Genomic DNA extraction from STERIVEX filter within capsule using QIAGEN´s DNeasy Blood and Tissue kit

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Protocol status: Working
We use this protocol and it's working

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ABSTRACT

This is our protocol for extracting environmental DNA from STERIVEX filters using QIAGEN´s Dneasy Blood and Tissue KIT. The protocol is a combination of previously described protocols from:

DNeasy Blood & Tissue Handbook (June 2023). QIAGEN

Kawato, M., Yoshida, T., Miya, M., Tsuchida, S., Nagano, Y., Nomura, M., Yabuki, A., Fujiwara, Y., & Fujikura, K. (2021). Optimization of environmental DNA extraction and amplification methods for metabarcoding of deep-sea fish. *MethodsX*, *8*, 101238. https://doi.org/https://doi.org/10.1016/j.mex.2021.101238

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Wong, M. K.-S., Nakao, M., & Hyodo, S. (2020). Field application of an improved protocol for environmental DNA extraction, purification, and measurement using Sterivex filter. *Scientific Reports*, *10*(1), 21531. https://doi.org/10.1038/s41598-020-77304-7

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MATERIALS

DNeasy Blood and Tissue kit for DNA extraction (QIAGEN)
Laminar flow hood with UV
2ml Luer Lock syringes
Petri dishes
Micropipets and tips
Incubator
Microcentrifuge
Pre and post PCR rooms

1 Clean the laminar flow hood surface and pipettes with DNAZAP and rinse with ddH_2O. 10m

2 Place gloves and 1.5 mL low bind tubes (for receiving DNA extract elution 1, elution 2 and one DNA extraction control) in the flow hood and expose to UV for 10 minutes. 10m

3 While exposing to UV in step 2 in the PRE-PCR room, in the POST-PCR room remove the parafilm fro 15m the sterivex filters and clean the outside of the sterivex filters with 10% bleach. Let them dry standing in a rack. Label the capsules with the label written on the sterile WhirlPack bag corresponding to each filter.

4 Heat the incubator to 56*C. 1m

5 Remove the red cap from the filter and remove the buffer EtOH 100% with a sterile 2mL Leur Lock 20m syringe (do not backflush) and leave it in a sterile 2mL LoBIND tube. Label the tube and store at -20*C or -80*C. (see other protocol for extracting eDNA from EtOH buffer)

6 Blot the sterivex filter gently on blotting paper to remove excess EtOH 100%. 5m

7 Prepare in a sterile 50ml eppendorf tube the lysis buffer mix of 990 µl of freshly prepared 1x PBS, 91(20m buffer AL (no EtoH added), 100 µl proteinase K per sample and inject it into the sterivex capsule using a sterile 2mL Luer Lock syringe and backflush (flush the capsule in reverse direction). Close with the red

cap, secure both sides with parafilm and place the capsule in a sterile petri dish.

8 Handshake or vortex the sterivex filter for 15s 5m

9 Incubate at 56*C for 2 hours and handshake/vortex in between. 2h

10 Handshake or vortex the filters for 15s 5m

11 Remove and discard the parafilm and red cap carefully. Remove the lysis buffer mix using a sterile 2r 20m
Luer Lock syringe, backflush, and put it into an sterile 1.5 mL Lobind eppendorf tube.

12 Discard the sterivex filter.

13 Add 1:3 EtOH 100% to the lysis buffer mix in the 1.5 mL LoBind tube. (eg if final lysis mix in tube is 1m 5m
than add 333 µl EtOH 100%).

14 Vortex well for 5s 5m

15 Pipet max. 600 µl of the mixture into the DNeasy Spin Column placed in a 2ml collection tube. Centrifu 5m
at 8000rpm for 1 min.

15.1 Discard flow-through. Repeat this step until the entire mixture has passed through the filter. Discar 5m
flow through and collection tube.

16 Place the DNeasy Spin Column in a new 2 ml collection tube and add 600 µl of Buffer AW1. Centrifuge 5m
8000rpm for 1min. Discard flow through and collection tube.

17 Place the DNeasy Spin Column in a new 2 ml collection tube and add 600 µl of Buffer AW2. Centrifug 10m
14.000rpm for 3min to dry the DNEasy membrane. Discard flow through and collection tube.

18 Place the DNeasy Spin Column in a sterile 1.5 ml LoBIND eppendorf tube and pipet 75 µl of buffer AE 5m
directly onto the DNeasy membrane. Incubate at room temperature for 1min and centrifuge at 8000rpm
for 1min to elute DNA.

19 Repeat step 18 in order to obtain a second elution of the DNA sample. 5m

20 Aliquote 30µl of the DNA samples for further analysis (QUBIT and PCR) and store at 4*C. Store the st 15m
DNA samples at -80*C.