Jun 17, 2024

DNA Extraction from 0.22µm Sterivex Filters - Qiagen Blood and Tissue with Inhibitor Removal

DOI
dx.doi.org/10.17504/protocols.io.bp2l6223dgqe/v1

Andreas Novotny[1]

[1]University of British Columbia

Hakai Genomics

Andreas Novotny
University of British Columbia, Hakai Institute, Stockholm U...

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.bp2l6223dgqe/v1

External link: http://hakai.org

Protocol Citation: Andreas Novotny 2024. DNA Extraction from 0.22µm Sterivex Filters - Qiagen Blood and Tissue with Inhibitor Removal. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bp2l6223dgqe/v1

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

Created: June 01, 2024

Last Modified: June 17, 2024

Protocol Integer ID: 101072

Abstract

This protocol is used to extract genomic DNA from 0.22µm sterivex filters using Quiagens Blood and Tissue Kit. The protocol was developed as an equivalent replacement for the Phenol Chloroform extraction protocol. An additional PCR inhibitor removal step has been added to increase reproducibility for PCR-based downstream methods. This is especially important for coastal waters influenced by land runoff of glacial debris. This protocol is developed to work across all domains of life, from viruses to prokaryotes to eukaryotes, allowing for both amplicon sequencing and shotgun sequencing.

Guidelines

MIOP: Minimum Information about an Omics Protocol

MIOP Term	Value
analyses	Nucleic Acid Extraction
audience	scientists
broad-scale environmental context	marine biome ENVO_00000447
creator	Andreas Novotny
environmental medium	sea water [ENVO:00002149]
geographic location	North Pacific Ocean [GAZ:00002410]
hasVersion	1
issued	2017
language	en
license	CC BY 4.0
local environmental context	coastal sea water [ENVO: 00002150]
materials required	Sterile workbench, Fume Hood, Centrifuge, Incubator
maturity level	Mature
methodology category	DNA extraction
personnel required	1
project	Urban Ocean Biodiversity
publisher	University of British Columbia, Pelagic Ecosystems Lab
purpose	DNA Extraction
skills required	sterile technique | pipetting skills
target	DNA
time required	1 day

AUTHORS

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 register)	DAT
Colleen Kellogg	Hakai Institute	https://orcid.org/0000-0003-4048-5316	201 7
Andreas Novotny	University of British Columbia	https://orcid.org/0000-0001-8910-6183	202 4

RELATED PROTOCOLS

PROTOCOL NAME AND LINK	ISSUER / AUTHOR	RELEASE / ACCESS DATE
Seawater filtration	Hakai Institute	

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

ACRONYM / ABBREVIATION	DEFINITION

GLOSSARY

SPECIALISED TERM	DEFINITION

BACKGROUND

This protocol is used to extract genomic DNA from 0.22µl sterivex filters using a modified version of the protocol developed by the Hakai Institute:

CITATION

Colleen Kellogg, rute.carvalho Carvalho, Matt Lemay. DNA Extraction from 0.22µm Sterivex Filters - Qiagen Blood and Tissue Kit. protocols.io.
LINK
https://protocols.io/view/dna-extraction-from-0-22-m-sterivex-filters-qiagen-der83d9w

This protocol was developed as an equivavelt to the Phenol Chloroform protocol for newly started projects that does not require comparable results to the weekly sampling series. The protocol was also modified to take extra precaution to PCR inhibitors originating from turbid coastal waters. We can see that adding PCR inhibition removal substantiallyincreases PCR efficiencies of all samples, but especially samples from turbid waters.

Spatial coverage and environments of relevance
This protocol was implemented as part of the Urban Ocean Bioidiversity project, a collaboration between University of British COlumbias Institute for the Oceans and Fistheries, and Tsleil-Waututh First Nation. In 2023 we sambled multiple stations in the Burrard Inlet BC, Canada (from Point Grey to Port Moody Arm, Including the Port of Vancouver). Samples were collected at four seasons. This protocol yielded DNA with successful 12S amplification rates for over 95% of the samples, including those from the glacial freshed in late spring.

Personnel Required
1 Technician

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

Training requirements
Sterile technique, pipetting skills. Work-safe laboratory practices.

Time needed to execute the procedure
1 Day (for 24 samples).

