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RNA extraction from Sterivex using phenol:chloroform - cutting off the filter from the Sterivex unit for low biomass samples

Forked from RNA extraction from Sterivex using phenol:chloroform

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1 Works for me dx.doi.org/10.17504/protocols.io.bh75j9q6

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ABSTRACT

This extraction protocol uses a modified filter extraction method and bead-beating technique. This verison uses a technique to attempt to increase the biomass recovered from low-biomass samples by cutting out the filter from the sterivex unit with a sterile razor blade or scalpel. The filter is then placed directly into the bead bashing lysis tube and is homogenized with the starting solution. The protocol has been tested and used on oligotrophic seawater samples that contain a low amount of biomass.

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GUIDELINES

All steps should be performed in a chemical fume hood.

MATERIALS TEXT

- Sterivex containing sample
- Zymo ZR BashingBead Lysis Tubes (S6012-50)
- Mini-bead beater/vortex of choice
- Forceps (2)
- Sharp scalpel or razor blade
- Pliers
- Clean/sterile surface, such as a petri dish or sterile tray
- RNase AWAY (or similar RNase/DNA decontaminating solution)
- 70% ethanol and flame for sterilization
- Refrigerated microcentrifuge
- 2 mL eppendorphs (nuclease free)
- 20% SDS
- 0.5M EDTA
- 3M sodium acetate
- Nuclease free water
- Acid phenol:chloroform:IAA (125:24:1; pH 4.8)
- Pure chloroform
- 100% ethanol (ice cold)
- 70% ethanol (ice cold)
- Heating block at 37 °C

SAFETY WARNINGS

Please review MSDS for all materials. Carcinogenic and corossive materials are used. Practice safe handling techniques when using a sharp razor blade or scalpel.

BEFORE STARTING

Set centrifuge to 4° C and allow to pre-cool.
Bring acid phenol:chloroform out of the -20°C freezer and allow to thaw in chemical hood.

RNA extraction

1 Make solution A: 750 µL of 20% SDS, 600 µL of 0.5M EDTA, 200 µL of 3M sodium acetate, 28.45 mL of nuclease free water

1.1 Make fresh weekly.

2 Sterilize surface with 70% ethanol. Flame forceps and razor blade/scalpel with ethanol. Clean pliers with 70% ethanol and flame the metal portion. Follow up with an RNase decontaminating spray (*e.g.,* RNase AWAY) on everything.

2.1 Holding the outward end of the Sterivex unit (****important****), carefully squeeze the plastic with the pliers all the wayaround the filter. Make sure to do this nearest to this end of the filter. The sterivex should pop out of the casing.

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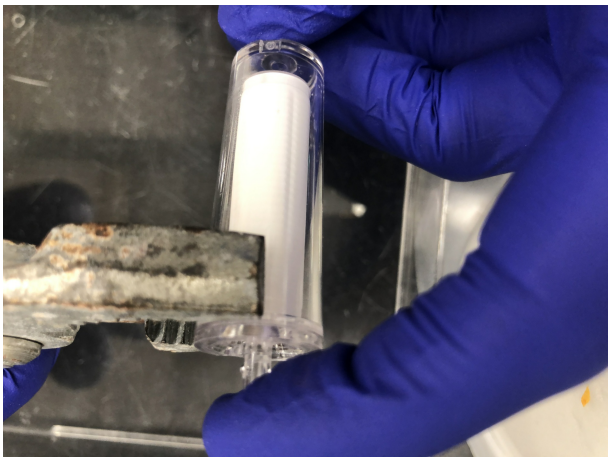
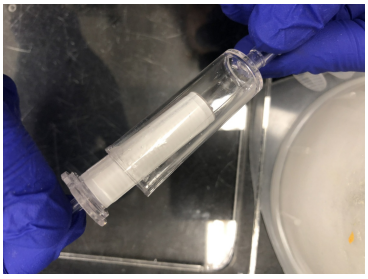
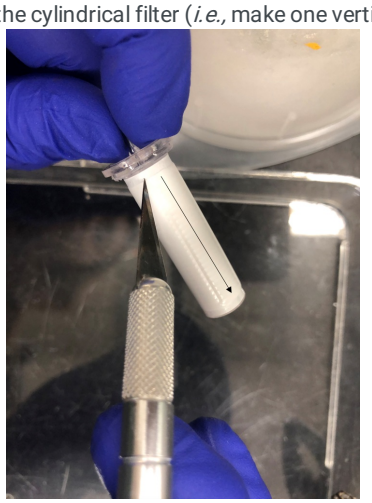




2.2 With the sterile razor blade/scalepel, cut two vertical lines down the filter, one cut on opposite sides of the cylindrical filter (*i.e.,* make one vertical cut, then turn the filter 180 degrees and make another cut).



