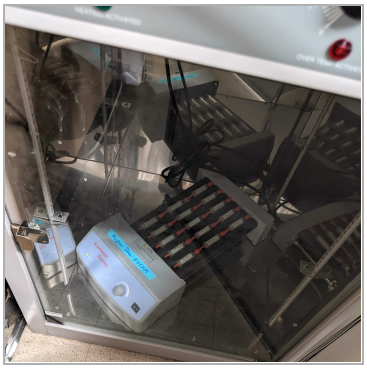

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MANUSCRIPT CITATION:
TBD

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

DNA Extraction Protocol from Sterivex Filters

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ABSTRACT

This is a modified protocol for extracting DNA from Sterivex filters using an adjusted procedure with the Qiagen DNeasy Blood and Tissue Kit, as first published by Spens et al. 2017.

This protocol originates with environmental DNA samples collected onto 0.22 µm capped Sterivex filters, e.g. through this sampling and filtration protocol:

Protocol

NAME
Coastal Environmental DNA Sampling & Gravity Filtration Protocol

CREATED BY
Meghan M. Shea

PREVIEW

ATTACHMENTS

HB-2061-003_HB_DNY_Blood_Tissue_0720_WW.pdf

IMAGE ATTRIBUTION

Meghan M. Shea

MATERIALS

General Laboratory Equipment:

	Equipment	Specific Model Used

Created: Jun 16, 2023

Last Modified: Aug 01, 2023

PROTOCOL integer ID: 83545

Keywords: environmental DNA, DNA extraction, Sterivex, Qiagen DNeasy Blood and Tissue Kit

	Equipment	Specific Model Used
	Incubator	VWR 1565B
	Tube roller shaker	Southwest Science (STL100)
	10% bleach solution in spray bottle	NA
	>70% ethanol solution in spray bottle	NA
	RNase Away solution in spray bottle	NA
	UV Crosslinker	UVP CL-1000 Ultraviolet Crosslinker
	Kimwipes	NA
	Gloves	NA
	1000 µL pipette with sterile tips (that fit the top of the Sterivex--ideally long & skinny)	Various
	200 µL pipette with sterile tips	Various
	100 µL pipette with sterile tips	Various
	10 µL pipette with sterile tips	Various
	Vortex	VWR Mini Vortexer
	Centrifuge	Eppendorf Centrifuge 5424
	Lab markers	VWR (52877-310): https://us.vwr.com/store/product/4597364/vwr-chemical-resistant-laboratory-marker
	Cryo-labels	USA Scientific (9187-0100): https://www.usascientific.com/cryo-tags-combo-sheets/p/9187-0100
	Feezer boxes	Cole-Parmer, via Fisher Scientific (3391550): https://www.fishersci.com/shop/products/polarsafe-81-place-polypropylene-storage-boxes-6/03391550
	Variety of sterile or sterilizable tubes for holding aliquots of reagents	Various, including some that fit in heat block
	Heat block	VWR Standard Heatblock
	Qubit Fluorometer	Invitrogen Qubit 2.0 Fluorometer

Note

There are many types of incubators and shakers (some combined) that could be used to heat and agitate the Sterivex filters overnight. The Southwest Rock & Roll Lab Tube Roller was by far the most cost effective option we found, and it fit (just barely) in an existing incubator.

Additional Materials:

	Material	Amount Needed	Source	Link	Approx. Cost
	Qubit dsDNA Broad Range assay kit	1 reaction/sample + additional for standards	Fisher Scientific (Q32850)	https://www.fishersci.com/shop/products/qubit-dsdna-hs-br-assay-kits/Q32850	$115/100 reactions
	Sterile 3 mL luer lock syringes	1/sample	BD, via Fisher Scientific (14-823-435)	https://www.fishersci.com/shop/products/bd-disposable-syringes-luer-lok-tips-3/14823435	$21.60/200 syringes
	Water, DNA Grade, DNASE, Protease free	Variable	Fisher Scientific (BP24701)	https://www.fishersci.com/shop/products/water-dna-grade-dnase-protease-free-fisher-bioreagents/BP24701	$30.05/1000 mL
	1.5/2 mL LoBind Eppendorf tubes (either size works; prefer 1.5 mL for storage)	3/sample	USA Scientific (4043-1021/4043-1048)	https://www.usascientific.com/dna-lobind-microcentrifuge-tubes/p/DNA-LB-Micro-Tubes	$38.95/250 tubes
	Ethanol, Absolute (200 Proof), Molecular Biology Grade	Variable	Fisher Scientific (BP2818500)	https://www.fishersci.com/shop/products/ethanol-absolute-200-proof-molecular-biology-grade-fisher-bioreagents-3/BP2818500	$61.77/500 mL
	5 mL LoBind Eppendorf Tubes	1/sample	USA Scientific (4011-8310)	https://www.usascientific.com/eppendorf-tube-5-0-ml-dna-lobind/p/4011-8310	$68.5/200 tubes