Protocol materials

QIAgen DNeasy Blood and Tissue Kit, 50 rxn **Qiagen Catalog #**69504	Step 2
OneStep PCR Inhibitor Removal Kit **Zymo Research Catalog #**D6030	Step 2
Proteinase K, 2mL **Qiagen Catalog #**19131	Step 2

Before start

Read background information, MIOP and BePOP-OBON information under the "Guidelines" tab.

PREPARATION

1

This protocol assumes that samples have been collected and preserved according to this procedure:

Protocol

NAME

Seawater Filtration for Microbial or Environmental DNA

CREATED BY

Andreas Novotny

PREVIEW

2

Note

We are aiming to do about 24 samples per day, but you can aim for less than that until you get comfortable with the protocol.

Materials needed:

Proteinase K, 2mL **Qiagen Catalog #**19131

- 0.5 mm glass beads, silica. Molecular grade
- Molecular grade ethanol (100%)

QIAgen DNeasy Blood and Tissue Kit, 50 rxn **Qiagen Catalog #**69504

The kit Includes:

- ATL (lysis buffer)
- AL (lysis buffer)
- RNAse
- AW1 (Wash buffer)
- AW2 (Wash buffer)
- AE (Elution buffer)
- Spin Columns

OneStep PCR Inhibitor Removal Kit **Zymo Research Catalog #**D6030

The kit Includes:

- ZYMO spin columns
- Prep solution

3 **UV for 30 minutes the following:** 30m

- Silica beads - you will need a 5mL tube full of beads to extract DNA from 12 samples

(*See bench top guide in the end of this protocol to check the amount of tubes you will need*)

- 2mL tubes
- 50mL falcon tubes
- Racks
- Pipettes
- pipette tips

Do not forget to UV tubes for that will be used to aliquot the buffers, and silica beads.

4 Wipe down the benches, centrifuge, and working areas using the PREempt solution/wipes. Turn on the incubator and set the temperature to 56°C.

5 Set aside ATL buffer, AL buffer, proteinase K, RNase A and anhydrous ethanol.
Put the ATL and AL buffers in the incubator to eliminate any precipitate that may be in the solution.

There is a cardboard box in the mini freezer with pro K aliquots. Each aliquot has enough volume for 12 samples. The anhydrous ethanol is stored in the CR1 mini fridge, and in the CR2 in the mini freezer.

6 Calculate the volume of each reagent you will need for each step and have a one-time-use tube to make an aliquot for that specific reagent. Try to add a little bit more than you need and dispose of the leftover. *Prepare the aliquots inside the workstation (with HEPA filter).*

7 Cut the parafilm to a size of 1 cm x 5 cm, two or three per Sterivex – sometimes you will need to replace the parafilm in the Sterivex.

BEADS BEATING AND INITIAL INCUBATION

8 Thaw the Sterivex filters.

9 Remove the parafilm and remove Longmire's buffer/SLB using a syringe. You can use a plastic "tripour" to dispose the buffer and then pour all of the volume in the sink. *Rinse the tripour with water after use, dry and wipe it down using a paper towel and PREempt solution.*

10 If needed, dry the Sterivex inlet using a Kimwipes (one wipe per Sterivex). Place the Sterivex on a clean Kimwipe while preparing the other one Sterivexes.

11 Add 0.1 mm silica beads (*about 0.3 g or three spoons**) into the Sterivex using a weighting paper (make a funnel with the paper to slide in the beads, use one per Sterivex). **It is a white spoon that is stored in the drawer with the silica beads.*

12 Seal the outlet port of the Sterivex filter unit with the parafilm.

13 Inject 720 µL ATL buffer into the Sterivex.

14 One more Sterivex +ATL buffer should be prepared for the extraction blank for detecting contamination during DNA extraction.

15 Place the Sterivex in the vortex adapter and do the "bead-beating" for 10 min to promote cell lysis.

16 Get one 50mL tube per sample, and put the Sterivexes inside the 50mL tube.
Incubate the Sterivex at 56°C for 30 min while tilting or rotating.

17 Repeat steps 15 and 16 one more time.
go to step #15

DIGESTION WITH PRO-K AND INCUBATION

18 Add 80 µL proteinase-K to each Sterivex.

19 Seal the outlet port of the Sterivex filter unit with a parafilm if you notice that some lysate is leaking.

20 Vortex tubes for ~10 s (or simply move it to mix the solution).
Place the Sterivexes back to the 50mL.
Incubate tubes at 56°C for 2h while rotating.

