RNA extraction protocol
9.10.2013

1. Thaw samples on ice or at 4C. Frozen tissues need not be totally thawed.
2. For each sample, label a 2 ml tube and add 1 clean BB and 1 ml Trizol or 750 µl Trizol-LS (for liquid samples). Be careful because Trizol is caustic.
3. Add samples to Trizol:
    a. For solid tissue samples, use a scalpel to cut a small chunk (<100 mg) of tissue. Dice it up some and add to tube. A t/c 6-well plate or 10 cm dish works well.
    b. For liquid samples, add 250 µl sample to the Trizol-LS.
4. Shake tubes to immerse tissue in Trizol and mix. Incubate at RT ≥ 5 min.
5. Shake the tubes in Tissue Lyzer for 1-3 min at 30 hz.
6. In fume hood, pulse down tubes then add 200 µl chloroform. Shake by hand 15 seconds then incubate ≥ 2 min.
7. Spin samples in cold room c'fuge 10 min 12,000g
8. During spin, label a 1.5 ml tube for each sample and add 900 µl of 1:1 Zymo RNA binding buffer and 100% ethanol (i.e. 450µl each).
9. Also prepare a zymo RNA CC-5 spin column for each sample (label and in collection tube).
10. After spin, in fume hood, transfer 450 µl aqueous phase to these new tubes w/ 900 µl. Mix and pulse down.
    a. The organic phase/interphase can be used for DNA extraction if so desired.
11. Return to T/C hood. Transfer RNA CC-5 column to vacuum manifold. In two steps, vacuum aqueous phase mixture through columns.
12. Wash each column w/ 400 µl Zymo RNA wash buffer on manifold.
13. Transfer columns to collection tubes. Prepare DNaseI master mix for N samples. Master mix should contain (for 1 sample):
    a. 24 µl RNA wash buffer (not H_2O!)
    b. 3 µl 10x DNaseI buffer (NEB)
    c. 3 µl DNaseI (NEB)
14. Add 30 µl master mix to each column, making sure it gets in column matrix.
15. Incubate 15-30 min at RT.
16. Spin columns (in collection tubes) 30 seconds 9000g. Leave flow-through.
17. Add 400 µl RNA prep buffer to each column. Spin 30 sec 9000g again.
18. Transfer columns back to vacuum manifold and discard DNase-containing collection tubes. Try to avoid contaminating things w/ DNase.
19. Wash columns on vacuum w/ 800 µl then 400 µl RNA wash buffer.
20. Transfer columns to new collection tubes.
21. Spin 1 minute at max speed to dry.
22. Label 1.5 ml tubes for each sample.
23. Transfer columns to labeled tubes. Elute RNA in 20-60µl nuclease-free H2O.
24. Store RNA on ice or at -80C.
25. Clean vacuum manifold by rinsing thoroughly inside and outside w/ distilled H_2O.