# Isolating RNA with RNA-Bee

1. Spin down up to 20 million cells at 1000 rpm for 5'
2. Pour off supernatant
3. Resuspend cell pellet with ~1 ml PBS and transfer to eppendorf tube
4. Spin eppendorf tubes in microfuge 1 minute at 3000 rpm
5. Pipet or aspirate supernatant from tubes
6. Resuspend cell pellet in 800 ul RNA-Bee. Put tubes on ice
7. Add 200 ul chloroform. Invert 2-3x and vortex 5-10 sec. Return to ice
8. Hold on ice for 10' inverting occasionally
9. Spin in microfuge 10-15 minutes at maximum speed at 4 degrees C
10. Meanwhile label fresh RNAse-free tubes and add 600 ul Isopropanol
11. Remove tubes from microfuge and place at room temp. Transfer clear supernatant to tubes with isopropanol. DO NOT carry over white interface.
12. Vortex tubes and either ice or freeze briefly (good if worried about yields) or spin immediately for 10-15 minutes at maximum speed at 4 degrees C
13. Decant supernatant and add 500 ul ice cold 70% ethanol
14. Spin in microfuge 2-3 minutes at maximum at 4 degrees C.
15. Decant supernatant (careful not to lose pellet!) and add 500 ul ice cold 70% ethanol.

16. Spin as in step 14

17. Decant as in step 15. Add 200 ul ice cold 70% ethanol.

18. Spin as in step 14

19. Remove and discard supernatant with pipet. Remove as much liquid as possible.

20. Speed-vac for 2-5 minutes

21. Resuspend in 12 ul H20.

22. Spec and adjust concentration to 0.5 mg/ml

OK to stop and store samples in –80 freezer after steps 6, 11 and 21