COLLECTING THE LYSATE

21 While warming the Sterivex filter unit to 56°C, prepare a 2.0 mL tube for DNA recovery (loBind DNA tubes). **Note:** write the necessary information on the cap of the 2.0 mL tube.

22 After completion of warming, carefully remove the parafilm and the luer fitting on the inlet port of the Sterivex filter unit, while preventing liquid inside from leaking.

23 Insert the inlet port of the Sterivex filter unit into the 2.0 mL tube and lightly push it down to the bottom of the 50mL conical tube. Then, close the cap of the conical tube firmly.

24 Put the conical tubes in the Megafuge adapters.
Centrifuge the conical tube containing the Sterivex filter unit at 6,000 x g for 1min and collect the extracted DNA in a 2 mL tube.

25 Remove the 50 mL conical tube from the centrifuge and remove the Sterivex filter unit and 2.0 mL tube using tweezers.
Note: The 2.0 mL tube is uncapped; handle it carefully. Wipe down the tweezers with PREempt wipes between samples.

26 Discard the used conical tubes (*keep one conical tube and use it as a provisory waste container)* and Sterivex filter unit.
Firmly cap the 2.0 mL tube and proceed with these vials and lysate

FINAL INCUBATION AND PRECIPITATION

12m

27 Add 4 µL of RNAseA (100 mg/mL).
Mix pipetting up and down (or vortexing).
Incubate at room temperature for 2 min.

28 Add 200 µL buffer AL.
Mix thoroughly pipetting up and down (or by vortexing). It may form some precipitate.
Incubate at 56°C for 10 min (it doesn't need to be in the rotisserie).

29 Add 200 µL ethanol (96-100%).
Mix thoroughly pipetting up and down (or by vortexing).
Total volume now is ~1.2 mL.

DNA BINIDNG AND WASHING

30 Pipette 600 µL the mixture into a DNeasy mini spin column placed in a 2 mL collection tube.
Place the tubes in the centrifuge and spin the tubes at 8,000 rpm (~6,000 x g) for 1 min.
Discard the flow through.

31 Repeat the previous step once more, using the remaining volume of the lysate.
go to step #30

32 Place the spin column in a new 2 mL collection tube. *If after spinning down you still can see some buffer in the spin column, centrifuge the tube again with a higher speed (about 10,000 x g). If this still not work, spin down with a higher speed.*
Add 500 µL buffer AW1.
Centrifuge for 1 min at 8,000 rpm (~6,000 x g).
Discard the flow through and collection tube.

33 Place the spin column in a new 2 mL collection tube. Add 500 µL buffer AW2.
Centrifuge for 30 s. at 14,000 rpm (~20,000 x g).
Discard the flow through and collection tube.

34 Transfer the spin column to a new 1.5 mL or 2 mL microcentrifuge tube.

35 Elute the DNA by adding 100 µL buffer AE to the centre of the spin column membrane.
Incubate for 1 min. at room temperature.
Centrifuge for 1 min at 8,000 rpm (~6,000 x g).

PCR INHIBITOR REMOVAL

2m

36 **Zymo-Spin™ III-HRC Columns need to be prepared prior to use:**
- Insert column into a Collection Tube. **Please note that* the *matrix in the column may appear dehydrated,*

or powdery. This is normal.
- Open the cap, add 600 µl of Prep-Solution, and centrifuge at 8,000 x g for 3 minutes.

37
- Transfer the prepared column to a clean, labeled 1.5 ml microcentrifuge tube.
- Add the eluted DNA from go to step #35 to the Zymo-Spin™ III-HRC Column.
- Centrifuge at 16,000 x g for 3 minutes.

38 Store the filtered DNA in -80.

Protocol references

This protocol is modified based on the manual of:

QIAgen DNeasy Blood and Tissue Kit, 50 rxn **Qiagen Catalog #**69504

Citations

Colleen Kellogg, rute.carvalho Carvalho, Matt Lemay. DNA Extraction from 0.22µm Sterivex Filters - Qiagen Blood and Tissue Kit
https://protocols.io/view/dna-extraction-from-0-22-m-sterivex-filters-qiagen-der83d9w