Harvesting for RNA

Filtering the cells is the quickest way to collect them for later RNA processing. I don't recommend spinning cells because of the chance you'll get a stress response or a temperature shock.

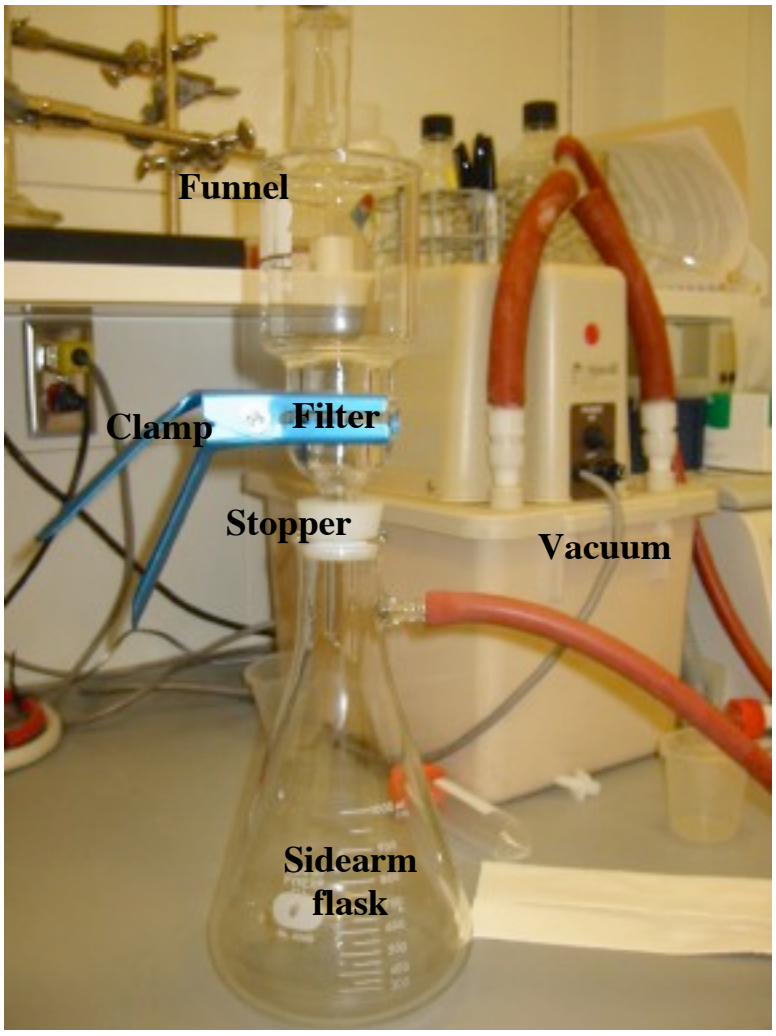
The following protocol is intended for ~10^8–10^{10} cells. If the filtering seems slow, harvest less culture or divide the cells over multiple filters. If you're harvesting less than ~10^8 cells, you should use 25 mm filters in 2 ml tubes with a smaller filter support (09-753G, Fisher).

Plug the filter support (K953805-0000, Fisher) in a sidearm flask hooked to vacuum. Center a 0.45 micron nylon filter (R04SP04700, GE Osmonics) on the filter support. Place the funnel on top and carefully clamp it all together. Start the vacuum. Listen for any whistling noises that may indicate a leak in the seal. If you do get whistling, make sure that the filter is centered and free of wrinkles. Some fraction of the filters have cracks or holes that will interfere with the harvest. Replace the filter with a new one if this seems to be the case.

Remove the top from a labeled 15 ml Falcon tube. Put the tube in a bucket of liquid nitrogen.

Once everything is ready, remove the culture from the shaker. Turn on the vacuum. Pour desired amount of culture into the funnel of the filter apparatus. Watch to make sure it is filtering properly and that no cells are making it into the flask. You can refilter if you have this problem, but try to avoid it.

Once the culture has completely filtered through, remove the clamp and then the funnel. Pull the vacuum hose off the sidearm. This order is important to keep cells from sticking to the funnel. Use forceps or a spatula to lift the edge of the filter, avoiding the cells in the center. Carefully roll up the filter. Dump out the liquid nitrogen in the 15 ml tube, pop the rolled up filter inside, loosely cap it, and dunk it back into the liquid nitrogen. Store the tubes at –80.



Large filter apparatus.

RNA prep

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

Use the small prep if you've used 25 mm filters and the large prep for 47 mm filters.

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

Lysis buffer

(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

Small RNA prep

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 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 or nanodrop.
Check the quality of the RNA on the Bioanalyzer or a gel.

Large RNA prep

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.
Ice 10 min.
While they are incubating, spin the 15 ml heavy phase lock gel tubes (made by Eppendorf, sold lots of places) 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 or nanodrop.
Check the quality of the RNA on the Bioanalyzer or a gel.