RNA Extraction from Filtered Vent/Crustal Fluids or Seawater Version 2

Julie Huber,Caroline Fortunato

Abstract

This is a RNA extraction protocol adapted from Byron Crump's lab, which was developed from both Zhou et al. 1996 and Crump et al. 2003. It is intended to be used with 47 mm 0.22 um PES filters or a 0.22 uM Sterivex filter, both preserved with RNALater after sample collection.

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Protocol

Step 1.
This protocol is adapted from mirVana RNA extraction kit (Ambion). Assumes a 47mm flat filter preserved in RNAlater in 50ml tube OR Sterivex filter. Wipe bench top, pipets, racks, with Nucleo-clean, 70% ethanol, and RNAzap wipes.If only extracting half the 47mm flat filter save the remaining half in the 50ml tube with half the RNAlater. If using half of a Sterivex, store remaining filter half in 2ml tube with fresh, filter-sterilized RNAlater.

Step 2.
Prepare 15ml tube, add 750ul Lysis Buffer and 0.5ml of RNA Powersoil beads (1/2 of tube).

Step 3.
Thaw 50 ml tubes with filter on ice.

Step 4.
Pipette RNAlater into a new 2ml tube, spin down cells at 12,000xg for 5min, pour off RNAlater, place on ice.- Might need to do a few rounds depending on amount of RNAlater in tubes.- If only extracting half of filter, only remove half of RNAlater volume .

Step 5.
Remove filter from tube and place on sterile/autoclaved piece of foil.

Step 6.
Using ethanol-flamed razor blade and forceps, cut filter in half (if only extracting half). Then cut remaining half into small pieces and place in 15ml tube. If extracting from a Sterivex, use pliers (ethanol-flamed) to crack cartridge, remove filter with razor blade, and cut into 6-8 pieces. Pour RNAlater from cartridge into 2ml tube and spin down as described in Step 3.

Step 7.
Add 250ul Lysis buffer to 2ml tube with spun down cells, resuspend by vortexting and add this to 15ml tube.

Step 8.
Using adaptor, vortex 15ml tube for 7-10min, at medium-high speed.

Step 9.
Add 100ul Homogenate additive and place on ice for 10min.

Step 10.
Centrifuge tube at 4000xg for 2min at 4ºC.10. Remove lysate and place in 2ml tube. Repeat centrifugation if necessary to remove all lysate.

Step 11.
Add 1 part acid:phenol chloroform to tube (equal to amount of lysate removed) and centrifuge at 10,000xg for 5min. Follow procedure for using phenol, use fume hood, double glove, etc.

Step 12.
Remove top aqueous layer and place in fresh 2ml tube. Be sure not to touch the bottom layer!

Step 13.
Heat the elution solution to 95ºC in heat block (use a small aliquot in a 1.5mL tube).

Step 14.
Add 1.25x 100% ethanol. Mix well, and add to filter cartridge in a collection tube provided. Centrifuge at 10,000xg for 15sec, discard the flow through. Filter cartridge can only hold 700ul, so might need to do a few spins.

Step 15.
Add 700ul Wash Solution 1, spin at 10,000xg for 15sec, discard flow through.

Step 16.
Add 500ul Wash Solution 2/3, spin at 10,000xg for 15sec, discard flow through.

Step 17.
Repeat Step 16

Step 18.
Put the filter cartridge back into the tube, centrifuge for 1.5 min at 10,000xg to remove residual fluid from the filter.

Step 19.
Transfer the filter cartridge into a fresh collection tube, apply 50 µl pre-heated (95 ºC) Elution solution to the center of the filter, spin for 30 sec at 10,000 X g; repeat with another 50 µl pre-heated (95ºC) Elution Solution20. Freeze at -80ºC long term. Short term (24-48hrs), RNA can be stored at -20ºC.

Step 20.
Carry out DNase Treatment: adapted from Ambion Turbo-DNAase kit. Set heat block to 37ºC.

Step 21.
Transfer 100ul of sample from mirVana collection tube to 1.5 ml centrifuge tube.

Step 22.
Add 10ul 10x Buffer to 100ul RNA.

Step 23.
Add 1ul Turbo DNAse, incubate @ 37ºC for 20min.

Step 24.
Add another 1ul of Turbo DNase, incubate for 20min more.

Step 25.
Add 10ul Inactivation Reagent and vortex on and off for 5 min.

Step 26.
Spin tubes @ 10,000xg for 1.5min.

Step 27.

Pipet RNA into new 0.5ml tube, be sure NOT to touch inactivation reagent.