eDNA extraction from buffer EtOH 100% using DNeasy Blood and Tissue Kit (QIAGEN)

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ABSTRACT

This is a protocol for extracting eDNA from the buffer EtOH 100% in which Millipore filters are stored.

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

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1 Clean the laminar flow hood and micropipettes with DNAZAP (solution 1 and 2) and rinse with ddH_2O.

2 Place disposable gloves, sleeves and 1.5ml sterilized tubes (as many as the number of samples to process + 1 negative extraction control) in the laminar flow hood and submit to UV light for 10 minutes.

3 In the meantime, sterilize the tubes containing the filters with bleach (10%). Let dry and then clean with Ethanol 70%.

4 Beadbeat the tube with the filter (stored in EtOH 99.9%) for 20s at 4.0M/S. Let tubes rest for 5min then beadbeat again.

5 In the flowhood, remove the EtOH buffer from the tube and pipet it in a new 1.5mL sterilized LoBind tube

6 Centrifuge the LoBInd tube with EtOH for 60 minutes at 8000rpm and 6°C (check if pellet has formed at bottom).

7 Refill the tubes with the filter with EtOH and store at -80°C.

8 In the flowhood, remove the supernatant and let the pellet dry with the fan on for 30 min.

9 Add 20µl proteinase K, 200µl PBS 1x, 200µl buffer AL to each pellet.
Freshly prepare 5ml of PBS 1x from PBS10X (mix 500µl PBS10x with 4500µl nuclease free water).

10 Vortex well.

11 Incubate samples at 56°C for two hours in a thermoshaker at 600rpm.

12 Vortex 15s.

13 In the flowhood, add 200 µl EtOH 100%.

14 Mix by vortexing.

15 Pipet the mixture (650 µl) into the DNeasy Mini spin column placed in a 2 ml collection tube. Centrifuge at 8000 rpm for 1 min. Discard flow- through and collection tube.

16 Place the DNeasy Mini spin column in a new 2 ml collection tube, add 600 µl Buffer AW1, and centrifuge for 1 min at 8000 rpm. Discard flow-through and collection tube.

17 Place the DNeasy Mini spin column in a new 2 ml collection tube, add 600 µl Buffer AW2, and centrifuge for 3 min at 14,000 rpm to dry the Dneasy membrane. Discard flow-through and collection tube.

18 Place the DNeasy Mini spin column in a clean 1.5 ml microcentrifuge LoBIND tube, and pipet 75 µl Buffer AE directly onto the DNeasy membrane. Incubate at room temperature for 1min, and then centrifuge for 1 min at 8000 rpm to elute.

19 Repeat step 18 in a new 1.5 ml microcentrifuge LoBIND tube for a 2nd elution.

20 Discard spin column and store eDNA samples at -20C.