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Environmental DNA (eDNA) extraction using Qiagen DNeasy 96 Blood and Tissue Kit

Forked from DNA EXTRACTION Protocol Template

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Better Biomolecular Ocean Practices (BeBOP)

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ABSTRACT

This protocol is a modified version of the Qiagen DNeasy 96-sample protocol: Purification of Total DNA from Animal Tissues.

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Protocol status: Working
We use this protocol and it's working

Created: Nov 07, 2023

Last Modified: Nov 13, 2023

PROTOCOL integer ID: 90592

MIOP: Minimum Information about an Omics Protocol

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MIOP Term	Value
analyses	DNA extraction [OBI:0000257]
audience	scientists
broad-scale environmental context	marine biome ENVO_00000447
creator	Jacoby Baker, https://orcid.org/0000-0002-0673-7535
environmental medium	sea water [ENVO:00002149] | filter paper [OBI:0000151]
geographic location	Monterey Bay [GAZ:00002509]
hasVersion	3
issued	2023-11-07
language	en
license	CC BY 4.0
local environmental context	oceanic epipelagic zone biome [ENVO:01000033]
materials required	centrifuge [OBI:0400106] | incubator [OBI:0000136]]
maturity level	Mature
methodology category	sample extraction and purification
personnel required	1

MIOP Term	Value
project	Marine Biodiversity Observation Network (MBON)
publisher	Monterey Bay Aquarium Research Institute, Chavez Lab
purpose	DNA extraction [OBI:0000257]
skills required	sterile technique | pipetting skills
target	DNA
time required	1320

See https://github.com/BeBOP-OBON/miop/blob/main/model/schema/terms.yaml for list and definitions.

AUTHORS

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PREPARED BY All authors known to have contributed to the preparation of this protocol, including those who filled in the template	AFFILIATION	ORCID (visit https://orcid.org/ to registe
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RELATED PROTOCOLS

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PROTOCOL NAME AND LINK	ISSUER / AUTHOR	RELEASE / ACCESS DATE
Qiagen DNeasy 96 Blood and Tissue Kit Protocol	Qiagen	2023-11-7
https://mbari-bog.github.io/MBON-Protocols/eDNA_extraction_V3.html	Jacoby Baker	2023-11-07

This is a list of other protocols which should be known to users of this protocol. Please include the link to each related protocol.

ACRONYMS AND ABBREVIATIONS

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ACRONYM / ABBREVIATION	DEFINITION
PVDF	polyvinylidene difluoride
GFF	Glass Microfiber Filters
art. comm	Artificial Community
eDNA	environmental DNA

GLOSSARY

5

SPECIALISED TERM	DEFINITION
Content Cell	Content Cell
Content Cell	Content Cell

BACKGROUND

6 **Summary**

This protocol is a modified version of the Qiagen DNeasy 96-sample protocol: Purification of Total DNA from Animal Tissues

7 **Method description and rationale**

This protocol was developed to extract environmental DNA from filtered seawater samples. It has been applied to 0.22µm PVDF and 0.7µm GFF filters.

8 **Spatial coverage and environment(s) of relevance**

This protocol has been used to extract DNA from filtered sea water samples taken from marine coastal stations.

sea water [ENVO:00002149]
http://purl.obolibrary.org/obo/ENVO_00002149

9 **Personnel Required**

1 Technician

10 **Safety**

Identify hazards associated with the procedure and specify protective equipment and safety training required to safely execute the procedure

11 **Training requirements**

Sterile technique, pipetting skills.

12 **Time needed to execute the procedure**

22 hours including an overnight incubation.

EQUIPMENT

13

DESCRIPTION e.g. filter	PRODUCT NAME AND MODEL Provide the official name of the product	MANUFACTURER Provide the name of the manufacturer of the product.	QUANTITY Provi
Durable equipment			
incubator	Gyromax 702	Amerex instruments	
centrifuge	Sorvall Legend XTR centrifuge	Thermo scientific	
bead beater	TissueLyser II	Qiagen	
Consumables			
Qiagen DNeasy 96 Blood & Tissue Kit	Qiagen DNeasy 96 Blood & Tissue Kit	Qiagen	
Chemicals			
ethanol (96–100%)			
RNase Away	RNase Displace	FoxWest sales	
EtOH wash	70% ethanol		
NF H2O	Nuclease-Free water	Invitrogen	

STANDARD OPERATING PROCEDURE

14 In the following SOP, please use the exact names of equipment as noted in the table above.

Provide a step-by-step description of the protocol. The identification of difficult steps in the protocol and the provision of recommendations for the execution of those steps are encouraged.

