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Cryogrinding protocol : mecanic lysis of planktonic filter for RNA/DNA extraction

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Cell Lysis can be difficult to conduct for envirronmental water samples collected on big filters, specially when big water volumes (20-100L) have been filtered on polycarbanate 142mm-diameter filters.
Here we developped a mecanic lyis protocol in liquid nitrogen conditions to get preserved DNA and RNA.
This protocol was developped for combined RNA and DNA extraction from environmental water samples for Biomarks (2009-2013) and TARA OCEANS projects (2009-ongoing).

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protocol

Alberti A., Poulain J., Engelen S., Labadie K., Sarah Romac S., [...], Wincker P. Viral to metazoan marine plankton nucleotide sequences from the TaraOceans expedition. Scientific Data (2017). dx.doi.org/10.17504/protocols.io.qv6dw9e

eDNA, plankton filter lysis, RNA-DNA extraction

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In steps of
RNA/DNA extraction from natural samples using NucleoSpin RNAII + RNA/DNA Buffer kits (Macherey Nagel)
RNA/DNA extraction from natural samples using NucleoSpin RNAL + RNA/DNA Buffer kits (Macherey Nagel)

Liquid nitrogen **should be handled in well-ventilated areas.**
Wear a labcoat, nitrile gloves and googles.
Put 2 glove pairs to be more protected from the cold.
Wear cold-specific gloves when you work in contact with liquid nitrogen.
To not degrade RNA, all work must be done with liquid nitrogen.

- FreezerMill 6770 (Spex) with grinding equipment;

0620-103306-FM_Brochure_2018_v4-1.pdf

- Becher 1L
- 2 tweezers;
- Cisor pair;
- Spoon or spatula;
- Falcon50 and rack;
- Teethbrush;
- Crab tweezer;
- Liquid Nitrogen;
- Decontaminants : 1% diluted bleach , milliQ water, RNase away and Ethanol 70%.

To not degrade RNA, all work must be done with liquid nitrogen.

Respect safety conditions when you work using liquid nitrogen.

Liquid Nitrogen.pdf

- Wash all the area and Becher 1L with EtOH 70% and RNase away.
- Put 2 glove pairs to be more protected from the cold.
- Put each material (tweezers, cisors, spoon, Falcon 50, FreezerMill equipment, teethbrush) at least 20min under UV light before and between each cryogrinding.

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FreezerMill Preparation

1 **Fill the Freezer Mill tank** : pour Liquid Ntrogen in the tank until the **"up limit"** graduation.

Always check the volume of liquid nitrogen inside the FreezerMill.

2 Close delicately the lid of the FreezerMill.

3 Check the level using a 10mL pipette (maximum level of liquid ntrogen should correspond to 5 mL on pipette's graduation).

4 Wait for 20-30 min until the Freezer Mill reachs in temperature.

5 Choose the format of the filter for next steps :
Step 5 includes a Step case.
142mm filters Cryogrinding
47mm filters Cryogrinding

FreezerMill Tube Assembly

step case

142mm filters Cryogrinding

6 Pour a half the 1 L Becher with liquid nitrogen.

Dive the sample tube containing the 142mm filter in the Becher to keep it deep-frozeen.

Always check the volume of liquid nitrogen inside the Becher to avoid degradation of sample.

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7 Assemble the bases for 2 FreezerMill cryogrinding tubes :

8 Put the 2 half-assembled tubes in the Becher .

9 Take the sample tube with the tweezers, open the tube and take the filter with the tweezers.

If the filter resists, delicately break the tube using the crab tweezer keeping holding the tube with the tweezer.

10 Take away the filter with the tweezer and cut the half of the filter using the cizors and put it in a Falcon50. Put this Falcon in the liquid Nitrogen.

Cut the other half-part filter again in twice and put these 2 pieces in one of the 2 frozen cryogrinding tube.

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11 Finish the assembly of the cryogrinding tube : insert the magnet inside the tube and close the tube with the other extremity containing the hole.

12 Put the tube in the FreezerMill chamber, and check the liquid nitrogen level and close the FreezerMill.

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13 Put a sterile Falcon50 correctly annotated (Sample code) in the Becher containing liquid Nitrogen for cooling.

This Falcon will be used to collect the final powder from the cryogrinded filter.

FreezerMill Run Conditions 3m

14 **Set up the FreezerMill cryogrinding conditions :**

Precool: **00:02:00**

Run time: **00:01:00**

Cycles number: **1**
Rate: **5 knocks per sec**. (respect roughly this condition!).

15 Start the run.

Powder Collection 5m

16 When the run is "complete", open delicately the FreezerMill and take the cryogrinding tube using the FreezerMill extractor.

Avoid to touch the cryoginding tube with the cryogenic gloves.

Preferably use some Kimwipe papers.

17 Put the cryogrinding tube in the Becher containing liquid nitrogen.

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18 Open delicately the cryogrinding tube using the FreezerMill extractor, and remove the magnet using tweezers.

19 Transfer the powder obtained in the Falcon50 tube by directly inversing the the cryogrinding tube inside the Falcon50.

Afterthat, use the spatula to collect a maximum of powder.

Recover most of material you can using the spatula.

20 Replace the Falcon50 in the liquid nitrogen in the Becher and process to the cryogrinding of the second part of the 142mm filter.

For that, take the Falcon50 containing the half-part left of the 142mm filter and **repeat steps 9 to 18**.

21 At the end of the 2 cryogrinding runs, store the Falcon50 tube containing the powder at -80°C until RNA/DNA extraction process.

Washing and Cleaning 30m

22 Disassemble the cryogrinding tubes.

Wash thoroughly all the parts of the cryogrinding tubes, and the FreezerMill extractor using a teethbrush to remove powder residues following this order :
- 1% diluted bleach ;
- tap water ;
- milliQ water ;
- EtOH 70% .

Decontaminate the bench, and Becher using RNase away for next sample cryogrinding.

Put all the small equipment cited above (including the teethbrush) on an alumin paper and incubate them into UV-light at least 20min. (in a PCR hood or a cross-linker for instance, https://www.dutscher.com/frontoffice/product?produitId=0O-78-39)

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