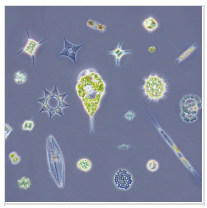

Apr 28, 2022

Metagenomic extraction of high molecular weight plankton from filters

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dx.doi.org/10.17504/protocols.io.5jyl89by8v2w/v1

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Filtration and extraction protocol of plankton metagenomic samples to obtain high molecular weight DNA for sequencing on Minion nanopore.

The protocol describes the following steps:
- filtration of the water on filters of different porosity
- Recovery and grinding of organisms
- HMW extraction
- Results

DOI

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Benoît Vacherie, Karine Labadie 2022. Metagenomic extraction of high molecular weight plankton from filters. **protocols.io** https://dx.doi.org/10.17504/protocols.io.5jyl89by8v2w/v1

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Use only wide-bore pipette tips

Reagent :

DNeasy PowerSoil Pro Kit (250) **Qiagen Catalog #Cat No./ID: 47014** Step 7

Liquid nitrogen **Contributed by users** Step 4

NucleoProtect RNA **Macherey-nagel Catalog #740400.500** Step 4

1X PBS (Phosphate-buffered saline) **Contributed by users** Step 5

Nylon Net filter 20 µm **Merck Millipore Catalog #NY2004700** Step 3

isopore membrane filter 3 µm **Merck Millipore Catalog #TSTP04700** Step 3

Isopore membrane filter 0.2 µm **Merck Millipore Catalog #GTTP04700** Step 3

Consumables :

2 mL Eppendorf **Contributed by users**

1.5ml Eppendorf DNA LoBind tubes **Contributed by users**

ART Wide-Bore tips 200µl **Contributed by users Catalog #2069GPK**

ART Wide-Bore tips 1000 µl **Contributed by users Catalog #2079GPK**

Equipment:

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Perilstatic Pump

Spectra Field Pro	SFP-100

Horizontal Microtube Holder for 24 Tubes	97060 -178

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Filtration 1h

1 From several liters of water (fresh or sea).
Cascade filtration on filters of different porosity with the help of a perilstatic pump.

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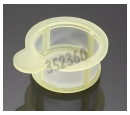
Perilstatic Pump

Spectra Field Pro SFP-100

2 Pre-filtration through a 100µm celular sieve to remove large debris and larger zooplanktonic organisms (not of interest in our case).

celular sieve falcon 100 µm

celular sieve falcon 100 µm 352360



3 Successive filtration on 3 filters of different porosities: 20 µm; 3 µm; 0.2 µm.

☒ Nylon Net filter 20 µm **Merck Millipore Catalog #NY2004700**

☒ isopore membrane filter 3 µm **Merck Millipore Catalog #TSTP04700**

☒ Isopore membrane filter 0.2 µm **Merck Millipore Catalog #GTTP04700**

4 Filters are transferred into 5 ml tubes and flash frozen in liquid nitrogen.
The tubes are stored at -80°C before extraction.

☒ Liquid nitrogen **Contributed by users**

Filters can be directly resuspended in nucléoprotect RNA and stored at 4°C.

☒ NucleoProtect RNA **Macherey-nagel Catalog #740400.500**

Recovery and grinding of organisms 1h

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5 Add 3 ml of 1X PBS to the tube with the filter.
Vortex at maximum power to loosen the organisms from the membrane.
Remove the filter with sterile forceps.
1X PBS (Phosphate-buffered saline) **Contributed by users**

6 5m
Centrifuge the tube to pellet the organisms.
5000 x g, Room temperature, 00:05:00
Gently remove the supernatant with a pipette.

6.1 If the filters have been stored in Nucleoprotect, make a second wash in 500 µl of 1X PBS (same centrifugation)

7 Resuspend the pellet with 800 µl of CD1 buffer (PowerSoil kit).
DNeasy PowerSoil Pro Kit
(250) **Qiagen Catalog #Cat No./ID: 47014**

8 Incubate 10 min at 65 °C with 300 rpm agitation.
300 rpm, 65°C, 00:10:00

9 3m
Transfer the suspension to a powerbeads tube (PowerSoil kit).
Vortex at maximum speed for 3 x 1 min (with 1 min pause between each grinding cycle) using the horizontal tube adapter.
00:03:00

Horizontal Microtube Holder for 24 Tubes	97060 -178

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10 Transfer the grinding material by dipping the tip at the bottom of the tube into a 2ml tube.

Powersoil extraction 30m

11 Add 200 µl of CD2 solution (stored at 4°C) to the grinding and vortex 5 sec. 1m
Centrifuge 1 min / 14 000 G / RT
14000 x g, Room temperature, 00:01:00

12 Transfer the supernatant (avoiding touching the pellet) to a 2ml tube.
Add 600 µl of CD3 solution and vortex 5 sec.

13 Load 700 µl of the lysate on a *MB spin column* (PowerSoil kit) 1m
Centrifuge 1 min / 14 000 G
14000 x g, Room temperature, 00:01:00

14 Discard the filtrate and repeat step 13 until the lysate is consumed

15 Place the column on a new collection tube and add 500 µl of EA Solution 1m
Centrifuge 1 min / 14,000 G
14000 x g, Room temperature, 00:01:00

16 Discard the filtrate and add 500 µl of Solution C5 1m
Centrifuge 1 min / 14,000 G
14000 x g, Room temperature, 00:01:00

17 Place the column on a new collection tube 2m
Centrifuge for 2 min / 14 000 G
14000 x g, Room temperature, 00:02:00

18 Place the column on a 1.5 ml eppendorf tube 6m
Add 25 µl of Solution C6 to the center of the column
Incubate 5 min at RT and Centrifuge 1 min / 14 000 G
00:05:00 **Room temperature**

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14000 x g, Room temperature, 00:01:00

19 Add 25 µl of Solution C6 to the center of the column
Incubate 5 min at RT and Centrifuge 1 min / 14,000 G
Remove the column.

6m

00:05:00 **Room temperature**

14000 x g, Room temperature, 00:01:00

20 Make the following quality controls:
- Qbit (concentration)
- Nanodrop (purity)
- Tapestation or Femto-pulse (molecule size estimation)

Store the tube at -20°C

Results

21

	size fraction µm	Qbit [c] ng/µl	Nanodrop A260/A280	Tapestation (kb)	MinION Yield (Gb)	N50 run MinION (kb)
1	20-100	33,3	1,87	17		
2	20-100	16,9	1,86	15		
3	3-20	97,9	1,87	52	14,6	12,3
4	3-20	65	1,86	20		
5	0,2-3	41,4	1,81	22		
6	0,2-3	10,4	1,66	17		

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