## RNA prep (12/20/10)

*The protocol for harvesting, preparing, and labeling RNA was taken from Maitreya Dunham's website*

Hybrid of the DeRisi protocol (www.microarrays.org) and a standard acid-phenol prep circulating around the Brown/Botstein labs circa 2001

Use RNase free reagents and glass-/plastic-ware throughout! Remember to use glass pipets with chloroform.

Lysis buffer (100 ml)

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

Remove a manageable set of samples from the -80. They should be in 2 ml eppendorf tubes.
Before they thaw, add 750 µl lysis buffer. Vortex, trying to get all the cells off the membrane.
Add 750 µl acid phenol. Vortex.
Incubate 1 hour 65C, vortexing every 20 minutes.
Fish out the filter and discard.
Ice 10 min.
While they are incubating, spin the 2 ml heavy phase lock gel tubes (made by Eppendorf, sold lots of places) for 30 sec full speed in a room temperature microcentrifuge. Set aside.
Spin lysate 5 min.
With a pipet, transfer the top aqueous layer to the PLG tube.
Add 750 µl chloroform. Invert to mix. Do not vortex!
Spin 5 min.
Pour aqueous layer into a new 15 ml falcon tube.
Add 75 µl (or 1/10 volume if you lost some) 3 M sodium acetate. Mix.
Add 1.5 ml (or 2 volumes) ethanol. Mix.
Incubate -20C >30 min (preferably overnight).
Spin 3000 rpm 10 min.
Wash pellet 2X with 70% ethanol, with 2 min 3000 rpm spins between washes.
Air dry inverted on the bench 30 min.
Dissolve pellet in 100 µl water.
Measure concentration of crude RNA with nanodrop.
Clean up the RNA prep with QIAGEN RNeasy kit. Elute in 30 µl water.
Measure concentration of clean RNA with nanodrop.
Optional: Check the quality of the RNA on the Bioanalyzer (the Bioanalyzer requires 2 µl of 50 – 500 ng/µl).