

Contents

NOAA/AOML DNA Extraction Protocol for Sterivex using KingFisher	1
1 PROTOCOL INFORMATION	1
1.1 Minimum Information about an Omics Protocol (MIOP)	1
1.2 Making eDNA FAIR (FAIRe)	1
1.3 Authors	1
1.4 Related Protocols	2
1.5 Protocol Revision Record	3
1.6 Acronyms and Abbreviations	3
1.7 Glossary	3
2 BACKGROUND	3
2.1 Summary	3
2.2 Method description and rationale	3
2.3 Spatial coverage and environment(s) of relevance	4
3 PERSONNEL REQUIRED	4
3.1 Safety	4
3.2 Training requirements	4
3.3 Time needed to execute the procedure	4
4 EQUIPMENT	4
5 STANDARD OPERATING PROCEDURE	8
5.1 DNA extraction Protocol	8
5.2 Quality control	9
5.3 Basic troubleshooting guide	9
6 REFERENCES	10
7 APPENDIX A: DATASHEETS	10

NOAA/AOML DNA Extraction Protocol for Sterivex using KingFisher

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
Luke Thompson	NOAA/AOML, MSU/NGI	https://orcid.org/0000-0002-3911-1280	2021-09-13
Sean Anderson	NOAA/AOML, MSU/NGI	https://orcid.org/0000-0003-3096-1120	2021-09-13
Sammy Harding	NOAA/AOML, MSU/NGI	https://orcid.org/0009-0008-8885-6140	2024-06-26

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: Protocols 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
NOAA/AOML Water Sampling Protocol using Sterivex with Zirconia Beads	https://github.com/aomlomics/protocols/blob/main/markdown/protocol_sampling_sterive_x_beads.md	1.2.0	2025-01-08
NOAA/AOML PCR Protocol 12S rRNA V5-V6 (MiFish)	https://github.com/aomlomics/protocols/blob/main/markdown/protocol_pcr_ssul2sv5v6_mifish.md	1.1.0	2024-11-16
NOAA/AOML PCR Protocol 16S rRNA V4-V5 (EMP)	https://github.com/aomlomics/protocols/blob/main/markdown/protocol_pcr_ssu16sv4v5_emp.md	1.1.0	2024-11-16
NOAA/AOML PCR Protocol 18S rRNA V9 (EMP)	https://github.com/aomlomics/protocols/blob/main/markdown/protocol_pcr_ssu18sv9_emp.md	1.1.0	2024-11-16
NOAA/AOML Metagenome Library Prep Protocol (Illumina DNA Prep)	https://github.com/aomlomics/protocols/blob/main/markdown/protocol_libprep_metag_illumina.md	1.1.0	2024-11-16

1.4.2 External Protocols

PROTOCOL NAME	LINK	ISSUER / AUTHOR	ACCESS DATE
ZymoBIOMICS Manual 96 MagBead DNA Kit	https://files.zymoresearch.com/ZymoBIOMICS_d4302_d430025d02085_zymobiomics-96_magbead_dna_kit.pdf	ZymoBIOMICS	

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	2024-06-26	Initial release
1.1.0	2024-11-16	Addition of FAIR eDNA terms in YAML front matter
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
MSU	Mississippi State University
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 DNA extraction of sea water eDNA samples from Sterivex filters using contents of a ZymoBIOMICS 96 Magbead DNA Kit and a KingFisher Flex robot. Optional use of Opentrons OT-2 Robot to prepare plates for KingFisher. This protocol is used by the Molecular and Computational Biodiversity group of NOAA's Atlantic Oceanographic and Meteorological Laboratory.

2.2 Method description and rationale

This protocol is optimized for high throughput DNA extractions of Sterivex filters. It is easily reproducible and generates sufficient DNA yields while minimizing contamination and time requirements through the use

of a KingFisher and optional use of an Opentrons OT-2 Pipetting Robot.

2.3 Spatial coverage and environment(s) of relevance

This protocol has been used to extract DNA from thousands of sea water eDNA samples taken from stations off of South Florida coasts (Atlantic Ocean and Gulf of Mexico). Sample collection depths range from 1m to 200m in the epipelagic zone (marine photic zone [ENVO:00000209]).

3 PERSONNEL REQUIRED

One person with molecular biology experience.