PREPARATION

15 **Important points before starting (from the original Qiagen DNeasy 96 Blood and Tissue Kit Protocol)**

- If using the DNeasy 96 Blood & Tissue Kit for the first time, read “Important Notes” (page 15).
- All centrifugation steps are carried out at room temperature (15–25°C).
- Optional: RNase Away may be used to digest RNA during the procedure. RNase A is not provided in the DNeasy 96 Blood & Tissue Kit (see “Copurification of RNA”, page 19).

16 **Things to do before starting**

- Buffer AL should be premixed with ethanol before use. Add 90 ml ethanol (96–100%) to the bottle containing 86 ml Buffer AL or 260 ml ethanol to the bottle containing 247 ml Buffer AL and shake thoroughly. Mark the bottle to indicate that ethanol has been added. (Please note that, for purification of DNA from animal blood, Buffer AL must be used

without ethanol. Buffer AL can be purchased separately if the same kit will be used for purification of DNA from animal blood.)

- Buffer AW1 and Buffer AW2 are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Buffer ATL and Buffer AL may form precipitates upon storage. If necessary, warm to 56°C for 5 min until the precipitates have fully dissolved.
- Mix Buffer AW1 before use by inverting several times.
- Preheat an incubator to 56°C for use in step 4.
- If using frozen tissue, equilibrate the sample to room temperature. Avoid repeated thawing and freezing of samples since this will lead to reduced DNA size.

17 **Pre-procedure**

0.1 Organize a 96-well sample layout spreadsheet to identify which samples are in each well location. Don't forget to include extraction blanks (EB; x1), PCR blanks (x2), and artificial communities (x1)
0.2 Preheat incubator to 56°C for later use
0.3 Place clean and sterile 3 or 5 mm TissueLyser beads into each of the collection microtubes.

Note: Be sure to label your plates along the plate skirt.

EXTRACTION

18 1. Place eDNA water filters into the collection microtubes. Be sure to have an organization chart to identify location of each sample in the 96-well-plate format.

Note: Use a plate template sheet to organize the location of the samples, blanks, and art. comm. Using two pairs of forceps, carefully roll the filter into a 'tube' shape so that it can fit within the collection microtube. When placing the rolled filter into the tube, make sure the eDNA filter residue is facing the inner portion of the tube so the bead can pass and properly lyse the material. It helps to keep the filter to the top of the collection microtube as the diameter is larger and the bead can more easily pass.

IMPORTANT: Between each filter, be sure to clean and sterilize forceps with a RNase away, 2X NF H2O, and EtOH wash.

Keep the clear covers from the collection microtube racks for use in step 3.

19 2. Prepare a proteinase K-Buffer ATL working solution containing 30 µl proteinase K stock solution and 270 µl Buffer ATL per sample, and mix by vortexing (we increased the overall volume to 300 µl so the filter would be submerged in the proteinase K-Buffer ATL working solution). For a set of 96 samples, use 3 ml proteinase K stock solution and 27 ml Buffer ATL (this has a 4-sample buffer for errors in pipetting). Pipette 300 µl working solution into each collection microtube containing the eDNA filter samples. Seal the microtubes securely using the caps provided.

Note: Move each column of tubes to a new box before uncapping and adding the proteinase K-Buffer ATL working solution. This is done to help prevent cross contamination between samples when uncapping the tubes after the filters are loaded. After the prot. K-ATL solution is added cap tubes with new caps.

Note: Check Buffer ATL for precipitate. If necessary, dissolve the precipitate by incubation at 56°C for 5 min before preparing the working solution.

