RNA Extraction Protocol from RNA-SIP Experiments

Julie Huber

Abstract

This is the protocol to extract RNA from Sterivex filters from RNA-SIP experiments carried out with seawater, vent fluids, etc.

Citation: Julie Huber RNA Extraction Protocol from RNA-SIP Experiments. **protocols.io**
dx.doi.org/10.17504/protocols.io.iumceu6
Published: 06 Jul 2017

Protocol

Step 1.

Wipe bench top, pipets, racks, with Nucleo-clean, 70% ethanol, and RNAzap wipes.

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

Step 3.
Thaw Sterivex on ice.

Step 4.
Using sterile pliers or pipe cutters (both ethanol-flamed) crack Sterivex cartridge.

Step 5.
Pipette or pour off RNAlater from cartridge into a new 2ml tube, spin down cells at 12,000xg for 5min, pour off RNAlater, place tube on ice.

Step 6.
Cut filter off of cartridge using razor blade (autoclaved, ethanol-flamed), and place on sterile/autoclaved piece of foil.

Step 7.
Using sterile, ethanol-flamed razor blade and forceps cut filter into 6-10 pieces and place into prepared 15ml tube.

Step 8.
Add 250ul Lysis buffer to 2ml tube with spun down cells, resuspend by vortexting, add this to 15ml tube.

Step 9.
Using adaptor, vortex 15ml tube for 7-10min, at medium-high speed.

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

Step 11.
Centrifuge tube at 4000 x g for 2min at 4ºC.10. Remove lysate and place in 2ml tube. Repeat centrifugation if necessary to remove all lysate.

Step 12.
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, respirator etc.

Step 13.
Remove top aqueous layer and place in fresh 2ml tube. Be sure not to touch the bottom layer!

Step 14.
Heat the elution solution to 95ºC in heat block (use a small aliquot in a 1.5mL tube).

Step 15.
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 16.
Add 700ul Wash Solution 1, spin at 10,000xg for 15sec, discard flow through.

Step 17.
Add 500ul Wash Solution 2/3, spin at 10,000xg for 15sec, discard flow through.

Step 18.
Repeat Step 16.

Step 19.
Put the filter cartridge back into the tube, centrifuge for 1.5 min at 10,000xg to remove residual fluid from the filter.

Step 20.
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 Solution.20. Freeze at -80ºC long term. Short term (24-48hrs), RNA can be stored at -20ºC.

Step 21.
DNase Treatment: adapted from Ambion Turbo-DNAase kit. Set heat block to 37ºC. 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.

Step 28.
Freeze at -80ºC long term. Short term (24-48hrs), RNA can be stored at -20ºC.