3.1 Safety

MagBinding Buffer from the ZymoBIOMICS 96 Magbead DNA Kit is classified as category 4 for acute toxicity and causes severe skin burns and serious eye damage. It is also noted that the MagBinding Buffer is harmful to aquatic life with long lasting effects. MagWash 1 from the ZymoBIOMICS 96 Magbead DNA Kit is classified as category 4 for acute toxicity, is highly flammable, causes skin and serious eye irritation and may cause drowsiness or dizziness. MagWash2 from the ZymoBIOMICS 96 Magbead DNA Kit is flammable, causes serious eye irritation and may cause drowsiness or dizziness. Additional details available on SDS (https://files.zymoresearch.com/sds/_d4302_d4308_zymobiomics-96_magbead_dna_kits.pdf). For other 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 94 Sterivex samples takes about 5 hours (300 minutes) including KingFisher run time.

4 EQUIPMENT

DESCRIPTION	PRODUCT NAME AND MODEL	MANUFACTURER	QUANTITY	REMARK
Durable equipment				
Vortex	Vortex Genie	Scientific Industries	as many as possible	# of vortexes will affect run time of protocol
Vortex Adapter Plate	Vortex Adapter for 6 x 5ml tubes	QIAGEN	depends on # of vortexes	# of vortex adapter plates will affect run time
Centrifuge	Centrifuge 5425 R - Microcentrifuge	Eppendorf	1	Can be substituted with generic
Opentrons OT-2 Robot	OT-2 Pipetting Robot	Opentrons	1	Optional equipment for protocol; 8-Channel pipette recommended

DESCRIPTION	PRODUCT NAME AND MODEL	MANUFACTURER	QUANTITY	REMARK
KingFisher	KingFisher Flex Purification System	ThermoFisher	1	
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	Can be substituted with any accurate pipette, optional for filling KingFisher plates		
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	1	Can be substituted with generic
Consumable equipment				
Sterivex Filter	Millipore Sterivex-GP Pressure Filter Unit, 0.22µm pore size	Millipore Sigma	94	
DNA/RNA Shield	Zymo DNA/RNA Shield	Zymo Research	94	(mL) only required if Sterivex are not pre-loaded or for adding an extraction blank
Zirconia Beads	Zymo ZR BashingBead Lysis Tubes (0.1 & 0.5 mm)	Zymo Research	94	only required if Sterivex are not pre-loaded or for adding an extraction blank
Filter Cap (Male)	MasterFlex Male Luer Lock Plug	VWR	94	Can be substituted with generic
Filter Cap (Female)	MasterFlex Female Luer Thread Style Cap	VWR	94	Can be substituted with generic

DESCRIPTION	PRODUCT NAME AND MODEL	MANUFACTURER	QUANTITY	REMARK
Parafilm	Parafilm M Lab Film	Generic	1	(roll) Can substitute with generic brand
Syringes	BD Disposable Syringes with Luer-Lock Tips, 5 mL	Fisher Scientific	94	Can be substituted with generic as long as they twist onto Sterivex
Microcentrifuge Tubes	NEST 2.0 mL Microcentrifuge Tube	Opentrons	94	Can be substituted with generic
KingFisher 96 deep-well plate	KingFisher Plastics for 96 deep-well format	ThermoFisher	7	
Kingfisher 96 well microplate (200uL)	KingFisher Plastics for 96 standard and PCR formats	ThermoFisher	1	
KingFisher 96 tip comb for deep-well magnets	Kingfisher Flex 96 Deep-Well Tip Combs	ThermoFisher	1	
96-well PCR plate	Armadillo PCR Plate, 96-well, clear, clear wells	ThermoFisher	3	
Aluminum Foil Sealing Tape	AlumaSeal II Sealing Foils for PCR and Cold Storage	VWR	3	Can be substituted with generic
1000µL Filter Tips	OT-2 Filter Tips, 1000µL	Opentrons	2	(boxes) Can be substituted with generic
200µL Filter Tips	OT-2 Filter Tips, 200µL	Opentrons	1	(box) Can be substituted with generic
2.5 mL Combitips	Combitips advanced, 2.5 mL, green, colorless tips	Eppendorf	1	(tip) Optional for MagBinding Beads with repeater pipette
10 mL Combitips	Combitips advanced, 10 mL, orange, colorless tips	Eppendorf	3	(tips) Optional for KF plate prep with repeater pipette
Gloves	Nitrile Gloves, Exam Grade, Powder-free	ULINE	1	(box) Can be substituted with generic
Kim Wipes	KimWipe Delicate Task Wipers	KimTech	1	(box) Can be substituted with generic