IMPORTANT: After preparation, the proteinase K–Buffer ATL working solution should be dispensed immediately into the collection microtubes containing the samples. Incubation of the working solution in the absence of substrate for >30 min reduces lysis efficiency and DNA purity.

19.1 2. 5 Bead beat the microtubes in the bead beater at 30hz for 2 minutes.

Note: Make sure that the bead beater is balanced.

20 3. Ensure that the microtubes are properly sealed to avoid leakage during shaking. Place a clear cover (saved from step 1) over each rack of collection microtubes, and mix by inverting the rack of collection microtubes. To collect any solution from the caps, centrifuge the collection microtubes. Allow the centrifuge to reach 3000 rpm, and then stop the centrifuge. It is essential that the samples are completely submerged in the proteinase K–Buffer ATL working solution after centrifugation.

If the proteinase K–Buffer ATL working solution does not completely cover the sample, increase the volume of the solution to 300 µl per sample (additional reagents are available separately; see page 56 for ordering information). Do not increase volumes above 300 µl as this will exceed the capacity of the collection microtubes in subsequent steps.

Keep the clear covers from the collection microtube racks for use in step 5.

21 4. Incubate at 56°C overnight. Place a weight on top of the caps during the incubation. Mix occasionally during incubation to disperse the sample, or place on a rocking platform.

After incubation the lysate may appear viscous, but should not be gelatinous as it may clog the DNeasy 96 membrane. If the lysate appears very gelatinous, see the "Troubleshooting Guide", page 47, for recommendations.

Note: It is important to make sure there is a weight or something holding the lids of the caps down as the head builds pressure in the collection microtubes and can lift the caps off of the tubes, potentially releasing DNA and cross-contaminating samples.

Note: Do not use a rotary- or vertical-type shaker as continuous rotation may release the caps. If incubation is performed in a water bath make sure that the collection microtubes are not fully submerged and that any remaining water is removed prior to centrifugation in step 5.

22 5. Ensure that the microtubes are properly sealed to avoid leakage during shaking. Place a clear cover over each rack of collection microtubes and shake the racks vigorously up and down for 15 s. To collect any solution from the caps, centrifuge the collection microtubes. Allow the centrifuge to reach 3000 rpm, and then stop the centrifuge.

IMPORTANT: The rack of collection microtubes must be vigorously shaken up and down with both hands to obtain a homogeneous lysate. Inverting the rack of collection microtubes is not sufficient for mixing. The genomic DNA will not be sheared by vigorous shaking.

Ensure that lysis is complete before proceeding to step 6. The lysate should be homogeneous following the vigorous shaking. To check this, slowly invert the rack of collection microtubes (making sure that the caps are tightly closed) and look for a gelatinous mass. If a gelatinous mass is visible, lysis needs to be extended by adding another 100 µl Buffer ATL and 15 µl proteinase K, and incubating for a further 3 h. It is very important to ensure that samples are completely lysed to achieve optimal yields and to avoid clogging of

individual wells of the DNeasy 96 plate.

23 6. Carefully remove the caps. Add 615 µl premixed Buffer AL–ethanol to each sample. For a 96-well plate, aliquot out 61.5 ml of Buffer AL-ethanol from the stock bottle to use for the 96 samples plus a 4-sample buffer for error.

Note: Ensure that ethanol has been added to Buffer AL prior to use (see "Buffer AL", page 18).

Note: A white precipitate may form upon addition of Buffer AL–ethanol to the lysate. It is important to apply all of the lysate, including the precipitate, to the DNeasy 96 plate in step 9. This precipitate does not interfere with the DNeasy procedure or with any subsequent application.

24 7. Ensure that the microtubes are properly sealed to avoid leakage during shaking. Place a clear cover over each rack of collection microtubes and shake the racks vigorously up and down for 15 s. To collect any solution from the caps, centrifuge the collection microtubes. Allow the centrifuge to reach 3000 rpm, and then stop the centrifuge.

Do not prolong this step.

IMPORTANT: The rack of collection microtubes must be vigorously shaken up and down with both hands to obtain a homogeneous lysate. Inverting the rack of collection microtubes is not sufficient for mixing. The genomic DNA will not be sheared by vigorous shaking. The lysate and Buffer AL–ethanol should be mixed immediately and thoroughly to yield a homogeneous solution.

