Contents

NOAA/AOML DNA Extraction Protocol for Sediment Trap Samples containing Formalin

1 PROTOCOL INFORMATION

1.1 Minimum Information about an Omics Protocol (MIOP)

- MIOP terms are listed in the YAML frontmatter of this page.
- See https://github.com/BeBOP-OBON/miop/blob/main/model/schema/terms.yaml for list and definitions.

1.2 Making eDNA FAIR (FAIRe)

- FAIRe terms are listed in the YAML frontmatter of this page.
- See https://fair-edna.github.io/download.html for the FAIRe checklist and more information.
- See https://fair-edna.github.io/guidelines.html#missing-values for guidelines on missing values that can be used for missing FAIRe or MIOP terms.

1.3 Authors

- All authors known to have contributed to the preparation of this protocol, including those who filled in the template.
- Visit https://orcid.org/ to register for an ORCID.
- Date is the date the author first worked on the protocol.

PREPARED BY	AFFILIATION	ORCID	DATE
Shiozaki et al.	NA	NA	2021
Santoro lab	UC Santa Barbara	NA	NA
Emily Osborne	NOAA AOML	0000-0001-9579-5851	2024-01-29
Gabriella Lirio	NOAA AOML, CIMAS	0009-0000-5574-1349	2025-01-29
Sammy Harding	NOAA AOML, MSU/NGI	0009-0008-8885-6140	2025-06-24

1.4 Related Protocols

- This section contains protocols that should be known to users of this protocol.
- Internal Protocols: Derivative or altered protocols, or other protocols in this workflow.
- External Protocols: Protcols from manufacturers or other groups.
- Include the link to each protocol.
- Include the version number (internal) or access date (external) of the protocol when it was accessed.

1.4.1 Internal Protocols

PROTOCOL NAME	LINK	VERSION	RELEASE DATE
AOML 'Omics Protocols	https://github.com/aomlomics/protocols	not applicable	ongoing

1.4.2 External Protocols

PROTOCOL NAME	LINK	ISSUER / AUTHOR	ACCESS DATE
DNeasy PowerSoil Pro Kit Handbook	https://www.qiagen.com/us/resources/resourcedetail?id=9bb59b74-e493-4aeb-b6c1-f660852e8d97&lang=en	QIAGEN	2025-06-24

1.5 Protocol Revision Record

- Version numbers start at 1.0.0 when the protocol is first completed and will increase when changes that impact the outcome of the procedure are made (patches: 1.0.1; minor changes: 1.1.0; major changes: 2.0.0).
- Release date is the date when a given protocol version was finalised.
- Description of revisions includes a brief description of what was changed relative to the previous version.

VERSION	RELEASE DATE	DESCRIPTION OF REVISIONS
1.0.0	2025-01-29	Initial release
1.1.0	2025-06-24	Minor formatting changes, changed name of NEBNext FFPE DNA Repair to NEBNext FFPE DNA Repair v2 Module
1.1.1	2025-12-15	Updated YAML front matter

1.6 Acronyms and Abbreviations

ACRONYM / ABBREVIATION	DEFINITION
NOAA	National Oceanic and Atmospheric Administration
AOML	Atlantic Oceanographic and Meteorological Laboratory
CIMAS	Cooperative Institute for Marine and Atmospheric Studies
NGI	Northern Gulf Institute
eDNA	environmental DNA
PPE	Personal protective equipment
PCR	Polymerase chain reaction
EtOH	Ethanol
NTC	No template control

1.7 Glossary

SPECIALISED TERM	DEFINITION
Extraction blank	A type of negative control to confirm there is no contamination during DNA extractions. Normally an empty filter extracted and PCR amplified alongside other samples.
No template control	A type of negative control during PCR to confirm there is no contamination during the PCR process. Normally nuclease-free water run in place of DNA on a PCR.

2 BACKGROUND

2.1 Summary

This protocol is used for {{analyses}} of {{environmental_medium}} eDNA samples from sediment-trap collected samples using contents of a {{nucl_acid_ext_kit}}. This protocol is used by the Ocean Biogeochemistry and Molecular and Computational Biodiverstiy groups of {{publisher}}.

2.2 Method description and rationale

This protocol was adapted from Shiozaki et al. (2021) and colleagues from the Santoro lab (UC Santa Barbara) to optimize eDNA extraction from sediment samples.

2.3 Spatial coverage and environment(s) of relevance

This protocol has been used to extract DNA from thousands of {{environmental_medium}} eDNA samples taken from the Northern Gulf of Mexico. Sample depths of 550m in the marine aphotic zone (ENVO:00000210).

3 PERSONNEL REQUIRED

One person with molecular biology experience.

