RNA Isolation
3/14/06

RNase Away everything

1. Thaw samples (in Trizol) at room temperature.
2. Label 2 sets of Falcon Tubes and 1 set of eppy's per sample.
3. Solutions: chloroform, isopropanol, 75% EtOH, and DEPC treated water.
4. Add 0.2 ml chloroform per 1 ml Trizol.
5. Cap tubes and shake for 15 seconds.
6. Centrifuge samples at 12,000 x g for 15 min at 2-8C.
7. Transfer aqueous phase to fresh tube (~1/2 original vol.)(save other phases for DNA and protein purification),
8. Precipitate RNA from aqueous phase by adding 0.5 ml isopropanol for every 1 ml Trizol (original vol.).
9. Vortex briefly and incubate at room temperature for 10 min.
10. Centrifuge at 12,000 x g for 10 min at 2-8C (Pellets at side and bottom of tube).
11. Pour off supernatant and wash RNA once with 75% EtOH at 1 ml per 1 ml Trizol (original vol).
12. Mix by vortexing and centrifuge at 7,500 x g for 5 min at 2-8C.
13. Pour off supernatant and allow RNA to air dry upside down in test tube rack.
14. Resuspend pellet in DEPC (~100ul) depending on size.
15. Pipette into eppie.
16. Incubate at 55-60C for 10 min.
17. Store at –80C.