



Sample collection and eDNA extraction from Sterivex filter units

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ABSTRACT

The following workflow covers several steps in the DNA analysis of environmental samples, from the water collection to the analysis back in the lab. The samples can be taken from several water systems (i.e. sea, lakes, rivers, streams) and collected in triplicate (1 L) in Sterivex sterile filter units (Merck, cat. no. SVGP01050). The DNA extraction protocol modifies the Dneasy PowerWater Sterivex kit (Qiagen, cat. no. 14600-50-nf).

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Sterivex, eDNA

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Sample collection

A portable peristaltic pump ([Vampire sampler](#); Buerkle) is used for sampling at an approximate 30-50 mL/min flow rate.

- 1 The tubing inserted in the unit's head has a suction hose ([Marprene](#); 4.8 mm inner diameter), connected to a flexible [silicone hose](#). A suitable adapter connects both hoses.



- 2 A [Sterivex](#) filter unit (0.22 µm pore size) is attached to the suction hose by a stainless steel male [Luer-lock ring](#) hose barb (1/4'). We recommend using a [hose clip](#) (9-10 mm; Buerkle cat. no. BURK8678-000) to firmly tight the Luer-lock ring to the hose. Each filter can process up to 2L of water depending on the amount of the suspended material.



- 3 Once the water sample is filtered, remove the remaining liquid from the Sterivex unit using a 5 mL sterilized syringe or similar by pushing air.



- 4 Cap both ends of the Sterivex. If the units have a male tip outlet, Parafilm can be used to cap them. Be aware of potential leakage, and make sure the Sterivex is dried and well sealed.



- 5 Now that the samples are ready and sealed, do not forget to identify them accordingly. A good rule of thumb is to write down ID, date, replicate number, filtered volume and station on the filter. Do the same on the sample bags, especially if there are more filters per site or replicate. The samples are store at **-20 °C** degrees.



DNA extraction

40m

- 6 Place the Sterivex vertically (with the inlet cap upward) and load **900 µl** of ST1B buffer. ST1B is stored at **84 °C**. Be careful putting the pipette tip through the inlet (orange cap). Dispense the buffer slowly; a fraction of the volume can be lost.



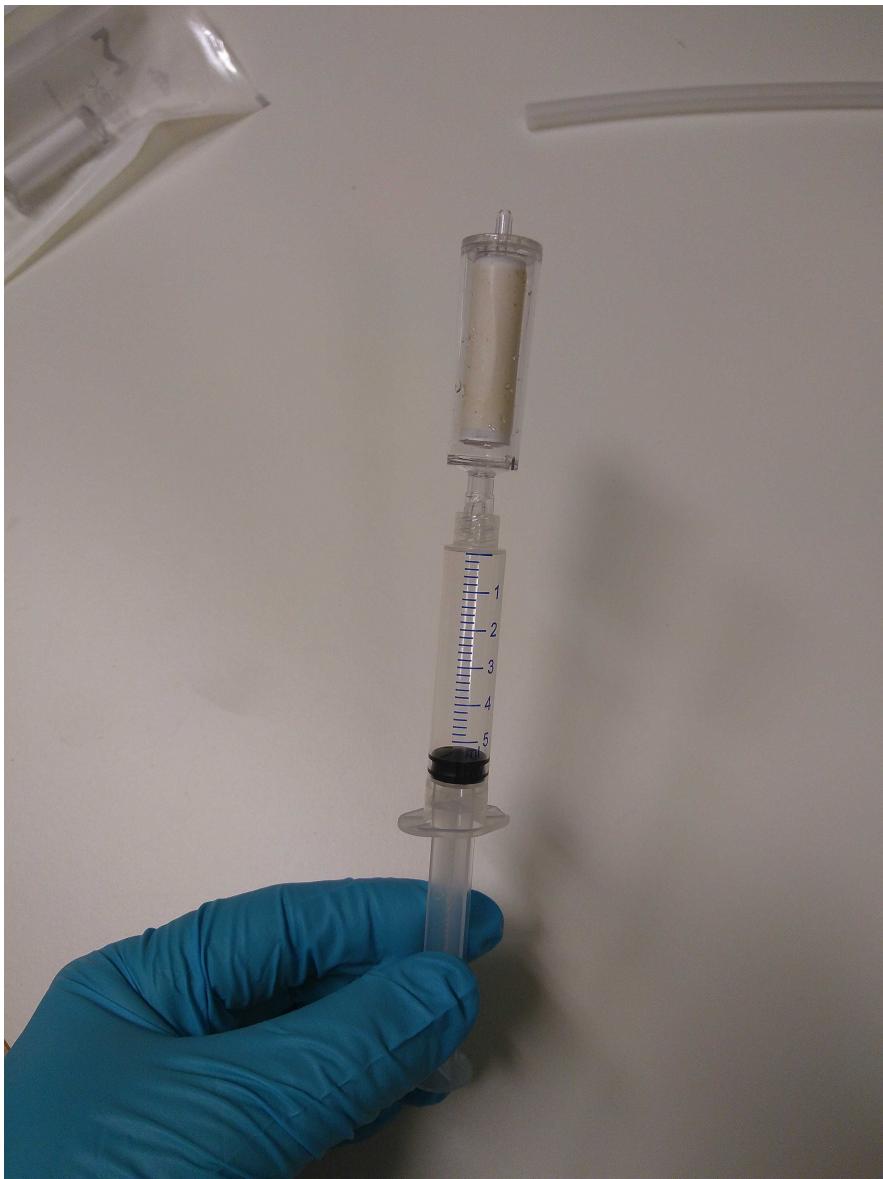
- 7 Allow mixing in a vortex with a horizontal [adapter](#) (Qiagen, Cat. No. 13000-V1-5) for 00:05:00 at the minimum speed. Set the filters with the inlet facing out and check for potential leakage from the outlet sealed with Parafilm.



