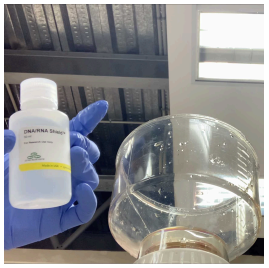
Nov 18, 2023 Version 1

eDNA Water Sample Collection, Preservation and Extraction (low-tech sampling, modified Qiagen PowerWater extraction) V.1



Forked from QIAGEN DNeasy Power Water SOP

DOI
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Protocol status: Working
We use this protocol and it's working. In the future, we would like to get an automated sampler, to reduce potential DNA contamination sources in the field, but this protocol works well when appropriate field negative controls are included in the analysis.

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Keywords: marine environmental dna, water sample collection, environmental dna, galápagos fieldwork, extraction protocol, edna, qiagen powerwater extraction, dna, gulf of california fieldwork, extraction, marine, preservation

Abstract

Marine environmental DNA (eDNA) collection, filtration, preservation, and extraction protocol used in Galápagos fieldwork 2021-2023, and Gulf of California fieldwork in 2023 by Eldridge Wisely.

Attachments

HB-2267-003_HB_DNY_P...
1.4MB

_r1100-50_r1100-250_...
320KB

Materials

Equipment

Reusable Filter Unit	NAME
Filter unit	TYPE
Nalgene	BRAND
NAL300-4100	SKU
https://www.fishersci.com/shop/products/nalgene-reusable-filter-holders-receiver/0974023E	LINK

Equipment

Oil-less vacuum pump	NAME
vacuum pump	TYPE
Fristaden Lab	BRAND
VP-10L	SKU
https://www.amazon.com/American-Fristaden-Lab-Portable-Diaphragm/dp/B0896VV35S?ref_=ast_sto_dp	LINK

Equipment

Wide-mouth HDPE bottle

NAME

sample collection bottle

TYPE

Nalgene

BRAND

N311-1000BPC

SKU

https://www.thermofisher.com/order/catalog/product/N311-1000BPC?SID=srch-srp-N311-1000BPC

LINK

Zymo DNA/RNA Shield **Fisher Scientific** **Catalog #**50-125-1706

Equipment

HAWP MF-Millipore Membrane Filter, 0.45 µm pore size

NAME

Membrane filter

TYPE

Millipore

BRAND

HAWP04700

SKU

https://www.sigmaaldrich.com/catalog/product/mm/hawp04700?lang=en&region=US&gclid=CjwKCAjw8pH3BRAXEiwA1pvMsdoaQbbYstapLy8iGgQMuPbpUIubisFSK9v3zg7Ab-Uv1HEHZmOhSBoCPx8QAvD_BwE

LINK

0.45 um 47 mm

SPECIFICATIONS

Equipment

NAME	TYPE	BRAND	SKU
PowerWater DNA bead tube	Tube	Qiagen	14900-50-NF-BT

Equipment

NAME	TYPE	BRAND	SKU
1.5mL sterile tubes	1.5 mL tube	Axygen	MCT150CS

Qiagen PowerWater kit **Qiagen Catalog #**14900-50-NF

Equipment

vortex adapter for 5mL tubes	NAME
vortex adapter	TYPE
Qiagen	BRAND
13000-V1-5	SKU
https://www.qiagen.com/us/products/instruments-and-automation/accessories/vortex-adapter?catno=13000-V1-5	LINK

Protocol materials

10% Bleach

Zymo DNA/RNA Shield **Fisher Scientific Catalog #**50-125-1706

Qiagen PowerWater kit **Qiagen Catalog #**14900-50-NF

DNA/RNA Shield **Zymo Research Catalog #**R1100-50

10% Bleach

Troubleshooting

Before start

- Solution PW1 must be warmed at 55°C for 5–10 minutes to dissolve precipitates prior to use.
- Solution PW1 should be used while still warm.
- Assume Solution PW3 has precipitated, and preemptively heat at 55°C for 5–10 minutes to dissolve precipitate.
- Shake to mix Solution PW4 before use.
- Perform all centrifugation steps at room temperature (15–25°C).

Water collection and filtration

23m

1 Collect water samples in a cleaned (see step 12) **1L Nalgene bottle** by submerging the bottle and then opening it and closing it underwater. This step can be adapted to your sampling logistics, while reducing as much as possible any contact between your skin or clothes or other equipment with the water being sampled. Label your samples, and take metadata including latitude and longitude , water temperature, and any other variables you would like to have for later analysis.

2 Store the sample bottles in a cooler On ice with icepacks to keep them cool and protected from UV light until they can be filtered (within 12 hours of collection).

3 Wear lab gloves for all subsequent steps, and change your gloves if any contact with your sample water occurs, so you don't cross contaminate your samples during the filtration step.

4 Clean the reusable filter funnel and included filter support pedestal with 10% Bleach and rinse 3x with remove the upper portion of the reusable filter funnel,

5 Rinse **3x** with purified water (drinking water if available, tap water if purified is not available or is cost prohibitive)

6 Assemble the reusable filter funnel with the **0.45 micron MCE filter** inside it.

7 Filter the water sample by pouring the contents of the 1 L Nalgene bottle into the reusable filter unit, while applying a vacuum with the **vacuum pump**.

