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Sterivex Water Filter eDNA Extraction Protocol

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We use this protocol and it's working

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Disclaimer

Abstract

An easy DNA extraction method for Sterivex^{TM} water filters using QIAGEN Blood and Tissue kit. May also be used for different filter papers.

Guidelines

It is recommended to perform the extraction in a PCR-free lab, in a UV laminar flow cabinet with the use of filter tips.

Materials

- QIAGEN Blood and Tissue Kit (Cat no. / ID. 69504)
- QIAshredder columns (Cat no. / ID. 79656)
- Bleach
- DNase-free ddH20
- Pipe cutter
- Tweezers and scalpel
- Petri-dish
- Sterile tissue paper
- Thermomixer
- Microcentrifuge

Safety warnings

Use Good Lab Practice and wear protective equipment. Avoid contact with skin.
All chemicals can be disposed of down the sink with copious amounts of water to dilute the ethanol down to >20% of the waste.

Day 1

1 Before starting, clean the area with 10% bleach. Fill one beaker with a 50% bleach solution and a second with ddH2O. Make sure the beakers have been cleaned and autoclaved prior to use or use disposable sterile containers to avoid DNA contamination.

2 Place the tweezer and the scalpel in 50% bleach for 1 min. Take out, swirl to rinse in the DNase-free ddH2O, and dry with sterile tissue paper. Clean the pipe cutter with 10% bleach, rinse with DNase-free ddH20, and dry with tissue paper.

3 Using the pipe cutter, crack open the filter unit on the outlet end.

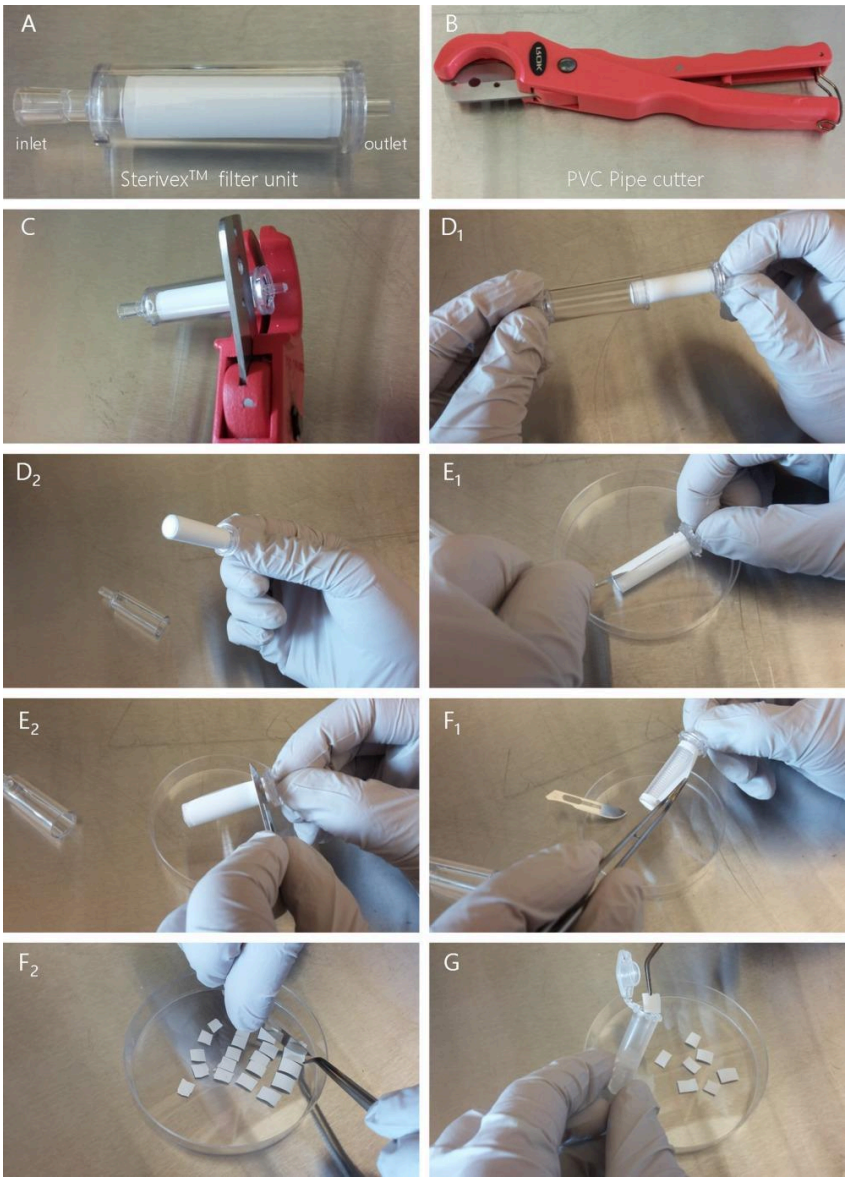

Figure 1: Illustrated protocol demonstrating the separation of the Sterivex™ filter from its casing (Cruaud *et al.* 2017).

4 Use the scalpel to cut longitudinally along the filter and around the filter paper and remove it from the plastic unit with the tweezer.

5 Place the filter paper onto a petri-dish, cut into small parts, and place these into a 2 ml microfuge tube.

6 Place the tweezers and scalpel in 50% bleach, clean the pipe cutter and bench with 10% bleach. Repeat this with all the samples in the batch.