	Material	Amount Needed	Source	Link	Approx. Cost
	Qiagen DNeasy Blood & Tissue Kit	1 reaction/sample	Qiagen (69506)	https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/genomic-dna/dneasy-blood-and-tissue-kit?catno=69506	$692.3/250 preparations
	Qubit tubes	1/sample + additional for standards	Unknown	NA	Unknown

Note

Due to the modifications to the Qiagen DNeasy Blood & Tissue Kit, some reagents are used in greater volumes than the manufacturer's protocol, and will run out before the kit is completed. Be prepared to order extra Proteinase K, Buffer ATL, and Buffer AL as needed.

SAFETY WARNINGS

Taken directly from the Qiagen DNeasy Blood & Tissue Handbook:
HB-2061-003_HB_DNY_Blood_Tissue_0720_WW.pdf

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view and print the SDS for each QIAGEN kit and kit component.

Safety information

Caution: DO NOT add bleach or acidic solutions directly to the sample preparation waste.

Buffers AL and AW1 contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with a suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Day 1 - DNA Lysing

1 Clean bench area with bleach, ethanol, and RNase away

2 Turn on incubator and set to 56°C

3 Wipe down 1000 µL and 200 µL pipettes with RNase Away and UV for 15 minutes on each side

4 Assemble materials and reagents needed:

- 1000 µL pipette tips (that fit in the inlet of the Sterivex)
- 200 µL pipette tips
- Proteinase K (from Qiagen DNeasy Blood & Tissue Kit)
- ATL Buffer (from Qiagen DNeasy Blood & Tissue Kit)

5 Remove sterivex filters from -15°C freezer

> Note
>
> The roller shaker used (see MATERIALS) only fits 15 Sterivex filters, which creates a 16 sample extraction batch with an additional extraction blank added on Day 2. 16 samples could be well-balanced in the centrifuge used, leading to easier subsequent processing.
>
> If processing a different number of filters, consider the subsequent processing steps (especially centrifuging) when deciding how many to extract at once.

