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# Collecting eDNA from marine water samples in the field

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1 Works for me dx.doi.org/10.17504/protocols.io.6yfhftn

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ABSTRACT

This protocol describes the process of collecting eDNA from marine water samples in the field and four different approaches for preservation of filters with eDNA as well as eDNA extraction:

1. Qiagen DNeasy kit
2. Zymo DNA/RNA shield
3. Longmire's solution
4. Alternative methods

ATTACHMENTS

Collecting eDNA from marine water samples in the field.docx

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KEYWORDS

eDNA extraction, Zymo Quick DNA Miniprep Plus Kit, Qiagen DNeasy kit, Preservation of filters with eDNA



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GUIDELINES

**Remarks/Tips:**

Water filtering:
The 250ml Nalgene unit is a nice size, but will clog quite rapidly if filtering lots of dirty water. You also need to empty and replace the bottom container more often, of just use a 1l (or 2l) scott/duran bottle as a replacement for the supplied 250ml bottle.

DNA Preservation method used:
For short preservation times, filters can be directly placed in ATL buffer (Qiagen DNeasy kit). This works well if you are able to process the samples within 48h or you have a freezer available within that time.
For preservation longer than a few days, I tested Longmire's solution. Works well, but only compatible with Phenol/Chlorophorm extraction, which is laborious and also does not yield the best and purest DNA.
Now I switched to using Zymo DNA/RNA shield. Have no test results back, but expect good results, and major advantage is that it directly fits into both the Zymo Quick-DNA miniprep Plus kits as well as the Qiagen DNeasy kit. And DNA will be stable at RT for 1 month.

MATERIALS

| NAME | CATALOG # | VENDOR |
|---|---|---|
| Thermo Scientific™ Nalgene™ Rapid-Flow™ Sterile Disposable Filter Units with CN Membrane | 10792351 | Thermo Scientific |

MATERIALS TEXT

Please refer to the steps for additional materials as each step-case requires different materials.

SAFETY WARNINGS

Please refer to the SDS (Safety Data Sheet) for safety warnings and hazard information.

BEFORE STARTING

Starting remarks:

All non-DNA free/not-gamma sterilized sampling devices **are cleaned with a bleach solution** or UV exposure before use to break down contamination DNA. Always wear gloves and minimize potential contamination with DNA from human handling and/or nearby food sources or animals.

Collect at least one **control sample on site of sampling collection. Filter 500 ml of DNA free Milli-Q (if not available, use tap water)** at each location, to monitor contamination during the filtering and subsequent DNA extraction.

## Collection of eDNA from the water on a filter membrane

1 Collect water in an appropriate sterile, DNA free container.

For marine samples collected during scuba diving, we use a small hand-pump and a punch-balloon.

2 Filter **1 L** - **2 L** with the 250 ml filters (0.8 micron, cellulose nitrate) from Nalgene.

3 Connect filter unit to vacuum pump, filter by vacuum.

I use a small vacuum-pump from ebay, powered with a 12V power pack. (DC12V Mini Vacuum Pump Negative Pressure Suction Pump)



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4 Using the vacuum, make sure the filter is dry, as to much transfer of liquid influences preservation and downstream DNA isolation.

5 Cut filter from filter unit, it is glued in place. No problem to cut off outer 0.5 cm of filter. Use sterile scalpel + scalpel holder. Fold filter twice using scalpel+tweezers.

6 Insert filter in 2 ml screwcap tube prefilled with **400 µl preservation solution** .

It is possible to use other tubes, but not when using 3d-fuge, as these do not fit and will snap open during centrifugation.

7 Shake tube with filter, to fully soak in the preservation solution. Repeat after **00:02:00** - **00:03:00** to re-soak.
Filter ready for storage.

Preservation of filters with eDNA

8 The DNA is now collected on the filter. Either directly continue with the DNA isolation protocol of choice, or store the preserved the DNA on the filter, depending on preservation method used.

**Qiagen DNeasy kit, short term (<48h) preservation:**

- Store filter in **400 µl ATL buffer** from the DNeasy kit, shake well, store at **Room temperature** or below.

**Zymo DNA/RNA shield, short+longterm preservation:**

- Store filter in **400 µl DNA/RNA Shield** , shake well, store at **Room temperature** or below.

**Longmire's solution: longterm preservation:**

- Store filter in **500 µl** - **1 mL longmire's solution** , shake well, store at RT or below
- *cheap, works ok, but only compatible with Phenol/Chlorophorm extraction, which is laborious and also does not yield the best and purest DNA.*

**Other alternative preservation methods**: ethanol, freezing, DMSO, DESS, dehydration, combinations, etc.

Step 8 includes a Step case.
**Qiagen DNeasy**
**Zymo DNA/RNA**
**Longmire**
**Alternative**

eDNA extraction

step case

### Qiagen DNeasy

Store filter in **400 µl ATL buffer** from the DNeasy kit, shake well, store at **Room temperature** or below.

9 When in the lab, add **20 µl protK** .

10 Incubate at **56 °C** for **00:10:00** - **00:30:00** .



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11 Follow standard protocol for blood samples: Since using **400 µl** volume, double amount of AL and Ethanol: add **400 µl Buffer AL** . Mix thoroughly by vortexing, add **400 µl Ethanol** . mix by vortexing, load on spin column, etc..

- *Pipetting is a bit of hassle with the filter in the tube, best to remove most buffer, spin shortly, remove rest. Also you will need to load the DNA filter tube twice to process all liquid.*
- *Elute in either recommended* **200 µl AE** *or use lower elution volume (down to* **50 µl** *) if more concentrated DNA is required.*

11.1 Add **400 µl Buffer AL** .

11.2 Mix thoroughly by vortexing.

11.3 Add **400 µl Ethanol** .

11.4 Mix by vortexing.

11.5 Load on spin column.



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