Opentrons Consumables

DESCRIPTION	PRODUCT NAME AND MODEL	MANUFACTURER	QUANTITY	REMARK
12-Well Reservoir, 15 mL	NEST 12-Well Reservoir, 15 mL	Opentrons	1	optional for KF plate prep on OT-2 Robot
Deep Single-Well Reservoir, 300 mL	Nalgene Disposable Polypropylene Robotic Reservoirs	ThermoFisher	2	optional for KF plate prep on OT-2 Robot
200µL Filter Tips	OT-2 Filter Tips, 200µL	Opentrons	1	(box) Optional for KF plate prep on OT-2 Robot
Chemicals				
70% EtOH	Molecular bio grade ethanol	Generic	1	(Wash Bottle) only used for cleaning lab surfaces
ZymoBIOMICS 96 MagBead DNA Kit				
Lysis Solution	ZymoBIOMICS Lysis Solution	Zymo Research	94 mL	(mL) Can be used in place of DNA/RNA Shield, only required if Sterivex are not pre-loaded or for adding an extraction blank (mL)
MagBinding Buffer	ZymoBIOMICS MagBinding Buffer	Zymo Research	57.6	(mL)
MagBinding Beads	ZymoBIOMICS MagBinding Beads	Zymo Research	2.4	(mL)
MagWash 1	ZymoBIOMICS MagWash 1	Zymo Research	48	(mL)
MagWash 2	ZymoBIOMICS MagWash 2	Zymo Research	86.4	(mL)
DNAse/RNase Free Water	ZymoBIOMICS DNAse/RNase Free Water	Zymo Research	14.4	(mL)

- Opentrons Consumables: If using Opentrons OT-2 Robot for KF Plate Prep.
- 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

1. Sterilize all workspaces and durable equipment with 70% EtOH.
2. Label 94 2-ml microcentrifuge tubes 1 through 94 and place in 96-well tube rack.
3. Fill large ice bin with ice.

5.1.2 Extraction

1. Remove Sterivex from freezer and immediately place on ice while randomizing the order of the filters.
2. If Sterivex are wrapped in aluminum foil, remove foil and ensure all Sterivex have filter caps on either end (male and female).
3. If Sterivex do not already contain zirconia beads and DNA/RNA Shield, add as follows:
 - Remove the male luer plug from the inlet port.
 - Remove any liquid from the inlet port using a sterile swab or twisted piece of KimWipe.
 - Pour beads from ZR BashingBead Lysis Tubes (0.1 & 0.5 mm) into the inlet port. If water has been removed, this should be easily done. If beads get stuck, use a pipette tip to unclog them.
 - Add 1 mL ZymoBiotics Lysis Buffer or 1:10 dilution of DNA/RNA Shield to the Sterivex using a pipette.
 - Replace the male luer plug in the inlet port.
4. Number the filters sequentially and use scanner to scan QR codes (cryo-babies labels) onto a google sheet or other form of processing log. If Sterivex do not have QR codes, record the numbers with their corresponding sample names in a google sheet or lab notebook.
5. Vortex for 40 minutes via Vortex Genie at speed ~6. Make sure the proper adjustable head is secured, which has brackets that fit the Sterivex filters. If brackets are loose and Sterivex are falling out, wrap a small portion of parafilm around the outside of the Sterivex and secure in bracket.
6. While vortexes are running, prepare KingFisher plates by hand using a repeater pipette:
 - Sample Plates (3): Add 600 µl of MagBinding Buffer and 25 µl of MagBinding beads to each well.
 - Wash 1: Add 500 µl of ZymoBiotics MagWash 1 to each well.
 - Wash 2-1: Add 900 µl of ZymoBiotics MagWash 2 to each well.
 - Wash 2-2: Add 900 µl of ZymoBiotics MagWash 2 to each well.
 - Elution: Add 150 µl of ZymoBiotics RNAse Free Water to each well.

OR

(OPTIONAL) Prepare KingFisher plates with Opentrons OT-2 Pipetting Robot:

- Turn on OT-2 Robot and computer, open Opentrons program and begin protocol setup.
- Place 200 uL filter tips in correct slot of deck.
- Carefully fill 12-well reservoir with reagents:
 - Fill the first two columns 1/2 way full with DNA/RNAse Free Water.
 - Fill columns five, six, seven and eight completely with MagWash 1.
 - Place reservoir in correct deck slot of Opentrons.
- Place a deep single-well reservoir in the MagBinding deck slot and carefully fill completely with Mag-Binding Buffer.
- Place a deep single-well reservoir in the MagWash 2 deck slot and carefully fill completely with Mag-Wash 2.