2.3 Cut around the top and bottom of the cylindrical filter.

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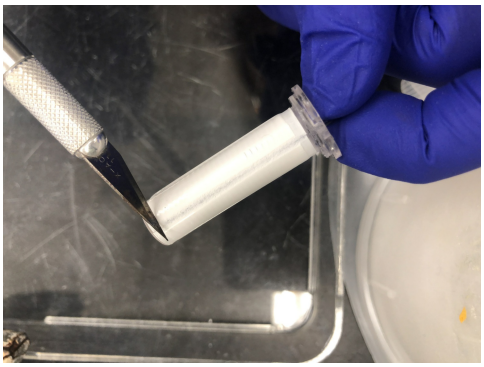
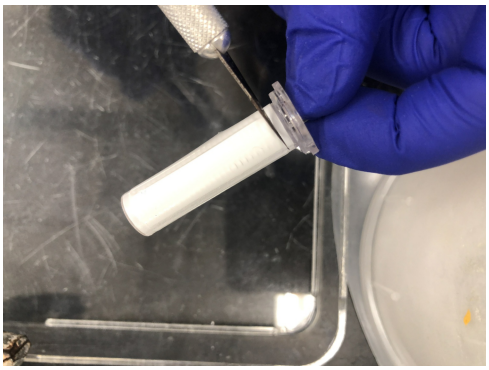
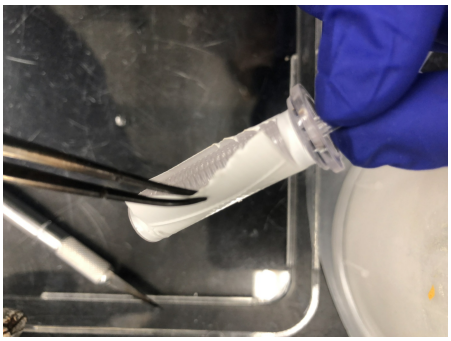
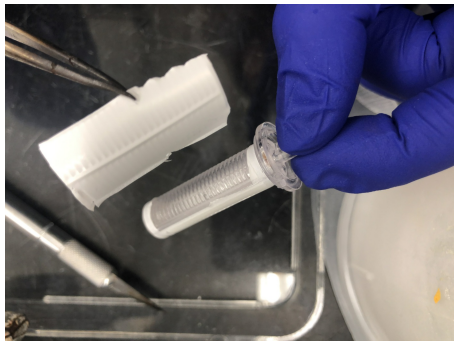




2.4 This should allow you to peel the filter off in two pieces. Using sterile forceps, peel the filter off the plastic and fold it up enough to fit nto the bead beating tube of choice (recommended tube: Zymo ZR BashingBead Lysis Tubes (S6012-50))





3 Add 700 µL of solution A to Bashing bead lysis tube.

4 Vortex Bashing bead lysis tube using vortex of choice. It is recommended to optimize the time and intensity of bead beating on practice samples. 20m

5 Return Bashing bead lysis tube to ice.

Breifly centrifuge the tubes to settle the liquid. Add 500 µL acid phenol:chloroform:IAA (125:24:1; pH 4.8) to the

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6 BashingBead tube.

6.1 You should be pulling from the bottom layer of the thawed phenol:chloroform mixture.

7 Beat on maximum speed for 40 s in bead beater and place on ice briefly to chill. 40s

8 Spin at maximum speed in a 4 °C benchtop centrifuge for 5 minutes. 5m

8.1 13,300 rpm for our centrifuge.

9 Pull off as much of the aqueous supernatant as possible from the beating tube and place in a new 2 mL centrifuge tube.

9.1 If there is a large white protein layer between your top and bottom layer, you may want to repeat the 500 µL acid phenol:chloroform:IAA addition, vortex, centrifuge, and add supernatant to new tube. You risk losing some RNA, but you can increase the quality. 5m

10 Add 500 µL chloroform to aqueous layer, vortex well, spin at max speed for 5 min. 5m

11 Repeat chloroform extraction to remove residual phenol. 5m

Ethanol Precipitation

12 Add sodium acetate to ~0.3 M and mix.

12.1 For 500 µL recovery, add 50 µL of 3M NaAc.

13 Add 2-2.5x volumes of ice cold 100% ethanol and mix well.

13.1 Typically fill the rest of the 1.5 mL microcentrifuge tube with ethanol.

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14 Place in -80 °C freezer for at least 1 hr. 1h

14.1 Can be stored at -80 °C overnight.

RNA pellet wash 45m

15 Spin at maximum speed at 0 °C for at least 30 min in refrigerated centrifuge. 30m

15.1 If you suspect low yields, spin for 1 hr at max.This seems to help in RNA (or DNA) recovery.

16 Carefully decant 100% ethanol without disturbing RNA (or DNA) pellet.

16.1 You may not be able to see your pellet so be careful to avoid where it would be located.

17 Wash pellet by adding 1 mL of ice cold 70% ethanol and gently pipetting up and down enough to dislodge pellet.

17.1 If you can see a pellet, you have at least ~2-5 µg nucleic acid. Do not panic if you cannot see pellet.

18 Spin at maximum speed at 0 °C for 15 minutes. 15m

18.1 Spin for 30 minutes if you expect low yields.

19 Gently and carefully aspirate as much of the 70% ethanol from the tube as possible taking caution not to accidently aspirate the pellet OR use a small pipette tip to suck out as much ethanol as you can.

20 Place the open tube in a 37 °C heating block for about 3-5 min to evaporate all ethanol. 5m

20.1 Try to place heating block in sterile environemnt to avoid falling contamination, given that the tubes need to remain open during this step. It may take longer if you did not remove all excess ethanol in previous step.

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21 Dissolve the RNA (or DNA) pellet in the appropriate amount of RNAse/DNase-free water depending on expected yields and needed final concentration.

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