Harvesting cells for RNA (12/5/07)

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

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.