

RNA extraction, small filters

Darach Miller

Abstract

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Protocol

Step 1.

Collect 5 to 15 e7 cells on a filter. Put filter immediately in a 1.5ml eppendorf, taking care to keep it neat and with the cells smoothly exposed to the center, ie not all wadded up where it's hard to get cells off the filter.

Flash freeze in liquid N2. Store at -80C.

Step 2.

PREPARE 1x TES (10mM Tris 7.5, 10mM EDTA, 0.5% SDS), get acid phenol (check color, make sure it's clear)

PROTOCOL

. TES

CONTACT: [Darach Miller](#)

Step 2.1.

TES

10mM Tris pH 7.5

10mM EDTA

0.5% SDS

water to 50ml

Step 3.

Take samples a few at a time out of the -80C, and quickly add 400ul TES and then 400ul acid phenol to the filter. Immediately vortex vigorously, then let sit on bench until you've got all your samples started to extract.

Step 4.

Put all samples at 65C on a heatblock, let incubate for 60min. Vortex occasionally. Every 20min seems to work.

Step 5.

PREPARE pre-spin the PLG tubes, get chloroform, glycogen, sodium acetate, and etOH reagents ready.

Step 6.

At end of incubation, vortex again. Remove the filter using a pipette tip (medium sized works) to waste. Put samples on ice for 5min.

Step 7.

Spin samples 5min max speed, RT.

Step 8.

Aspirate aqueous layer to a new tube. Add 400ul acid phenol to new tube, and vortex well. Spin 5min max speed RT.

Step 9.

Aspirate aqueous layer to a new tube. Add 400ul chloroform to new tube, and vortex well. Spin 5min max speed RT. Be very careful not to get any phenol across in this step.

Step 10.

Aspirate aqueous layer to a pre-spun PLG. Add 400ul chloroform and invert. Spin 5min 16000rcf RT.

Step 11.

Aspirate aqueous layer to a new tube. Add 10% volume of 3M Na Acetate, and > 10ug of glycogen for carrier (if need be). Add 2 - 2.5 volumes of 100% etOH.

Step 12.

Incubate this mix on ice for 30min to an hour. Longer is better, 30min is enough. Going below freezing is apparently counter productive, so avoid using -80C except for the initial chill.

Step 13.

Spin samples at max speed in the 4C centrifuge for 15min.

Step 14.

Check for pellets, and aspirate the supernatant. Add 500ul 80% etOH, invert a few times to mix gently. Spin 2min max speed RT.

Step 15.

Aspirate all of the etOH. Quickspin on bench, and aspirate ALL of the etOH.

Step 16.

Let dry for 10min on the bench.

Step 17.

Resuspend in 50ul nuclease-free H₂O. Run sample on nanodrop to check for a 260/280 > 1.7 but preferably 2.2. Check for a 260/230 > 2, preferably 2.4 or 2.5. If 260/230 is high, you should repeat a phenol chloroform and precipitation to get rid of the phenol, being more careful this time.