- 8 Rotate the Sterivex in 90 degrees and vortex for **⌚00:05:00** at the minimum speed. Repeat the previous step 2 more times (4 times x **⌚00:05:00** each filter) 10m
- 9 Add **900 µl** of solution MBL. Dispense slowly; a fraction of the volume can be lost. 10m
Before use, heat the MBL solution at 65°C for **⌚00:10:00** as is suggested by the manufacturer.
- 10 Incubate the filter units vertically with the inlet upward at **8 90 °C** for **⌚00:05:00** in an oven. Before and after the incubation, check for any leakage in the Parafilm; replace it if needed. 5m
- 11 Let the filters cool down at room temperature for **⌚00:02:00**, and re-tight the caps and check the Parafilm. Then, 7m

vortex at maximum speed for **00:05:00**. While mixing, check that the filters stay in place. If not, lower the speed.

- 12 Transfer the lysate from the filter unit to a **3-5 mL** syringe; push 1 mL of air into the filter while it is vertical, and then release the plunger. Continue pulling back until the lysate is recovered in the syringe.



- 13 Pour the lysate into a **5 mL** Powerbeat tube and vortex horizontally for **00:05:00** at maximum speed 5m



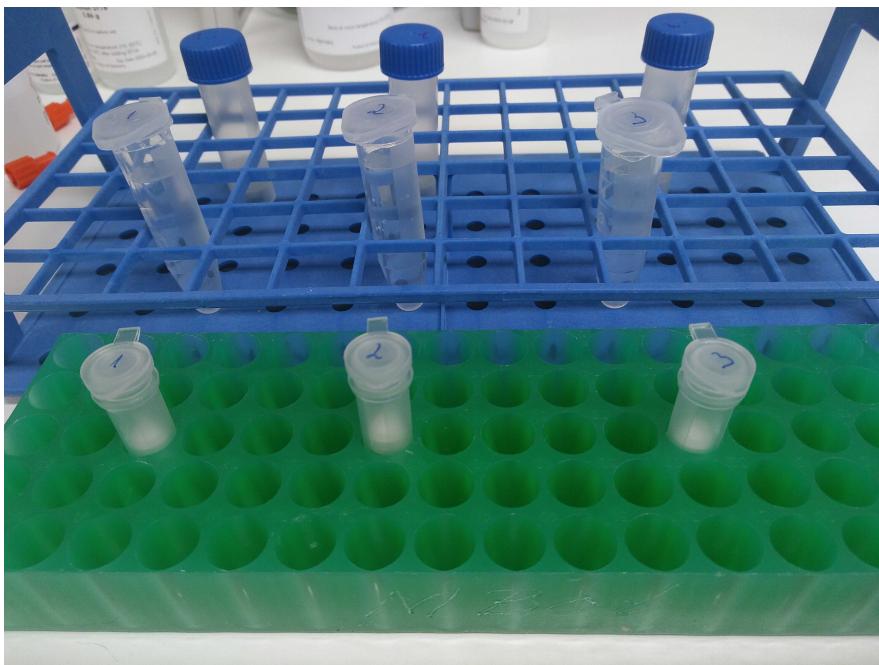
14 Centrifuge the tube at $\textcircled{S} 4000 \times g$ for $\textcircled{C} 00:01:00$ 1m