25 8. Place two DNeasy 96 plates on top of S-Blocks (provided). Mark the DNeasy 96 plates for later sample identification.

26 9. Remove and discard the caps from the collection microtubes. Carefully transfer the lysate (transfer a maximum of 800 µl of lysate so the flow-through doesn't come in contact with the bottom of the spin column) of each sample from step 7 to each well of the DNeasy 96 plates.

Take care not to wet the rims of the wells to avoid aerosols during centrifugation. Do not transfer more than 800 µl per well.

Note: Lowering pipet tips to the bottoms of the wells may cause sample overflow and cross-contamination. Therefore, remove one set of caps at a time, and begin drawing up the samples as soon as the pipet tips contact the liquid. Repeat until all the samples have been transferred to the DNeasy 96 plates.

Note: If the volume of proteinase K–Buffer ATL working solution was increased in steps 3 or 5, transfer no more than 800 µl of the supernatant from step 7 to the DNeasy 96 plate. Larger amounts will exceed the volume capacity of the individual wells. Discard any remaining supernatant from step 7 as this will not contribute significantly to the total DNA yield.

27 10. Seal each DNeasy 96 plate with an AirPore Tape Sheet (provided). Centrifuge for 10 min at 4700 rpm (our centrifuge cannot reach 6000 rpm).

AirPore Tape prevents cross-contamination between samples during centrifugation. After centrifugation, check that all of the lysate has passed through the membrane in each well of the DNeasy 96 plates. If lysate remains in any of the wells, centrifuge for a further 10 min.

28 11. Remove the tape. Carefully add 500 µl Buffer AW1 to each sample.

Note: Ensure that ethanol has been added to Buffer AW1 prior to use. It is not necessary to increase the volume of Buffer AW1 if the volume of proteinase K–Buffer ATL working solution was increased in steps 3 or 5.

29 12. Seal each DNeasy 96 plate with a new AirPore Tape Sheet (provided). Centrifuge for 5 min at 4700 rpm.

30 13. Remove the tape. Carefully add 500 µl Buffer AW2 to each sample.

Note: Ensure that ethanol has been added to Buffer AW2 prior to use.

It is not necessary to increase the volume of Buffer AW2 if the volume of proteinase K–Buffer ATL working solution was increased in steps 3 or 5.

31 14. Centrifuge for 15 min at 4700 rpm.
Do not seal the plate with AirPore Tape.
The heat generated during centrifugation ensures evaporation of residual ethanol in the sample (from Buffer AW2) that might otherwise inhibit downstream reactions.

31.1 14.5 If there is still liquid in the spin-column or flow-through touching the bottom of the spin column, repeat the drying step (Centrifuge for an additional 10 minutes at 4700 rpm.)

32 15. Place each DNeasy 96 plate in the correct orientation on a new rack of Elution Microtubes RS (provided).

33 16. To elute the DNA, add 100 µl Buffer AE to each sample, and seal the DNeasy 96 plates with new AirPore Tape Sheets (provided). Incubate for 1 min at room temperature (15–25°C). Centrifuge for 3 min at 4700 rpm.

34 17. After DNA is eluted, transfer 50 µl into a new skirted 96-well plate (what we have been using for bead clean-ups) and seal with the heat sealer, and label plate for archiving (plate_name archive DNA date).

35 18. Transfer the remaining DNA into a second new skirted 96-well plate. This aliquot will be used for further lab work. Label plate (plate_name lab DNA date).

QUALITY CONTROL

36 Total DNA is quantified using a Quant-iT kit on a plate reader.

BASIC TROUBLESHOOTING GUIDE

37 Identify known issues associated with the procedure, if any.

Provide troubleshooting guidelines when available.

REFERENCES

38 Qiagen Inc., November 2023 DNeasy Blood and Tissue Handbook; available online from Qiagen Inc.

APPENDIX A: DATASHEETS

39 Link templates (e.g. preformatted spreadsheets) used to record measurements and report on the quality of the data as well as any documents such as manufacturer specifications, images, etc that support this protocol. Please include a short note describing the document's relevance.