Total Yeast RNA Prep

- Grow ~ 100 ml cells to OD600 ~1.0. Heat shock or treat if desired. Streak cells on plates to confirm relevant phenotypes at the time of harvest.

- Pellet cells 6K X 5 min in GSA rotor; quick freeze in liquid nitrogen (or dry ice/ethanol) and store pellets at –80 C or go to next step.

- Resuspend cells in 4 ml lysis buffer (10mM Tris-Cl pH 7.5, 10 mM EDTA, 0.5 % SDS)

- Transfer cell suspension to sterile 15 ml conical tube; add 4 ml acid phenol (water saturated, pH 4.3; Fisher BP1751I-400), parafilm around cap and vortex well.

- Incubate at 65 C for one hour with occasional vortexing.

- Place tubes on ice for 10 min, spin in clinical centrifuge at 4 C for 10 min.

- Remove aqueous layer, transfer to new sterile 15 ml conical tube, re-extract with phenol (room temp, no incubation).

- Extract aqueous phase once with chloroform. Transfer aqueous phase to chloroform washed, autoclaved 15 ml COREX tubes.

- Add 0.4 ml 3 M sodium acetate and 8 ml 200 proof ethanol. Incubate at –20 C for ~30 mins. Spin 8K X 20 min in SS34 rotor (with adaptors) at 4 C.

- Wash RNA pellets 3X with 8 ml 70% ethanol (store ethanol at –20 C).

- Dry RNA pellets briefly in speed-vac (need to remove speed vac rotor, parafilm tube tops and then pierce parafilm a few times with needle) Leaving tubes at room temperature for a few hours also works.

- Resuspend RNA pellets in 500 μl autoclaved water. Transfer RNA to sterile screw cap Eppendorf tubes.

- Quantitate RNA by adding 5 μl RNA to 1 ml sterile water in chloroform washed quartz cuvette. Use Ultrospec RNA program to obtain concentration (multiple by 200) and calculate 260/280 ratio (ratio should be 1.9-2.1)

- Store RNA at -80C.