Candida RNA isolation

1. Centrifuge cells from a 5 ml culture, decant, and resuspend pellet in 400 ul AE buffer.
2. Add 40 ul 10% SDS.
3. Add equal volume acid phenol (440 ul) warmed in heat block to 65˚C.
4. Incubate at 65˚C for 4-6 minutes, vortexing every 30 seconds.
5. Centrifuge at top speed for 2 minutes at room temperature.
6. Transfer aqueous phase to new 1.5 ml tube.
7. Repeat steps 3 through 6.
8. To aqueous phase, add equal volume phenol:chloroform:isoamyl alcohol, vortex (what ratio of phenol:chloform:isoamyl?).
9. Centrifuge at top speed for 5 minutes.
10. Transfer aqueous phase to new tube, and add 1/10 volume 3 M NaOAc, pH 5.2, and 2.5 volumes 100% EtOH (-20˚C).
11. Store at -20˚C for 15 minutes to precipitate.
12. Centrifuge at top speed for 5 minutes, carefully remove supernatant.
13. Resuspend pellet in 400 ul DEPC-treated H2O.
14. Add 40 ul 3 M NaOAc, pH 5.2, 1 ml 100% EtOH (-20˚C), and place at -20˚C for 15 minutes.
15. Centrifuge at top speed for 5 minutes, carefully remove supernatant.
16. Wash pellet with 500 ul 80% EtOH, place at -20˚C for 15 minutes.
17. Centrifuge at full speed for 3 minutes, carefully remove as much supernatant as possible.
18. Air dry pellet, 10-15 minutes or as needed.
19. Resuspend in 20 ul H20.
20. Store at -80˚C.

AE Buffer:
50 mM NaOAc, pH 5.3
10 mM EDTA

Acid Phenol: Ambion Cat. No. 9720

Phenol:CHCl3:IAA: Ambion Cat. No. 9730 (do NOT raise pH with provided buffer)