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Collection and preservation of eDNA from marine water samples

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DISCLAIMER

ABSTRACT

This protocol is designed for water collection from Niskin bottles and filtration at sea using reusable filter cups.

Aim: to collect and filter 2.5 L of water at each depth from each CTD cast and preserved the filter at room temperature.

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LICENSE

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GUIDELINES

Water should be filtered immediately after collection.
Only DNA-free forceps should touch the filter!

MATERIALS TEXT

- DNA away
- Kimwipes
- gloves
- 69 oz Whirlpack bags (2.5 L is at 9.5” of the bag from the bottom)
- 2L pitchers (for whirlpack bags stability)
- utensils for getting membrane from filter (i.e. forceps)
- 500 ml filter cups with arubber stopper fitted with anadapter for filter cup
- mixed cellulose ester sterile filters (1 µm, 47 mm diameter) (Advantec® Cat. A100H047A)
- pump
- 3-port manifold
- tubing adaptors
- wastewater container carboy
- labeled tubes containing 2 ml of Longmires’s buffer
- bleach (5%) and bleach bucket
- distilled water
- 2 buckets (for bleach and rinsing)
- mesh bags
- drying racks
- bungees
- absorbent towelsµ
- distilled water from the evaporator onboard and from the lab

Lysis buffer recipe (Longmire et al 1997):
To make 1 liter add in numerical order:
1. 975 ml double-distilled water
2. 100 ml of 1 M Tris-HCL, pH 8.0
3. 200 ml of 0.5 M EDTA, pH8.0
4. 2 ml of 5 M NaCl
5. 25 ml of 20% SDS (w/v)

Filter the buffer with an autofill PES bottle top filtration device (sterile 500ml, 0.22 µm)

DISCLAIMER:

Filter cups cleaning and assemblage

1 Set up wash station (two buckets, one with 0.5% bleach, one with clean distilled H2O)

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2 Replace bleach and water stations at regular intervals (at least every other day, 0.5% bleach = 1.2L bleach in 20L bucket)

3 Place all used small items in a small mesh bag and the filter cups in a large mesh bag in bleach for at least 30 minutes

4 Rinse the abovementioned items for at least 10 minutes

5 Allow to dry on the drying racks

6 Wearing a fresh pair of gloves, mount filter cups with new sterile filter

Collection of water from Niskin bottles

7 Let the water from the hull (“surface water sample”) run for > 3 min while the CTD is being deployed

8 Collect 2 samples of 2.5 L each of “surface water” for each CTD cast

9 Once the rosette has been brought back to the surface, water needs to be extracted from each Niskin bottle and transported into the lab for filtration. Each Niskin contains 10L of seawater. We want to collect 2.5L of water from each Niskin.

Wipe the spigot on the Niskin with DNAway to remove potential contaminants on the outside of the Niskin

10 Flush water (count to 10) from the Niskin to further reduce risk of contamination and collect 2.5 L of water in a whirlpack bag and place in 2L pitcher

11 Include a sampling negative control by collecting 2.5L of distilled H2O (at least daily)

Filtration of water samples

12 Wipe working area with DNAway

13 Switch on the pump and set vacuum in the middle range between-8 and -12 bars (keep an eye on the gauge & adjust if necessary)

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14 Pour water from whirlpack bag (supported by pitcher) into the filter cup, repeat until finished (2.5L of water)

15 Using DNA-free forceps fold filter and place in pre-labeled tube pre-filled with preservative buffer (2ml, Longmire Buffer in a 5 ml LoBind tube).

16 Filter 2L of ship distilled H2O every time the cleaning distilled H2O is replaced as a sampling negative control

17 Every 2-3 days 2L of lab diH2O should be filtered as sampling negative controls

18 Note tube label in notes. Also note time filtered, membrane type, place filtered (field/lab, which lab), etc.

19 Store samples at room temperature away from UV light

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