3.1 Safety

Borate-NaOH is classified as category 5 acute oral toxicity and should not be swallowed. SDS solution has category 2 acute aquatic toxicity and category 3 chronic aquatic toxicity. It may cause serious eye damage, harmful if swallowed or inhaled, and may cause skin irritation. Proteinase K is classified as category 2 for skin corrosion/irritation and serious eye damage/eye irritation, category 1 for respiratory sensitization, and cateogry 3 for specific target organ toxicity (single exposure). A mixture within the AMpure XP beads is

classified as category 2 acute toxicity. Ethanol is categorized as a category 2 flammable liquid and for serious eye damage/eye irritation. For all reagents, standard precautions for handling chemicals including wearing PPE is recommended to avoid skin and eye exposure.

3.2 Training requirements

Basic molecular biology training (including sterile technique, pipetting) is required to conduct this protocol.

3.3 Time needed to execute the procedure

Extracting DNA from 21-cup sediment trap samples (64 triplicates + 3 blanks) is suggested to be completed over 2-3 days.

4 EQUIPMENT

DESCRIPTION	PRODUCT NAME AND MODEL	MANUFACTURER	QUANTITY	REMARK
Durable equipment				
Vortex	Vortex Genie	Scientific Industries	as many as possible	# of vortexed will affect run time of protocol
Water bath	Content Cell	Content Cell	Content Cell	Content Cell
Microcentrifuge	Centrifuge 5425 R - Microcentrifuge	Eppendorf	1	Can be substituted with generic
Magnetic stand for DNA repair	MagRack 6	Cytiva	8	# of magnetic stands will impact run time
100-1000 uL Pipette	Eppendorf Research Plus Adjustable-Volume Pipette	Eppendorf	1	Can be substituted with any accurate pipette
10-100 uL 8 Channel Pipette	Eppendorf Research Plus 8 Channel Pipette	Eppendorf	1	Can be substituted with any accurate pipette
1 uL-10 mL Repeater Pipette	Repeater M4 Pipette, 1 uL-10 mL Dispensing Volume for use with Combitips Advanced Syringe Tips	Eppendorf	1	Can be substituted with any accurate pipette, optional
Tube Racks	2 mL tube 96-well tube racks	Generic	1	Can be any brand, must fit 2 mL tubes
Wash bottles	Safety wash bottles 500 mL for EtOH	Generic		

DESCRIPTION	PRODUCT NAME AND MODEL	MANUFACTURER	QUANTITY	REMARK
Metal spatula	Eisco Stainless Steel Laboratory Spatula with Scoop	Eisco	1	Can be substitued with generic
Consumable equipment				
50 mL falcon tubes	Nunc 50 mL Bioreactor Tube	Thermo Scientific	21	Can be substituted with generic
1000 uL tips	OT-2 Filter Tips, 1000 uL	Opentrons	6 boxes	Can be substituted with generic
200 uL tips	OT-2 Filter Tips, 200µL	opentrons	7 boxes	Can be substituted with generic
10 uL tips	10 µL XL graduated	TipOne	1 box	Can be substituted with generic
5 mL Combitips	Combitips advanced, 5.0 mL, blue, colorless tips	Eppendorf	1 box	optional for MagBinding Beads with repeater pipette
Aluminum Foil Sealing Tape	AlumaSeal II Sealing Foils for PCR and Cold Storage	VWR	1	Can be substituted with generic
96-well PCR plate	Armadillo PCR Plate, 96-well, clear, clear wells	ThermoFisher	1	
Parafilm	Parafilm M Lab Film	Generic	1 roll	Can be substituted with generic
Gloves	Nitrile Gloves, Exam Grade, Powder-free	ULINE	1 box	Can be substituted with generic
Chemicals				
NaOH pellets		Generic	10 g	Used to create Borate-NaOH buffer
Borate powder		Generic	0.7725 g	Used to create Borate-NaOH buffer
SDS (10%)		Generic	5 mL	Used to create working solution
Proteinase K		Generic	4125 uL	
AMPure XP Beads	AMPure XP Bead-Based Reagent	Beckman Coulter	12.28 mL	
80% EtOH	Molecular bio grade ethanol	Generic	13.2 mL	Used in protocol

