Yeast RNA Extraction

Hybrid of the DeRisi protocol (www.microarrays.org), a standard acid-phenol prep circulating around the Brown/Botstein labs circa 2001, a protocol from Cold Spring Harbor, and steps developed by Benjy Neymotin and other members of the Gresham lab 2010-2011.

Harvesting Cells

In most circumstances, vacuum filtration is the best way to filter cells for RNA preps. Spinning down cells may induce a stress response.

Collection Supplies:

- Vacuum flask, w/ vacuum source
- Rigid tube connecting liquid pump to vacuum
- Filter support, collection funnel, clamp ("glass microanalysis filter holder assembly", small)
- Filters (use appropriate size), tweezers for moving filters
- Liquid nitrogen
- 2ml eppendorf and/or 15ml Falcon tube for each sample

**Do not harvest samples into our standard 1.5ml eppendorf tubes**, as the volume of liquid within the tube will be at capacity, some liquid will leak out, and the phenol smell will penetrate the tubes into the laboratory, and that is **really bad**. Use 2ml eppendorf for small prep and 15ml eppendorf for large prep.

Collection Procedure:

1. Assemble vacuum flask with filter support, turn on vacuum source.
2. Place filter on filter support, attach funnel, and clamp. Check filter and setup using DI water, before each sample collection.
3. Pour sample into funnel and allow to drain through filter.
4. Once drained, remove the clamp and funnel, and use tweezers to remove filter.
5. Roll up filter without touching cells, and put in tube. Drop tube into liquid nitrogen.
6. Rinse funnel, and repeat for other samples.

If doing a time series, try to harvest cells as fast as possible. Switching filters takes time, but with practice, it becomes manageable. Depending on the number of samples and size of prep, it may be worth asking someone to help with the harvest.

RNA Extraction

The protocols for the large and small preps are similar. Use RNase free reagents and glass-/plastic-ware throughout! If using glassware, make sure it was baked overnight. Remember to use glass pipets with chloroform for large extractions, otherwise the pipets will melt and spill chloroform all over.

**Lysis buffer for RNA (100 ml)**

- 0.5 M EDTA 2ml
- 10% SDS: 5ml
- 1 M Tris pH 7.5: 1ml
- RNase-free water: 92ml

EDTA needs to be around pH 8.5 for all of the solute to dissolve at the required concentration. Use 10M NaOH to adjust the pH, and not the standard 5M NaOH. Add EDTA in steps, first putting some EDTA and then NaOH. Each time NaOH is added, wait for most of the EDTA in the container to dissolve before adding more EDTA.

Do not vortex to get SDS into solution. This leads to many bubbles and SDS becomes difficult to use.

**Supplies/Reagents for RNA extraction:**

- Lysis buffer
- Acid phenol (Acid phenol may be labeled “saturated phenol” in the 4C fridge. Make sure it has an acidic pH.)
- Heavy phase lock gel (PLG) tubes
- Pipets and tips (which ones depend on small or large prep)
- Eppendorfs or Falcon tubes (depends on small or large prep)
- Ice
- Chloroform
- Sodium Acetate 3M
- Ethanol 95% (Use the ethanol in the red container in fume hood)
- Ethanol ~70% (Dilute 95% ethanol 35ml in 15ml RNase free water)
• RNase free water

**Small RNA prep (for samples of 2-10 ml)**

Any steps involving phenol or chloroform MUST be done in the fume hood.

Set waterbath or heatblock to 65°C. Bring vortex into fume hood.