15 Carefully transfer the lysate to a clean $\textcircled{V} 2.2 \text{ mL}$ collection tube. Then, add $\textcircled{V} 1.6 \mu\text{l}$ of RNase (final concentration $[M] 100 \text{ mg/ml}$). Incubate the samples at $\textcircled{A} 37^\circ\text{C}$ x $\textcircled{C} 00:30:00$ in a heating block or a water bath. 30m

16 Add $\textcircled{V} 300 \mu\text{l}$ IRS solution to the tube and vortex. Then incubate at $\textcircled{A} 4^\circ\text{C}$ for $\textcircled{C} 00:05:00$. 5m

17 Centrifuge the tube at $\textcircled{S} 13000 \times g$ for $\textcircled{C} 00:01:00$ 1m

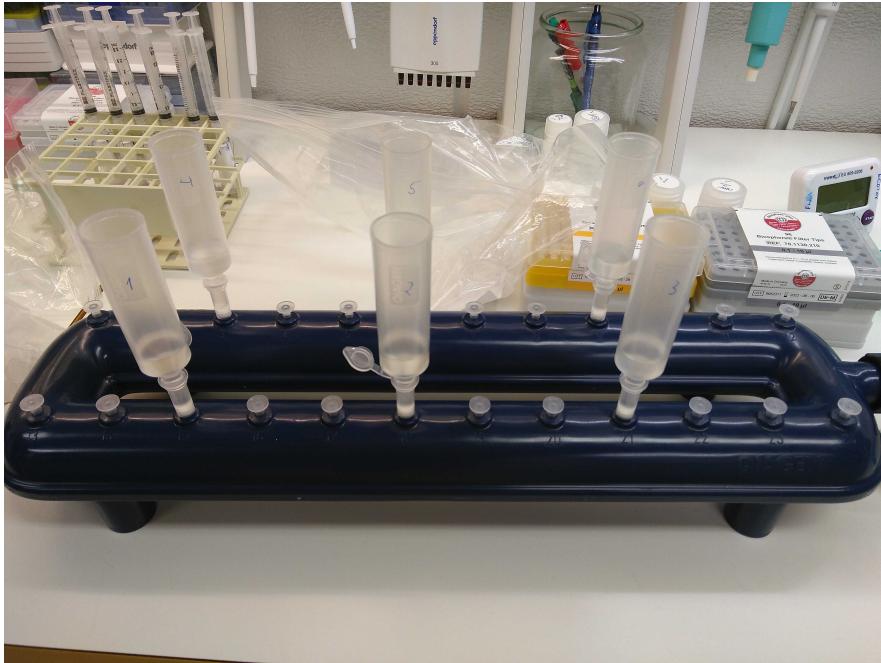
- 18 Transfer the supernatant to a **5 mL** collection tube, avoiding the pellet, and add **3 mL** of MR solution and vortex. Heat the MR solution at **65 °C** for **10:00** before using it.



- 19 A VacValve vacuum system and a vacuum pump are used for the following steps.



- 20 Load the supernatant (**4.5 mL**) into a tube extender and MB spin column. Filtrate at low pressure. The column is attached to the VacValve vacuum system, as shown below.



- 21 Once the lysate passes through, the column extender is carefully removed. Next, wash the spin column with \square 800 μ l of ethanol. Keep the columns open.



- 22 Now, wash the spin column with \square 800 μ l of PW solution. Keep the columns open.
Allow the membrane to dry by keeping the vacuum pump running for 1 min.
- 23 Repeat step 21, and wash the spin column adding \square 800 μ l of ethanol. Allow the membrane to dry as before.

- 24 Place the spin column in a clean collection tube and dry it out by centrifugation at **13000 x g** for **00:02:00** 2m
- 25 Transfer the spin column to a new clean tube, and add **50-100 µl** of solution EB (or DNA-free grade water). Centrifuge at **13000 x g** for **00:01:00**. 1m
- 26 Voila! The DNA is in the collection tube, ready for further processing.
- DNA quality check
- 27 The DNA purity is checked with a Nanodrop.
- 28 The DNA concentration is quantified by Qubit.