DESCRIPTION	PRODUCT NAME AND MODEL	MANUFACTURER	QUANTITY	REMARK
70% EtOH	Molecular bio grade ethanol	Generic	1 Wash Bottle	Used for cleaning lab surfaces
RNase Away	Research Products International Corp RNase Away, 475ml Spray Bottle	Thermo Fisher	1 bottle	
0.1X TE	TE, pH 8.0, RNase-free	Invitrogen	2.64 mL	
Qiagen DNeasy PowerSoil Pro Kit				
Microcentrifuge tubes	Microcentrifuge tubes (2 mL)	Qiagen		Can be substituted with generic
Elution tubes	Elution tubes (1.5 mL)	Qiagen	132	Can be substituted with generic
MB Spin Column	MB Spin Columns	Qiagen	66	Can be substituted with generic
Bead beating beads	PowerBead Pro Tubes	Qiagen	66 tubes	
Solution CD2		Qiagen	13.2 mL	
Solution CD3		Qiagen	39.6 mL	
Solution EA		Qiagen	33.0 mL	
Solution C5		Qiagen	33.0 mL	
Solution C6		Qiagen	6.6 mL	
NEBNext FFPE DNA Repair v2 Module				
FFPE DNA Repair Buffer v2	NEBNext FFPE DNA Repair Buffer v2	New England Biolabs	429 uL	
FFPE DNA Repair Mix v2	NEBNext FFPE DNA Repair Mix v2	New England Biolabs	132 uL	

- 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: Provide quantities necessary for one application of the standard operating procedure (e.g., number of filters).
- Remark: For example, some of the consumable may need to be sterilized, some commercial solution may need to be diluted or shielded from light during the operating procedure.

5 STANDARD OPERATING PROCEDURE

5.1 DNA extraction Protocol

5.1.1 Preparation • Stock solution: NaOH solution = 10 g NaOH pellets + 250 mL MilliQ (MQ)(make in hood)

• Stock solution: Borate-NaOH buffer = 0.7725 g borate + 250 mL MQ - Add small amount of NaOH until pH, 1 mL additions = 11 (check using pH strips)

• Working solution (50 mL centrifuge), make fresh each time - Borate-NaOH (45 mM), SDS (1%) = 9 mL 50-mM Borate-NaOH + 1 mL 10% SDS. Makes 10 mL, make more based on the desired number of samples (~1 mL per sample needed). - For a full sediment trap extraction with triplicates, ~70 mL of of working solution needed (65 mL Borate-NaOH + 5 mL 10% SDS)

5.1.2 Extraction Day 1: Separate sediments into sample vials (Shiozaki et al., 2021 and Santoro Protocol, unpublished) and begin Qiagen PowerSoil Kit

1. Centrifuge 50 mL sediment trap sample falcon tubes to concentrate/pellet the sediment (maximum speed, 4000rpm for 4 minutes – may need to do this twice if the sample does not fullt pellet)
    a. Decant off the supernatant gently.
        i. If the sediment trap sample is large, reserve supernatant in secondary centrifuge tube to add back to remaining sample falcon tube for long term storage.
    b. If there are larger organisms or shells present, remove them and make note in the sample log.
        i. Ideally, remove these large organisms/shells before centrifuging.
2. Prepare Samples in 2 mL sample tubes
    a. Clean off all scooping tools with RNase Away
    b. Tare weight of 2-mL sample tube and then scoop/load sediments from 50 mL into 2-mL sample tube (provided in Qiagen kit), aiming for ~250 mg (as little as 200 mg and as much as 300)
    c. In cases of small samples, use eyedropper with Milli-Q to disturb the sample to pour into 2 mL tubes, wait to settle, then remove supernatant with dropper.
    d. Record exact sediment weight in each discrete tube
    e. Ideally, prepare samples in triplicate and use additional splits of centrifuged sediment trap samples as needed
3. Rinse samples with MilliQ
    a. If after loading, the sample vial already has high water content, centrifuge (30 seconds, 10,000 x g) and remove supernatant.
    b. Add at least two sample blanks, label two microcentrifuge tubes to sample rack
    c. Add 1 mL MilliQ to vials, vortex for 5-10 seconds to mix, centrifuge (1 minute at 10,000 x g), and discard supernatant
4. Cell Lysis: Add reagents and bead beating beads to each sample (these reagents replace CD1 in kit)
    a. Add 980 ul of working solutiom (Borate-NaOH-SDS)
    b. Add 62.5 ul of 800U/ml Proteinase K to achieve 50 U/ml
    c. Add the Qiagen bead beating beads (pouring beads from tube into sample tube OR move solution into the bead pro tubes)
    d. Vortex briefly (5-10 seconds) confirming that the beads and sediment pellet are mixing. If not, use a clean metal spatula to aggressively disturb sediment pellet to mix with beads. Vortex to check.
    e. Once mixed, clip onto vortexer and run at maximum speed for 10 mins. Carefully remove tubes from vortexer clips as tops can easily pop open resulting in sample loss.
5. Incubate samples in a water bath (65 °C) for 24 hours (for Isotemp bath, setting 7 on temperature control (below) = 65 deg)

Day 2: Continue extraction using Qiagen PowerSoil Kit

6. Remove vials from bath and check for cracks. If a vial is cracked, transfer all contents to a new vial.
7. Centrifuge incubated samples + beads at 15,000 x g for 1 minute

