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We use this protocol and it's working

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Protocol for eDNA extraction within STERIVEX capsule based on Spens et al. 2017

delphine.vanhaecke[1]

[1]Universidad de Aysén

delphine.vanhaecke

ABSTRACT

This protocol for eDNA extraction from STERIVEX filters within the capsule is modified from the protocol published by Spens et al. 2017. (DOI:10.1111/2041-210X.12683) based on the DNeasy® blood & tissue kit(QIAGEN, Stockach, Germany) handbook pp.28-30, ver. 07/2006 (Qiagen®).
This protocol has given us the highest concentrations of eDNA, quantified by QUBIT, from freshwater samples in Chilean Patagonia, compared to other tested protocols.

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DAY 1

1 Clean the laminar flow hood and micropipettes with DNAZAP, wipe with ddH2O and 70% Ethanol using tissue paper.

2 Preheat incubator to 56°C.

3 Prepare 10 ml of molecular grade ethanol (99.9%) in a 25 mL sterile eppendorf tube and place at -20°C .

4 Place gloves, sleeves, 1.5 mL (8) and 2.0 ml (24) sterilized LoBind tubes in the laminar flow hood and expose for 10min to UV light. (1.5 mL tubes for receiving eDNA eluate, 2.0 mL tubes for storing the EtOH buffer in the STERIVEX filter and 2.0 mL tubes for receiving the Lysis mix for eDNA extraction after the 56°C heating step)

5 Carefully wipe the outer surfaces of all the filter capsules with 5% bleach using clean tissue paper. Dry and wipe with 70% Ethanol using tissue paper.

6 Remove the yellow inlet caps from the capsule and transfer the buffer from the filter capsule into a 2mL sterile LoBind tube, with a 3 mL Luer- Lock syringe. Be careful not to apply too much pressure. In a tube rack placed inside a fume hood, dry the filters by placing them vertically with the inlet side (yellow cap side) facing down. Let them blot on clean laboratory tissue paper placed underneath the rack.

7 Label and store the EtOH in the LoBind tubes at -80C for future analysis as EtOH buffer also contains eDNA from the sample.

8 After removal of the ethanol buffer, consider the SX capsules as test tubes. The filter will remain intact in the capsules to avoid loss of DNA and contamination risk by unnecessary handling.

9 Immediately before the lysis step make a premix of Lysis working solution by adding 900 µL ATL buffer and 100 µL proteinase K per sample provided by the kit in a 10 mL sterilized tube. Add 1 mL lysis premix to each sample. Process 7 sterivex filters and 1 negative DNA extraction control at a time (max. of 8 samples at once). So for 8 samples prepare lysis mix: 900*9 (8+1)= 8100 uL ATL buffer and 100*9 (8+1)=900 uL Prot K).

10 Keep the outlet end closed with the red outlet cap. Carefully add 1000 µL Lysis working solution to the filter by using a 1,000 µL micropipet and sterile filter tips. Pipet the solution between the outside of the filter and the capsule walls. Close with a yellow inlet cap and seal with parafilm.

11 Handshake vigorously for a few seconds. Transfer the Sterivex filter cartridge to a sterile petri dish.

12 Place the sterivex filters in petri-dishes in an incubator preheated at 56 °C for 24 h without rotating. Handshake filters vigorously in between.

DAY 2

13 Preheat thermomixer to 70°C.

14 Handshake SX filter capsules vigorously before processing.

15 Remove ALL the liquid from yellow inlet end of capsule by using a 2 mL Luer Lock syringe. Measure the volume, transfer to two separate 2 mL LoBind tube (sample 1a and 1b - each with e.g. 500 uL of lysis mix). Vortex for a few seconds. Spin down for 2 seconds to seed out excess debris.

16 Add Buffer AL **and ice cold molecular** grade 99% ethanol (Thermo Fisher Scientific, Waltham, MA, USA) to the sample in equal volumes. Buffer AL and ethanol can be premixed. Sample:Buffer:Ethanol = 1:1:1. e.g. 500ul lysis mix + 1000ul AL+EtOH mix (or 500ul EtOH + 500 ul AL buffer).

17 Vortex vigorously.

18 Pipet the mixture (max 600 µL at a time) into a DNeasy Mini Spin column in a 2 mL collection tube provided in the kit.

19 Spin in microcentrifuge preferably at 4°C at 8000 rpm for 1 min.

20 Discard flowthrough.

21 Repeat previous 3 steps until all sample is filtered through DNeasy Mini spin column.

22 Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW1, and centrifuge for 1 min at 8,000 rpm. Discard flow-through and collection tube.

23 Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW2, and centrifuge for 3 min at 14,000 rpm to dry the DNeasy membrane. In the meantime label the 8 1.5 mL Lo Bind tubes.

24 Transfer spin column to a new 1.5 mL DNA LoBind tube with caps and walls labeled.

25 Heat AE buffer to 70°C on thermomixer. Place tubes with spin columns, four at a time, on a 70°C heating plate, add 75 µl 70°C Buffer AE (pH 8.0) to the membrane, immediately remove spin column with filter from thermomixer and let stand at Room Temperature for 10 minutes.

26 Centrifuge for 1 min at 8,000 rpm. Discard the spin column.

27 Aliquot 30 µL of the eluate eDNA in a separate labeled PCR tube for DNA measurement and PCR and store in dark room refrigerator at 4°C for immediate use (max. 6 months).

28 Store the other 45 µL of the eluate eDNA (STOCK) at -80°C for long term storage.