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DNA Extraction from Sterivex Filters - Qiagen Blood and Tissue Kit

Forked from DNA EXTRACTION Protocol Template

This protocol is a draft, published without a DOI.

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Protocol status: In development
We are still developing and optimizing this protocol

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Last Modified: May 24, 2024

Protocol Integer ID: 99155

Disclaimer

Draft!

Abstract

Draft!

Guidelines

MIOP: Minimum Information about an Omics Protocol

MIOP Term	Value
analyses	
audience	
broad-scale environmental context	
creator	
environmental medium	
geographic location	
hasVersion	
issued	
language	
license	
local environmental context	
materials required	
maturity level	
methodology category	
personnel required	
project	
publisher	
purpose	
skills required	
target	
time required	

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

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
Content Cell	Content Cell	Content Cell	yyyy-mm-dd
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RELATED PROTOCOLS

PROTOCOL NAME AND LINK	ISSUER / AUTHOR	RELEASE / ACCESS DATE
Content Cell	Content Cell	yyyy-mm-dd
Content Cell	Content Cell	yyyy-mm-dd

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
Content Cell	Content Cell

GLOSSARY

SPECIALISED TERM	DEFINITION
Content Cell	Content Cell
Content Cell	Content Cell

BACKGROUND

This document describes the required protocol to conduct insert name of the method/protocol.

Summary

Insert a short description of the background for the method/protocol (e.g. why and for which purpose do you perform water sampling).
Please provide a brief summary of your method including, as appropriate, a brief description of what techniques your best practice is about, which ocean environments or regions it targets, the primary sensors covered, what type of data/measurements/observing platform it covers, limits to its applicability.

Method description and rationale

Insert a short description of the functioning principal of the methodology used in the protocol (i.e. how does the method work?). Please note that this is different from the step-by-step description of the protocol procedure.
Insert a short statement explaining why the specific methodology used in the protocol has been selected (e.g. it is highly reproducible, highly accurate, procedures are easy to execute etc.…).

Spatial coverage and environment(s) of relevance

If applicable, please specify the region where the protocol is applied. For regional term guidance see here. If applicable, please indicate here the environment(s) of relevance for the protocol, e.g. Abyssal plain. Select from the ENVO terminology.

Personnel Required
Insert the number of technicians, data managers, and scientists required for the good execution of the procedure

Safety

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

Training requirements

Specify technical training required for the good execution of the procedure.

Time needed to execute the procedure

Specify how much time is necessary to execute the procedure.

Materials

	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 qua
	Durable equipment			
	Content Cell	Content Cell	Content Cell	Content Cell
	Content Cell	Content Cell	Content Cell	Content Cell
	Consumable equipment			
	Content Cell	Content Cell	Content Cell	Content Cell
	Content Cell	Content Cell	Content Cell	Content Cell
	Chemicals			
	Content Cell	Content Cell	Content Cell	Content Cell
	Content Cell	Content Cell	Content Cell	Content Cell

Protocol materials

QIAgen DNeasy Blood and Tissue Kit, 50 rxn **Qiagen Catalog #**69504 Step 1

Before start

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

PREPARATION - must be done in Clean Room 1 or Clean Room 2

1

Note

We are aiming to do about 20 samples per day, but you can aim for less than that until you get comfortable with the protocol. # of samples that can fit in the Clean room 1 centrifuge – Megafuge = 16 (50 mL canonical tubes), Froggabio microcentrifuge = 12 (1.5 mL eppendorf tubes). # of samples that can fit in the Clean room 2 centrifuge – 16 x 50 mL conical tubes, 30 x 2 mL tubes.

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

2 **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.

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

4 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.

5 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 left over. *Prepare the aliquots inside the workstation (with HEPA filter).*

6 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

7 Thaw the Sterivex filters.

8 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.*

9 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.

10 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.*

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

12 Inject 720 µL ATL buffer into the Sterivex.

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

14 Place the Sterivex in the vortex adapter and do the “bead-beating” for 00:10:00 to promote cells lysis.

15 Get one 50mL tube per sample, and put the Sterivexes inside the 50mL tube.
Incubate the Sterivex at 56 °C for 00:30:00 using the rotisserie.

16 Repeat the steps 8 and 9 once again.

DIGESTION WITH PRO-K AND INCUBATION

17 Add 80 µL proteinase-K to each Sterivex.

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

19 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 02:00:00 using the rotisserie.
2h

COLLECTING THE LYSATE

20 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.

21 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.

22 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.

23 Put the conical tubes in the Megafuge adapters.
Centrifuge the conical tube containing the Sterivex filter unit at 6,000 x g for 00:01:00 and collect the extracted DNA in a 2 mL tube.

24 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.

25 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

26 Add 4 µL of RNAse A ([M] 100 mg/mL).
Mix pipetting up and down (or vortexing).
Incubate at Room temperature for 00:02:00 .
2m

27 Add 200 µL buffer AL.
Mix thoroughly pipetting up and down (or by vortexing). It may form some precipitate.
Incubate at 56 °C for 00:10:00 (it doesn’t need to be in the rotisserie).
10m

28 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

29 Pipette 600 µL the mixture into a DNeasy mini spin column placed in a 2 mL collection tube. 1m
Place the tubes in the centrifuge and spin the tubes at 8,000 rpm (~6,000 x g) for 00:01:00 .
Discard the flow through.

30 Repeat the step 22 once again using the remaining volume of the mixture from step 21.

31 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.* 1m
Add 500 µL buffer AW1.
Centrifuge for 00:01:00 at 8,000 rpm (~6,000 x g).
Discard the flow through and collection tube.

32 Place the spin column in a new 2 mL collection tube. Add 500 µL buffer AW2. 3m
Centrifuge for 00:03:00 at 14,000 rpm (~20,000 x g).
Discard the flow through and collection tube.

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

DNA ELUTION

34 Elute the DNA by adding 200 µL buffer AE to the center of the spin column membrane. 2m
Incubate for 00:01:00 at Room temperature .
Centrifuge for 00:01:00 at 8,000 rpm (~6,000 x g).

35 OPTIONAL: repeat the step 27 for increased DNA yield.

36

Note

1. Place the tubes in the appropriate boxes (glass door freezer). Each project has its own boxes labeled.
2. Update the metadata spreadsheet by adding date of DNA extraction, responsible for the extraction, comments, etc.

Protocol references

Insert all references cited in the document.

Please insert full DOI address when available, e.g. http://doi.dx.org/10.1007/s11258-014-0404-1