8. Transfer supernatant of samples to a clean 1.5 mL microcentrifuge tube, some sediment may be suspended in the supernatant
9. Inhibitor Removal Technology: Add 200 uL of Solution CD2 and vortex for 5s
    a. CD2 will precipitate non-DNA organic and inorganic material including humic substances, cell debris, and proteins
    b. Centrifuge at 15,000 x g for 1 min.
    c. Avoiding the pellet, transfer up to 700 uL of supernatant to a clean 2 mL microcentrifuge tube. Expect 500-600 uL.
10. Bind DNA: Add 600 uL of Solution CD3 and vortex for 5s. Incubate for 1 min.
11. Load 650 uL of the lysate onto an MB Spin Column and centrifuge at 15,000 x g for 1 min

a. Discard the flow-through and repeat previous centrifuge step to ensure that all of the lysate has passed through the MB spin column
b. Carefully place the spin column into a clean 2 mL collection tub. Avoid splashing any flow-through onto the MB spin column.

12. Wash: Add 500 uL of Solution EA to the spin column. Centrifuge at 15,000 x g for 1 min.

a. Discard the flow-through and place the spin column back into the same 2 mL collection tube

13. Wash: Add 500 uL of Solution C5 to the spin column. Centrifuge at 15,000 x g for 1 min

a. Discard the flow-through and place the spin column into a new 2 mL collection tube
b. Centrifuge at up to 16,000 x g for 2 min. Carefully place the spin column into a new 1.5 mL elution tube

14. Elute: Add 100 uL of Solution C6 to the center of the white filter membrane (don't use repeater pipette for this step)

a. Centrifuge at 15,000 x g for 1 min. (Samples may need to be split into batches to fit elution + spin column into the centrifuge). Discard the spin column. DNA is now ready for downstream applications.

15. Aliquot DNA extract: Remaining sample stock, can store samples in 4 °C for days to weeks, -20 °C for long term weeks to months.
16. Measure extract concentrations of pre-repaired DNA.

Day 3: DNA Repair (can be combined with Day 2)

17. Mix the following components in a sterile nuclease-free tube:

a. FFPE DNA 53.5 uL
b. FFPE DNA Repair Buffer v2 6.5 uL
c. NEBNext FFPE DNA Repair Mix v2 2 uL
    i. Total volume= 62 uL
d. Mix by pipetting followed by a quick spin to collect all liquid from the sides of the tube.
e. Incubate at 20 °C for 15 minutes.

18. Cleanup using AMPure XP Beads

a. Vortex AMPure XP Beads to resuspend.
b. Add 186 uL (or 3x the volume of the sample) of resuspended AMPure XP Beads to the repair reaction. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.
c. Incubate for 5 minutes at room temperature.

19. Put the tubee on an appropriate magnetic stand to separate beads from supernatant.

a. After the solution is clear (about 5 minutes, but can take up to 20 minutes), carefully remove and discard the supernatant. Be careful not to disturn the beads that contain the DNA targets.

20. Add 200 uL of 80% freshly prepared ethanol to the tube/PCR plate while in the magnetic stand. (20 mL ethanol needed = 16 mL concentrated ethanol + 4 mL ultra pure MQ)

a. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant
b. Repeat this step once (ethanol addition and removal)

21. Air dry beads for up to 5 minutes while the tube is on the magnetic stand with the lid open.

a. Caution: Do not over-dry the beads. This may result in lower recovery of DNA target.
b. Elute the samples when the beads are still dark brown and glossy looking, but when all visible liquid has evaporated. When the beads turn lighter brown and start to crack, they are too dry.

22. Remove the tube from the magnet. Elute DNA target by adding 40 uL 0.1X TE to the beads. (2 mL needed = 200 uL 1X TE + 1.8 mL ultra pure MQ)

a. Mix well on a vortex mixer or by pipetting up and down, and incubate for 2 minutes at room temperature. Put the tube in the magnetic stand until the solution is clear.

23. Without disturbing the bead pellet, carefully transfer 32 uL of the supernatant to a fresh, sterile microfuge tube.
24. Proceed to library construction using end-user supplied reagents.

5.2 Quality control

- Extraction blank (negative control) included in every extraction.
- Qubit repaired samples to confirm DNA concentrations.

5.3 Basic troubleshooting guide

Not applicable.

6 REFERENCES

Shiozaki T, Itoh F, Hirose Y, Onodera J, Kuwata A, et al. (2021) A DNA metabarcoding approach for recovering plankton communities from archived samples fixed in formalin. PLOS ONE 16(2): e0245936. https://doi.org/10.1371/journal.pone.0245936

7 APPENDIX A: DATASHEETS

Not applicable.