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# Seawater eDNA extraction for Sterivex - rocky intertidal habitats

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**We use this protocol and it's working**

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## Abstract

This protocol describes DNA extractions from filtered 1-L seawater samples from rocky intertidal habitats for environmental DNA (eDNA) metabarcoding. Optimized for samples collected from rocky intertidal monitoring sites in southern and central California (USA), filtered using Sterivex-HV 0.45um units (SVHVL10RC, EMD Millipore), and preserved with Longmire's lysis buffer. Adapted from:

DNeasy® Blood & Tissue Kit Handbook, Purification of Total DNA (Spin-Column Protocol), QIAGEN, Stockach, Germany, ver. 07/2020.d

Spens, J., Evans, A.R., Halfmaerten, D., Knudsen, S.W., Sengupta, M.E., Mak, S.S., Sigsgaard, E.E. and Hellström, M., 2017. Comparison of capture and storage methods for aqueous macrobial eDNA using an optimized extraction protocol: advantage of enclosed filter. Methods in Ecology and Evolution,8(5), pp.635-645.

## Materials

You will need the following reagents and consumables (per sample):

Reagents (*included in Qiagen DNeasy Blood and Tissue kit):
100ul proK*
900ul buffer ATL*
1000ul buffer AL*
1000ul molecular-grade 99% (pure 200 proof) ethanol
500ul buffer AW1*
500ul buffer AW2*
200ul buffer AE*

Consumables:
2 - 5ml sterile Luer lok syringes
2 - 2ml sterile LoBind tubes (Eppendorf)
1 - 5ml sterile LoBind tube (Eppendorf)
2 - sterile Luer lok caps
1 - DNeasy spin column*
2 - additional DNeasy collection tubes*

Instruments: incubator, rotator with tube rack, microcentrifuge, vortexer, laminar flow hood with UV lamp, freezer (-20 or -80)

Other materials: single-channel pipettors, sterile filter pipet tips, sterile 15ml & 50ml falcon tubes, parafilm, tube racks in various sizes, tube cap openers, KIM wipes, tube labels and markers, 10% bleach, 70% ethanol

## Safety warnings

Qiagen Buffers AL and AW1 contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with a suitable laboratory detergent and water.

Follow general lab safety protocols when working with hazardous chemicals.

## Before start

Wipe all work surfaces with 10% bleach followed by 70% ethanol. If possible, briefly UV-irradiate tubes, pipettors, racks, and reagent aliquots. Label tubes according to sample IDs. You may also carefully wipe pipettors with 10% bleach and 70% ethanol, taking care to ensure there is no residual bleach. Set incubator to 56C and place 99% ethanol on ice or in freezer until needed.

*NOTE: In this protocol, a sample will be physically split into two components - $SX_{tube}$ and $SX_{capsule}$ - prior and during incubation. After incubation, sample components will be re-combined through the end of the protocol.

## Sample preparation

1 $SX_{TUBE}$
Transfer the Longmire's buffer from the Sterivex filter capsule and into a 2mL sterile LoBind tube using a 5 mL Luer-Lock syringe. Be careful not to apply too much pressure. The extractions from the buffers are hereafter referred to as $SX_{TUBE}$. Spin at 6,000 ∗ g (8,000 rpm) for 30-45 min in a micro-centrifuge 24 ∗ 2mL. Discard liquid.

$SX_{CAPSULE}$
After removal of Longmire's buffer (above), 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. Remove and discard the inlet and the outlet caps. In a tube rack placed inside the hood, dry the filters by placing them vertically with the 'inlet end' facing down. Let them blot on clean lab tissue paper placed underneath the rack. The extractions from the filters are hereafter referred to as $SX_{CAPSULE}$. Let the filters dry out while $SX_{TUBE}$ are centrifuging.

## Lysis prep and addition

2 Prepare lysis working solution by adding 900 µL ATL buffer and 100 µL proteinase K **per sample**.

3 $SX_{TUBE}$
Dissolve the dried pellet by adding 200 µL working solution per sample. Close tube and seal with parafilm. Vortex for 5 s.

$SX_{CAPSULE}$
Close the outlet end with a sterile end cap. Carefully add 800 µL Lysis working solution to the filter by using a 1,000 µL pipet and extended-length sterile filter tips. Pipet the solution between the outside of the filter and the capsule walls. Close open end with another sterile inlet cap. Handshake vigorously for 5 s.

## Start incubation

4 Place all the tubes and capsules on the rotator – make sure they are secure and will not fall out or hit anything when the rotator is on.

Incubate, while rotating, at 56°C for up to 24 hours. Record incubation start time.

## Stop incubation

5 Remove samples from the incubator, record the time, and set incubator to 70°C. Heat Buffer AE at 70°C until elution step.

## Re-combine samples

6 $SX_{TUBE}$
Transfer 200 µL to a labeled 5 mL LoBind tube.

$SX_{CAPSULE}$
Remove ALL the liquid from inlet end of capsule using a Luer Lock syringe and transfer to 5 mL LoBind tube.

Note the approximate volume in each of the 5 mL tubes.

## Bind, wash, and elute

7 Add Buffer AL and ice-cold 99% ethanol to the sample in equal volume ratios. Sample:Buffer:Ethanol = 1:1:1. Add AL to all tubes first, then add cold ethanol. Vortex vigorously for 5 s.

8 Pipet the mixture (max 650 µL at a time) into a labeled DNeasy spin column in a 2 mL collection tube provided in the kit. Spin in micro-centrifuge at 6000 ∗ g (8000 rpm for rotor max capacity 24 ∗ 1.5-2 mL tubes) 1 min. Discard flow-through and keep collection tube. Repeat until all sample is filtered through DNeasy spin column.

9 Place the DNeasy spin column in a new 2 ml collection tube, add 500 µl Buffer AW1, and centrifuge for 1 min at 6000 ∗ g (8,000 rpm). Discard flow-through and collection tube.

10 Place the DNeasy spin column in a new 2 ml collection tube, add 500 µl Buffer AW2, and centrifuge for 3 min at 20,000 ∗ g (14,000 rpm) to dry the DNeasy membrane. Discard flow-through and re-use the collection tube. Centrifuge 1 min at 17,000 ∗ g (13,000 rpm).

11 Transfer spin column to a new labeled 2 mL DNA LoBind tube with caps removed.

12 Add 100 µl 70°C Buffer AE to the membrane. Incubate at RT for 10 min. Centrifuge for 1 min at 6,000 ∗ g (8,000 rpm). Repeat.

13 Discard the spin column and keep the LoBind tube with DNA eluted in 200 µl AE. Store at -20°C.

## Protocol references

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