7 Add **450 µl Buffer ATL** and **25 µl Proteinase K** to each microfuge tube. Ensure that the filter is fully immersed, using the filter tip to push all filter material down into the liquid if necessary.

8 Vortex thoroughly and following this ensure that the filter remains fully submerged in the lysis solution.

9 Incubate at **56°C overnight** in a thermomixer at 900rpm.

Day 2

10 Clean the area and centrifuge with 10% bleach. Remake the 50% bleach solution and ddH2O beakers and place the tweezer in the bleach solution. After one minute remove the tweezers, rinse with ddH2O and dry with tissue paper.

11 Vortex each microfuge tube containing the filter sample for 15 seconds. Pipette as much solution as possible into a new 2 ml microfuge tube. Place the remaining filter paper into a Qiashredder spin column using the tweezers. Clean the tweezers with 50% bleach and ddH2O between samples.

12 After removing the filter paper, if there looks to be remaining lysis solution in the original microfuge tubes, give them a quick spin down and pipette any solution into the 2ml microfuge tube with the rest of the lysis solution.

13 Place the Qiashredder spin columns in a centrifuge and centrifuge at **11,000rpm for 2 minutes**. Combine the product (liquid that passes through the column to the collection tube) with the rest of the solution within the 2ml tube.

14 Dispose of the Qiashredder, and empty microfuge tubes used for lysis.

15 Add **500 µl Buffer AL** to the 2ml microfuge tubes with the lysis solution, vortexing immediately. Incubate at **56°C for 10 minutes** in a thermomixer at 900rpm.

16 While waiting, pipette out sufficient AE buffer (50 µl x sample) into 1.5 or 2ml tubes and place in the thermomixer. This will be used in a later step.

17 Remove the samples from the heatblock and add **500 µl 100% ethanol** (analytical reagent grade), vortex immediately.

18 For each sample, add **700 µl of the solution** to a DNeasy spin column. Centrifuge at **8000rpm for 1 minute.** Place the spin column in a new collection tube and repeat this until all the solution has passed through the spin column.

19 Place each spin column into a new collection tube and add **500 µl Buffer AW1**. Centrifuge at **8000rpm for 1 minute**.

20 Place each spin column in a new collection tube and add **500 µl Buffer AW2**. Centrifuge at **11,000rpm for 3 minutes**.

21 Place the spin columns in 1.5 ml tubes labelled with sample name and date on the lid and side. Elute the DNA with **50 µl Buffer AE** (preheated to 56°C). Incubate at **room temperature for 5 minutes** then centrifuge at **8000rpm for 1 minute**.

22 (Optional) To increase DNA recovery further, put the elute in the 1.5ml back on the spin column and centrifuge again at **8000rpm for 1 minute**.

23 Discard the DNeasy spin column. Freeze the 1.5 ml tube containing the DNA sample at -20C (short-term) or -70 (long-term).

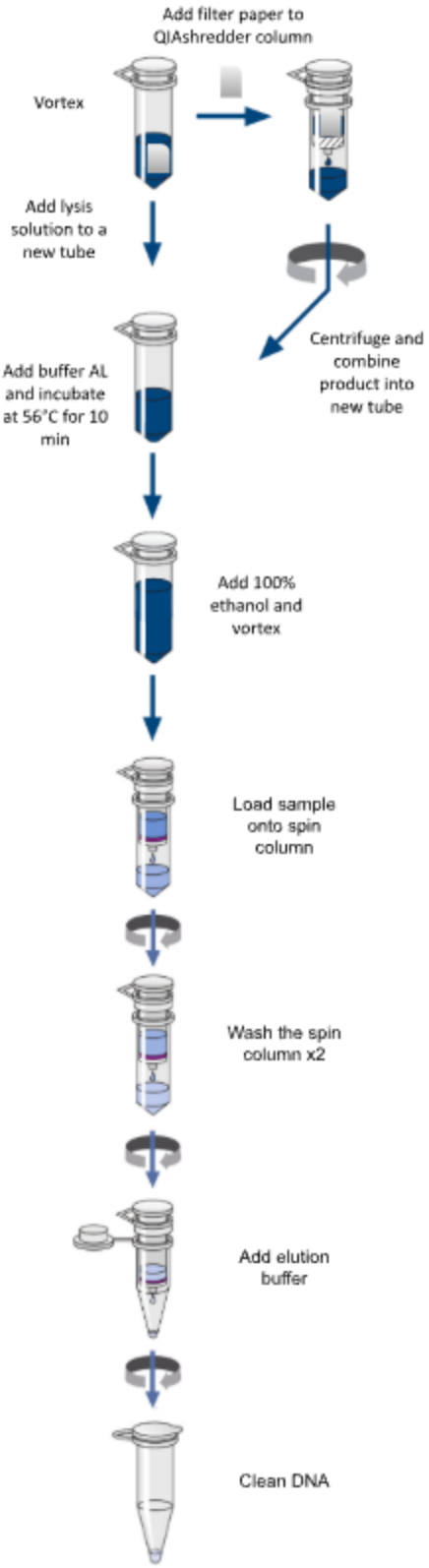

Figure 2: Illustrated protocol demonstrating Day 2 of the filter DNA extraction method.

Protocol references

Cruaud, P. *et al.* (2017) 'Open the STERIVEXtm casing: An easy and effective way to improve DNA extraction yields', *Limnology and Oceanography: Methods*, 15(12), pp. 1015–1020. doi:10.1002/lom3.10221.