6 For each filter:

6.1 Remove Sterivex from Whirl-Pak bag and remove cap from inlet end of Sterivex filter

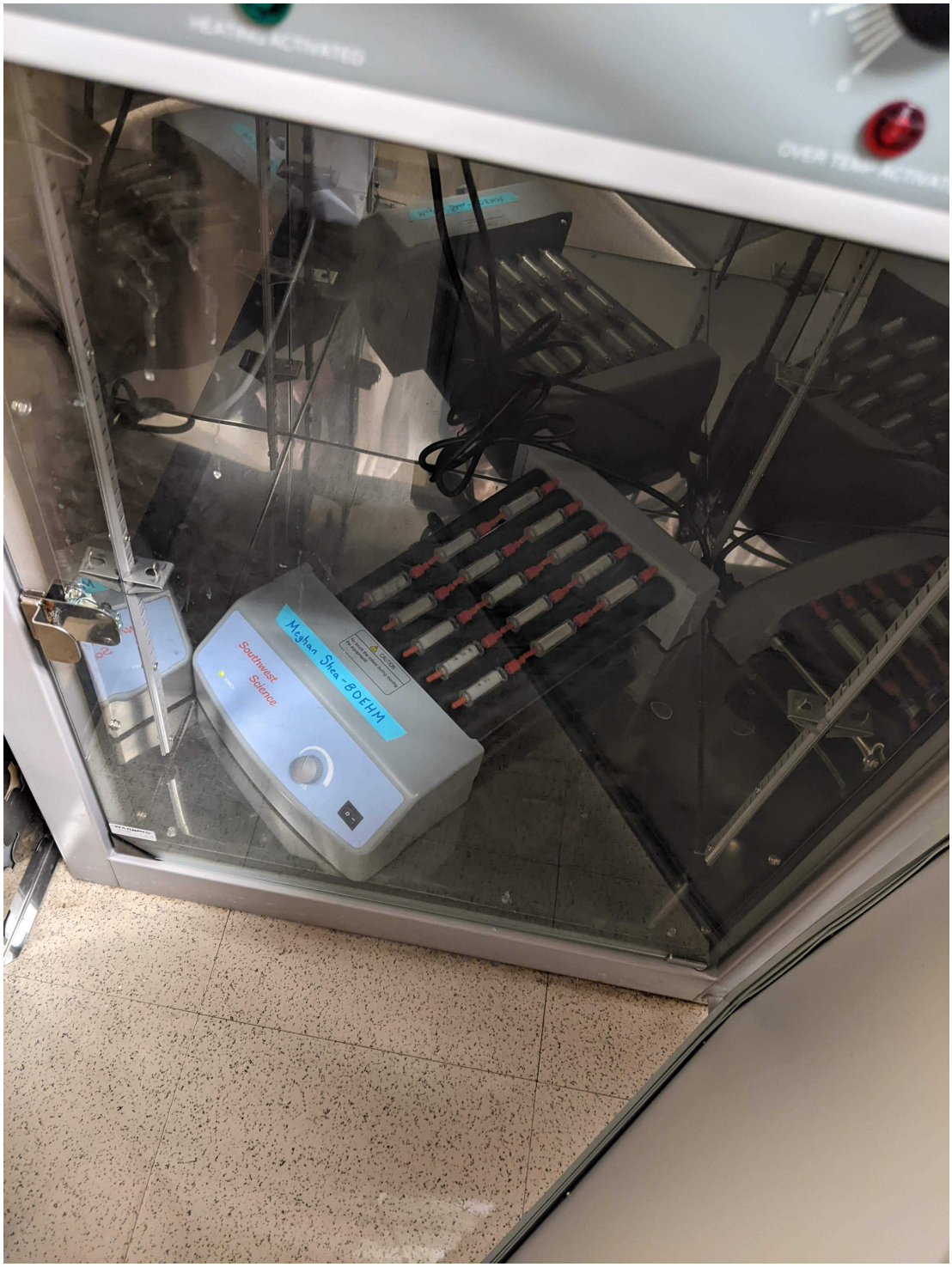
6.2 Slowly pipette 80 µL of Proteinase K directly on top of the filter through the inlet, avoiding backsplash by expelling slowly

6.3 Slowly pipette 720 µL of ATL buffer directly on top of the filter through the inlet, again avoiding backsplash

6.4 Secure Sterivex with same luer cap

6.5 Handshake vigorously for several seconds

7 Place all filters onto roller shaker in incubator



Sterivex arranged on roller shaker in incubator (Photo Credit: Meghan M. Shea)

8 Incubate at 56°C for ~24 hours (minimum of 12 hours; try to incubate for the same amount of time for all filters for a particular project) while rotating at approximately 6 rpm

Day 2 - DNA Extraction

9 Clean bench area (including vortex), centrifuge area, and centrifuge with bleach, ethanol, and RNase away.

10 Soak all tube racks in a 10% bleach solution, followed by 3 rinses with DI water. Let dry and UV for 15 minutes

11 UV Qubit and LoBind tubes for 15 minutes (in pre-sterilized tube racks) and label with sample numbers:

- Qubit: (# of samples+ extraction blank) + 2 for standards
- 1.5/2 mL LoBind: (# of samples + extraction blank) * 3
- 5 mL LoBind: # of samples + extraction blank
- Enough tubes (any type, does not need to be LoBind) to hold AE Buffer that fit in heat block (see Step 15), and to hold Qubit working solution (see Step 23.1)

12 Open and label additional tubes needed from Qiagen DNeasy Blood & Tissue Kit:

- Packaged spin columns: # of samples + extraction blank
- Additional collection tubes: (# of samples + extraction blank) * 2

13 Wipe down 1000 µl pipette, 200 µl pipette, 100 µl pipette, 10 µl pipette with RNase Away and UV for 15 minutes on each side

