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

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delphine.vanhaecke<sup>1</sup>

<sup>1</sup>Universidad de Aysén



delphine.vanhaecke Vanhaecke

Universidad de Aysén

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**Protocol status:** Working

We use this protocol and it's working

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## Abstract

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



- 1 Clean the laminar flow hood and micropipettes with DNAZAP (solution 1 and 2) and rinse with ddH<sub>2</sub>O.
- 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  $\mu$ l) 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  $\mu$ l 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  $\mu$ l 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  $\mu$ l 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.