Reverse Transcription
4/27/06

1. Measure 1 ug for all RNA samples. Bring up to 8.5 ul with DEPC H2O.
2. Make a master mix of 0.5 ul of DNAase (1 u/ul) and 1 ul of 10x DNase Reaction buffer per sample.
3. Add 1.5 ul of Master mix for every sample.
4. Let samples incubate at room temperature for 15 minutes.
5. Add 1 ul of 25 mM EDTA to each sample.
6. Incubate samples at 65C for 10 minutes (PCR Machine).
7. Store at –80C or continue to next step.
8. Calculate master mix of 1 ul of Oligo (DT) and 1 ul dNTP mix per sample.
9. Add 2 ul of Master Mix to each sample.
10. Run program OligoDT (70C for 10 min and hold at 4C).
11. Store at –80C or continue to next step.
12. Calculate Master Mix at 4 ul of 5x First Strand Buffer, 1 ul DTTT, 0.25ul RT enzyme, 2.75 ul Sterile DI water per sample.
13. Add 8 ul of Master mix to each sample.
14. Run in program cDNA (42C for 1 hour, 72C for 5 min, hold at 4C).
15. Dilute samples with 100ul sterile DI water.
16. Store at –80 or –20C.