RNA Isolation from Chlamydomonas reinhardtii

Stock Solutions (Use RNA free chemicals and baked glassware!)

1. H_2O saturated Phenol
    - Add Milli-Q water to a new bottle of phenol
2. 24:1 Chloroform : Isoamyl alcohol
    - Add 20 ml Isoamyl alcohol to a new bottle (500 ml) chloroform

Procedure

1. Before beginning, make sure one water bath is at 65°C
2. Place tubes with frozen cells/lysis buffer in water bath for approximately 3 minutes to thaw.
3. Add 2ml phenol and shake vigorously for 1 minute.
4. Add 2 ml Isoamyl/chloroform and shake vigorously for 1 minute.
5. Centrifuge @ 10,000 rpm for 10 minutes at 10C (JA 20 or JA 17) (Use rubber adapters).
6. Collect upper, aqueous phase
    a. Do not remove any interface material and it is okay to leave some aquoues phase behind
7. Repeat steps 3 - 6. (Total of 4 Phenol/ CHCl3 extractions). Do not worry if phase is pink.
8. Extract twice with CHCl3/Isoamyl Alcohol (you can reduce centrifuge time to 5 minutes each).
9. Remove upper, aqueous phase and precipitate with 2.5 volumes of cold 100% ethanol overnight at -20ºC. (Use extra pure ethanol in the freezer in 5069)
10. Collect pellet by centrifugation at 10,000 rpm for 30 minutes (JA 20, JA 17) at 4ºC our off supernatant – be careful not to lose pellet.
11. Wash pellet in 70% cold ethanol, do not resuspend pellet. Spin down again.
12. Remove supernatant.
13. Spin down again and remove any remaining liquid.
14. Allow the pellet to dry on bench or in hood.
15. Once dry, add 100 – 200 ul milli-Q water and leave on ice for 30-45 minutes.
16. At this point, pellet should be resuspended.
17. Transfer to an o-ring sealed tube.
18. Measure OD260 and OD280 of a 1:99 dilution. (or use nanodrop)
19. mg/mL = OD260 X 2.0 (1 OD260 = 40 µg/mL RNA)
20. Adjust concentration to 1 ug/ul
    a. You can remove an aliquot now for RNA gel (8ul), Northern (8ul) or DNase treatment (40ul)
21. Label and store @ -80ºC (RNA has been quite stable >= 1 year).