RNA prep

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

The protocols for the large and small preps are more or less the same with minor volume and centrifuge differences. The phase lock gel makes everything so much easier, but if you aren't using it, add in 1 or 2 extra chloroform extractions to clean things up.

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

Lysis buffer for RNA
(100 ml)

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

RNA prep for 5 ml daily samples

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 (PLG) tubes 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 25 µl water. You can speed up the dissolution by heating the sample or by pipetting the pellet up and down, but I prefer a gentler room-temperature-with-frequent-flicking approach.
Measure the concentration with the spectrophotometer. You should get about 50-60 ug, just enough for an array. If using the nanodrop, use the RNA undiluted.
Check the quality of the RNA on the Bioanalyzer or a gel. You will probably have to dilute it ~1/10 to run on the Bioanalyzer.

RNA prep for 100 ml harvest

Remove a manageable set of samples from the –80. They should be in 15 ml Falcon tubes.
Before they thaw, add 4 ml lysis buffer. Vortex, trying to get all the cells off the membrane.
Add 4 ml acid phenol. Vortex.
Incubate 1 hour 65C, vortexing every 20 minutes.
Fish out the filter and discard if you wish. I generally don't for the larger samples, but the filter can sometimes interfere with the phase separation.
Ice 10 min.
While they are incubating, spin the 15 ml heavy phase lock gel (PLG) tubes for 2 min 1500xg in a room temperature swinging bucket centrifuge. Set aside.
Spin lysate 10 min 3000 rpm.
With a pipet, transfer the top aqueous layer to the PLG tube.
Add 4 ml chloroform. Invert to mix. Do not vortex!
Spin 5 min 1500xg.
Add another 4 ml chloroform to the same tube, invert to mix, and spin again.
Pour aqueous layer into a new 15 ml Falcon tube.
Add 400 µl (or 1/10 volume if you lost some) 3 M sodium acetate. Mix.
Add 8 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 ~250 µl water, adding more if necessary. You can speed up the dissolution by heating the sample or by pipetting the pellet up and down, but I prefer a gentler room-temperature-with-frequent-flicking approach.
Measure the concentration with the spectrophotometer. You should get several mg. If using the nanodrop, you will almost certainly have to dilute the RNA. 1/2 is a good guess, or dilute 1/20 and use the same sample for the Bioanalyzer. You could also run a gel to check the quality.