Total RNA extraction using Fast Prep

Wellinger Lab

1. Grow 10 mL of yeast cells to OD = 0.8
2. Pellet cells in 15 mL tubes and wash with H_2O; transfer to 1.5 mL tubes
3. Resuspend in 300 µl 1X LETS buffer (keep everything on ice from now on)
4. Add ~300 µl glassbeads (or up to 2 mm below the meniscus)
5. Seal the tubes with parafilm
6. Put in Fast Prep for 45 seconds, 4.0 m/sec
7. Place on ice for 2 minutes
8. Redo Fast Prep for another 45 seconds
9. Remove the tubes. In order to remove the glassbeads, pierce a small hole with a **flamed** 23G1 needle (blue). To avoid the liquid to go out of the hole, uncap the tube to release the pressure before piercing. Place the pierced tube on top of another 1.5 mL tube and reclose the cap.
10. Spin at 2000 rpm for 1 minute (the Eppendorf centrifuge works best). Leave one space between each tube (easier to remove them from the centrifuge). You should now have the glassbeads on top and the extract on the bottom.
11. To the 300 µl of extract, add 200 µl of 1X LETS buffer.
12. Under the hood, extract 2X with phenol-chloroform-isoamyl (25:24:1). Each time, keep upper, aqueous phase and transfer to new tubes.
13. Extract 1X with 500 µl chloroform-isoamyl (24:1), vortex and spin.
14. To the upper layer, add 30 µl 3M NaOAc pH 5.2 and 1 ml cold ethanol 100%. Vortex; place at -80°C for 30 minutes.
15. Spin 15 minutes at 4°C. Remove supernatant.
16. Wash pellets with 1 mL of 70% ethanol.
17. Dry pellets and resuspend in nuclease-free H_2O.
18. Quantify RNA using your favorite method.

2X LETS Buffer (50 mL):

20 mM Tris-Cl pH 7.5	1 mL of 1M
200 mM LiCl	2 mL of 5M
20 mM EDTA pH 8.0	2 mL of 0.5M
0.4% SDS	1 mL of 20%

- Complete to 50 mL with RNAse-free H_2O