1. Remove sample filters from the -80°C. They should be in 2 ml eppendorf tubes.
2. Before they thaw, add 750 ul lysis buffer. Vortex vigorously until solution is foamy and opaque.
3. Add 750 ul acid phenol. Vortex vigorously until foamy and opaque.
4. Incubate 1 hour @ 65°C, vortex vigorously at 20, 40, and 60 minutes.
5. Fish out the filter and discard. (Use a clean pipet tip for each sample.)
6. Ice 10 min. While incubating, spin the 2 ml heavy phase lock gel (PLG) tubes for 30 sec full speed.
7. Spin sample lysate 5 min, 5kRPM at RT.
8. With a pipet, transfer the top aqueous layer to the PLG tube.
9. Add 750 ul chloroform. Invert to mix. Do not vortex!
10. Spin 5 min at 13k rpm.
11. Pour or pipette aqueous layer to new 2ml tubes.
   a. If you're concerned about small amounts of RNA, you can use glycogen to help precipitate it. See here for more info.
12. Add 75 ul (or 1/10 volume if you lost some) 3 M sodium acetate. Vortex.
13. Add 1.2 ml 100% ethanol. Vortex.
14. Incubate -80C for 30 min (this is a good time to get lunch), or if you plan to finish the prep tomorrow, incubate samples in the -20C overnight.
15. Spin 5k rpm 10 min in cold room, decant supernatant.
16. Wash pellet with 350ul of 70% ethanol. Vigorous pipetting is not needed or recommended, just make sure the surface of the pellet is exposed to EtOH.
17. Spin 2 min 5k rpm. Discard supernatant.
18. Wash and spin one more time. Discard supernatant.
19. Air dry inverted on the bench 30 min. Do not leave too long past 30 min as the RNA can degrade.
20. Dissolve pellet in 25 ul water (or more or less if necessary) by frequent up and down pipetting.

Check the concentration of RNA on the nanodrop. Depending on how you plan to use the RNA, run the RNA through the RNeasy and Bioanalyzer. Dilutions may be required, depending on the final concentration of RNA.

**Large RNA prep (for 10 ml cultures or more)**

Any steps involving phenol or chloroform MUST be done in the fume hood. Ensure there is enough water in water bath and set to 65°C. Bring vortex into fume hood.

1. Remove samples from the -80C. They should be in 15 ml Falcon tubes.
2. Before they thaw, add 4 ml lysis buffer. Vortex, trying to get all the cells off the membrane.
3. Add 4 ml acid phenol. Vortex until opaque and foamy.
4. Incubate 1 hour 65C, vortexing at minutes 20, 40 and 60.
5. Fish out the filter using tweezers and discard the filter.
6. Ice 10 min. (unlike the small prep, we do not use phase lock gel tubes for large preps)
7. Label new 15ml Falcon tubes.
8. Spin lysate 10 min 3000 rpm. With a pipet, transfer the top aqueous layer to a new falcon tube.
   a. If supernatant is very cloudy, let warm to RT and see if it clears up. Most PP 15ml tubes can be spun at 4000rpm, check the documentation to see what the max is.
9. Add 4 ml chloroform. Invert to mix. Do not vortex!
10. Spin 5 min 3000 rpm in the large centrifuge.
11. Add another 4 ml chloroform to the same tube, invert to mix, and spin again for 5 min 3000 rpm.
12. Label new 15ml Falcon tubes.
13. Pipette aqueous layer into new 15 ml Falcon tube. Careful not to mix polystyrene pipettes with the chloroform layer.
14. Add 400 ul (or 1/10 volume if you lost some) 3 M sodium acetate, and glycogen if you expect less than 15ug RNA. Mix.
15. Add 8 ml (or 2 volumes) ethanol. Mix.
16. Incubate -80C for 30 min (this is a good time to get lunch), or if you plan to finish the prep tomorrow, incubate at -20C overnight.
17. Spin 3000 rpm 10 min.
18. Wash pellet with 2-3ml of 70% EtOH. Vigorous pipetting is not needed or recommended, just make sure all sides of the surface of the pellet are exposed to EtOH.
20. Wash and spin one more time. Discard supernatant.
21. Air dry inverted on the bench 30 min. Do not leave too long past 30 min as the RNA can degrade.
22. Dissolve pellet in ~250 ul water (or more or less if necessary). The amount of water used in this step depends on the volume and cell density of the original culture you used.

Check the concentration of RNA on the nanodrop. Depending on how you plan to use the RNA, run the RNA through the RNeasy and Bioanalyzer. Dilutions may be required, depending on the final concentration of RNA.