for 00:01:00 at 13.000 x g** .

Discard the flow through and **wash with 0.7 mL of ethanol** and **centrifuge for 00:01:00 at 13.000 x g** . **Continue with Step 27.**

2m

26.2 **Place the MB spin column in a 2.2 ml tube** (provided for Step 27) and **load 700 µl of supernatant** onto the binding column and **centrifuge for 00:01:00 at 13.000 x g** . **Discard the flow through** and **repeat** until all the supernatant has been loaded onto the binding column. Seven loads for each sample processed are required. **Wash with 0.7 mL of Solution PW** and **centrifuge for 00:01:00 at 13.000 x g** . **Discard the flow through** and **wash with 0.7 mL of ethanol**. **Centrifuge for 00:01:00 at 13.000 x g** . **Continue with Step 27**.

3m

27 **Remove the MB spin Column** and **place in a 2.2 mL Collection Tube**. Pull the lid of the Collection Tube forward so that the cap fits completely over the Binding Column. **Centrifuge the tube at 13.000 x g for 00:02:00** to completely dry the membrane.

2m

Note: The spin removes residual ethanol. It is critical to remove all traces of wash solution because the ethanol can interfere with many downstream DNA applications.

28 **Transfer the MB spin Column to a new 2.2 ml Collection Tube** and **add 100 µl of Solution EB or DNA-Free PCR Grade Water** to the center of the white filter membrane. Pull the lid of the Collection Tube forward so that the cap fits completely over the Binding Column.

29 **Centrifuge at room temperature for 00:01:00 at 13.000 x g** .

1m

30 **Discard the Binding Column**. The **DNA in the tube is now ready for any downstream application**.

Note: We recommend storing DNA frozen (-20°C to -80°C).

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