14 Place 50 mL tube of molecular-grade ethanol in freezer or on ice to chill for later use

15 Heat aliquot (volume calculation below) of AE buffer (from Qiagen DNeasy Blood & Tissue Kit) on heating block at 70°C

*Volume: (# of samples including blank * 100 µl) * 1.1*

16 Make cryo-labels for storage tubes (2 of 3 1.5/2 mL LoBind tubes already sterilized)

17 Assemble other reagents & materials needed:

- Sterile 3 mL syringes (one per sample)
- AL Buffer (from Qiagen DNeasy Blood & Tissue Kit)
- Buffer AW1 (from Qiagen DNeasy Blood & Tissue Kit)
- Buffer AW2 (from Qiagen DNeasy Blood & Tissue Kit)

18 Remove Sterivex filters from incubator

19 Remove liquid from each filter:

19.1 Handshake vigorously for several seconds

19.2 Remove caps from filter

19.3 Using a sterile 3 mL syringe attached to the inlet end of the Sterivex, remove all liquid from filter, record the volume, and transfer into a 5 mL LoBind tube

20 Create an extraction blank by adding 1000 uL nuclease free water subbed in for extracted liquid

to a 5 mL LoBind tube

21 For each sample and extraction blank, add AL buffer and 0°C ethanol to the extracted liquid in a 1:1:1 ratio and vortex vigorously for 10 seconds

22 For each sample and extraction blank, filter the mixture through a spin column:

22.1 Pipet the mixture (650 µl at a time) into a DNeasy Mini Spin column placed in a collection tube

22.2 Spin in micro-centrifuge for 1 minute at 6000 x g (8000 rpm)

22.3 Discard flow throw, and dab the rim of the spin column dry on a Kimwipe

22.4 Repeat sub-steps of 22 until all sample is filtered through spin column

23 For each sample and extraction blank follow the remaining Qiagen DNeasy Tissue and Blood Kit steps:

23.1 Place the spin column in a new collection tube, add 500 µl Buffer AW1

23.2 Centrifuge for 1 minute at 6000 x g (8000 rpm). Discard flow through and collection tube.

23.3 Place in new collection tube and add 500 µl Buffer AW2

23.4 Centrifuge for 3 min at 20,000 x g (14000 rpm) to dry the membrane

23.5 Discard flow through and dab rim of spin column on a clean Kimwipe

23.6 Place the spin column back in collection tube and centrifuge for 1 min at 20,000 x g (14000 rpm)

23.7 Transfer spin column to new 1.5/2 mL LoBind tube with cap left open

23.8 Place samples in 70°C heat block

23.9 Add 100 µl Buffer AE (4 samples at a time) directly over membrane

23.10 Immediately transfer tubes to room temperature

23.11 Incubate at room temperature for 10 minutes

23.12 Centrifuge for 1 min at 6000 x g (8000 rpm)

23.13 Re-elute DNA from DNA LoBind tube (apply eluate back on spin column while tubes are in heat block)

23.14 Incubate at room temperature for 10 minutes

23.15 Centrifuge for 1 min at 6000 x g (8000 rpm)

23.16 Discard the spin column

Note

Sample tubes should now contain a final volume of 100 µl of DNA extract

24 Aliquot 1 µl of each sample into labeled Qubit tubes

25 Transfer ~50 µl of remaining DNA extract to 2 pre-marked DNA LoBind tubes with lids intact:
- 1 archive tube to store at -80°C
- 1 working tube to store at -15°C

Note

Aliquot to archive tube first, so that archive volumes are consistently 50 µl. Working tube volumes may be slightly less than 50 µl due to Qubit aliquot, etc.

26 Use Qubit to measure DNA concentrations in all samples:

26.1 Make Qubit working solution:
- Reagent: ((# of samples + extraction blank + 2) * 1)*1.1
- Buffer: ((# of samples + extraction blank + 2) * 199)*1.1

26.2 Vortex Qubit working solution

26.3 Add 199 µl of working solution to Qubit tubes containing sample DNA

26.4 Add 190 µl of working solution to Qubit tubes for 2 standards

26.5 Add 10 µl of respective standards to Qubit tubes for 2 standards

26.6 Mix all tubes gently by vortexing, being sure not to introduce bubbles

26.7 Allow tubes to incubate at room temperature for 2 minutes

26.8 Read samples with Qubit fluorometer and record DNA concentrations