RNA extraction from tough cells

Applied on extracting RNA from stationary phase cells.

1. Pellet cells in a 1.5ml lo-bind tube and resuspended in
   a. 270ul of sodium acetate buffer (50mM sodium acetate, PH-5.2) and 10mM EDTA, PH-8.0 and
   b. 30ul of 10% SDS and
   c. 0.3 to 0.4 g of glass beads (0.5~0.6mM diameter)
   d. 1ul RnaseOUT.

2. (optional) For spike in experiments, add expected spike-ins (ul) if you want to normalize at cell level

3. Add 300ul of acid phenol (preheat) to each sample

4. Vortex at RT for 1 min at full speed and then incubate the tubes @ 65 degree for 5 mins. This was repeated for a total of six vortex mixings over an approximately 36mins in total.

5. Check the cell lysis under the light microscope to make sure all cells are broken down. If not, repeat step 4 for couple more times

6. Cool on ice for 10 min.

7. While incubating of cell lysis, spin the 2 ml heavy phase lock gel (PLG) tubes for 30 sec full speed (RT).

8. Add 300ul of chloroform/isoamyl alcohol (24:1), mixed by vortexing for 1 min, and spin for 5 min at 17,000g @ 4 degree

9. With a pipet, transfer the top aqueous layer to the PLG tube.

10. Add 300ul chloroform. Invert to mix. Do not vortex!

11. Spin 5 min at 13k rpm.

12. Pipette the aqueous layer to new 1.5ml lo-bind tubes.

13. Add 30ul (or 1/10 volume if you lost some) 3 M sodium acetate (PH~5.2). Vortex

14. Add 10ug glycojen (optional), then add 800ul 100% ethanol (final conc. of ethanol=70%). Vortex.

15. Incubate -80C for 30 min (this is a good time to get lunch), or if you plan to finish the prep tomorrow, incubate samples in the -20C overnight.

16. Spin @ Max speed 10 min in cold room, decant supernatant.

17. Wash pellet with 150ul of 70% ethanol. Vigorous pipetting is not needed or recommended, just make sure the surface of the pellet is exposed to EtOH.

18. Spin 2 min @ Max speed. Discard supernatant.

19. Wash and spin one more time. Discard supernatant.

20. Air dry inverted on the bench 30 min. Do not leave too long past 30 min as the RNA can degrade.

21. Dissolve pellet in 25 ul water (or more or less if necessary) by frequent up and down pipetting.

Check the concentration of RNA on the nanodrop. Depending on how you plan to use the RNA, run the RNA through the RNAeasy and Bioanalyzer. Dilutions may be required, depending on the final concentration of RNA.