8 After sample has been filtered, stop the vacuum and release the pressure by loosening the tubing to the reusable filter unit, then add 500uL of DNA/RNA Shield **Zymo Research Catalog #**R1100-50 to the filter so that the filter is covered by the buffer, let the buffer sit on the filter for at least 00:00:05 , then re-apply the vacuum again briefly, until the liquid has been pulled through the filter.

5s

9 Remove the filter funnel portion of the reusable filter unit so that the filter is exposed.

10 Using two sets of sterile forceps (tweezers cleaned with bleach solution and then 70% EtOH, and allowed to air dry), pick up the white filter membrane at opposite edges and roll the filter into a cylinder with the top side facing inward.

11 for each subsequent liter of water to be processed, go to step #4

12 Clean the sample bottle (**1L Nalgene bottle**)

Equipment

Wide-mouth HDPE bottle	NAME
sample collection bottle	TYPE
Nalgene	BRAND
N311-1000BPC	SKU
https://www.thermofisher.com/order/catalog/product/N311-1000BPC?SID=srch-srp-N311-1000BPC	LINK

with 10% Bleach and rinse 3x with purified water (drinking water if possible).

Take at least one negative control of the rinse water as described in step 13, in accordance with your sampling and processing logistics and budget.

13 Each time sampling logistics change, or rinse water availability changes, take a sample of 1L of rinse water and process according to this protocol, as a laboratory negative control. Also, take a field negative control by leaving rinse water in one of the Nalgene bottles after cleaning and take it to the field with the rest of the bottles and handle the same as the sampling bottles, except don't change the water in the bottles, as a negative field control sample.

eDNA Extraction 23m

14 Heat Solutions PW1 and PW3 to 55 °C and Buffer EB to 70 °C .

15 Add 1 mL of Solution PW1 to the PowerWater DNA Bead Tube.

16 Secure the tube horizontally to a

Equipment	
vortex adapter for 5mL tubes	NAME
vortex adapter	TYPE
Qiagen	BRAND
13000-V1-5	SKU
https://www.qiagen.com/us/products/instruments-and-automation/accessories/vortex-adapter?catno=13000-V1-5	LINK

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17 Vortex at maximum speed for 00:05:00 . 5m

18 Transfer the supernatant to a clean 2 mL Collection Tube (provided). Draw up the supernatant using a 1 mL pipette tip by placing it down into the beads. (**Note**: Placing the pipette tip down into the beads is required. Pipette until you have removed all the supernatant. Expect to recover 650-800 µL of supernatant.)

19 Centrifuge at 13000 x g, Room temperature, 00:01:00 1m

20 Avoiding the pellet, transfer the supernatant to a clean 2 mL Collection Tube (provided).

21 Add 200 µL of Solution IRS and vortex briefly to mix. Incubate at 2-8 °C for 00:05:00 . 5m

22 Centrifuge the tubes at 13000 x g, Room temperature, 00:01:00 . 1m

23 Avoiding the pellet, transfer the supernatant to a clean 2 ml 2 mL Collection Tube (provided).

24 Carefully, add 650 µL of Solution PW3 and thoroughly pipette mix or vortex briefly to mix.

25 Load 650 µl of supernatant onto an MB Spin Column. Centrifuge at 13000 x g, Room temperature, 00:01:00 . Discard the flow-through. Repeat until all the supernatant has been processed. 1m

26 Place the MB Spin Column Filter into a clean 2 mL Collection Tube (provided).

27 Add 650 µL of Solution PW4 (shake before use). Centrifuge at 13000 x g, Room temperature, 00:01:00 . 1m

28 Discard the flow-through and add 650 µL of ethanol (provided) and centrifuge at 13000 x g, Room temperature, 00:01:00 . 1m

29 Discard the flow-through and centrifuge again at 13000 x g, Room temperature, 00:02:00 . 2m

30 Place the MB Spin Column into a clean 2 mL Collection Tube (provided).

31 Add 100 µL of 70 °C Solution EB to the center of the white filter membrane.

31.1 Incubate at Room temperature for a minimum of 00:10:00 10m

32 Centrifuge at 13000 x g, Room temperature, 00:01:00 . 1m

33 Discard the MB Spin Column, and place eluted liquid containing the eDNA into a new 1.5mL DNAse- , RNAse-free, sterile 1.5mL tube.

Equipment	
1.5mL sterile tubes	NAME
1.5 mL tube	TYPE
Axygen	BRAND
MCT150CS	SKU

The DNA is now ready for downstream applications.

33.1 QIAgen recommend storing DNA frozen (-90 °C to -15 °C) as Solution EB does not contain EDTA

Protocol references

https://www.qiagen.com/us/resources/resourcedetail?id=75765ef9-2a6f-4f5d-a36b-dbd9beb43079&lang=en