- Place sample plates (3), wash plates (3), and elution plate in respective deck slots.
 - Confirm all plates are correctly placed in deck slots and begin the run.
 - Once the run is complete, use a repeater pipette to add 25 uL of Magbinding Beads to each well of the 3 sample plates. Make sure to shake or vortex the MagBinding Beads before pipetting to resuspend the beads.
7. After 40 minutes of vortexing, expel lysate from Sterivex filters as follows:
 - Remove the male filter cap and insert a 3-ml syringe into the inlet port (syringe should twist and fit tight).
 - Gently expel the lysate into the syringe. Some bead carry-over is common.
 - Transfer lysate to a labeled and sterile 2-ml microcentrifuge tube (liquid and beads will reach ~0.75 ml mark ideally).
 8. Centrifuge tubes at 10,000 x g for 1 min to pellet the beads and leave the sample as supernatant.
 9. Gently pipette 250 uL of sample supernatant into respective well of KingFisher Sample Plate 1, Sample Plate 2 and 3. Repeat for each sample. If there is less than 750 uL of sample lysate in a microcentrifuge tube, attempt to pipette even amounts into each of the 3 sample plates.
 10. Turn on the KingFisher instrument and computer and open up the BindIt software for KingFisher.
 11. Choose correct protocol for a 3-plate run.
 12. Press start and save a new run file (you can create a new file or save over a previous file that used the same protocol).
 13. Follow along with instructions on the KingFisher screen to load the appropriate plates onto the KingFisher carousel and ensure all plates are in the correct position. Once the last plate has been added, the run will begin.
 14. Run will last ~1hr 15min for a 3-plate protocol.
 15. Once the run is complete, discard the wash, sample and tip plates. DO NOT THROW OUT ELUTION PLATE.
 16. Transfer samples in Elution plate (~150 uL per well) to a permanent 96-well PCR plate for long term storage of DNA at -20 to -80 °C.
 - Specific to NOAA/AOML: Transfer 50 uL of sample to a 96-well PCR plate labeled "Working Stock", another 50 uL of sample to a 96-well plate labeled "Archive 1", and the remainder 50 uL of sample to a third 96-well plate labeled "Archive 2". (see quality control for more information)
 17. Turn off KingFisher instrument and computer.

5.2 Quality control

- An extraction blank (negative control) is included in every extraction set (93 samples + 1 extraction blank). All samples and controls are then quantified to confirm DNA concentration. Samples are also PCR amplified with a NTC and positive controls (Zymo Mock Community).
- Specific to NOAA/AOML: Extracted samples are transferred into 3 aliquots stored at -20°C: a working stock for use in lab (~50 uL), Archive 1 (~50 uL), and Archive 2 (~50 uL).

5.3 Basic troubleshooting guide

- Issue 1: There have been issues with cracks in the ring around the inlet ports of Sterivex (usually only one side) and leaks occurring after thawing.
 - Solution: To make the Sterivex usable, wipe off excess leakage/condensation on the outside of the Sterivex using a KimWipe and firmly press putty into the concave ring surrounding the inlet ports of the Sterivex. Then use parafilm to tightly wrap the areas where putty was applied.

- Issue 2: Depending on the type of bracket used on Vortex Genies, Sterivex may fall out of the brackets as soon as vortexes are turned on.
 - Solution: Use a KimWipe to wipe off excess condensation on the Sterivex then wrap a small strip of parafilm around the outside of the Sterivex and clip back into the bracket.
- Issue 3: (Opentrons OT-2 Robot) Occasionally the Opentrons OT-2 Robot will have an issue with dispensing liquid because of bubbling occurring inside the tips dispensing MagBinding buffer due to the “soapy” nature of the solution.
 - Solution: Press the “Pause” button on the computer and replace the bubbly tip manually with gloved hands. Then unpause the run and watch to see if the tip correctly dispenses MagBinding buffer. If a sample plate has empty wells, use a pipette to manually add MagBinding buffer after the plate comes off the Opentrons.

6 REFERENCES

Not applicable.

7 APPENDIX A: DATASHEETS

Not applicable.