5/22/08 JD

cDNA Synthesis from total RNA Protocol – total Reaction volume is 20uL. Final concentrations are in parentheses.

1. Thaw on ice: RNA samples, random primers, dNTPs, 5X First Strand Buffer, and 0.1M DTT.
If you are in a hurry, DTT and Buffer can be thawed faster at room temp, but the RNA, dNTPs, and random primers must be thawed on ice.

2. Set Heatblock 1 to 65°C and Heatblock 2 to 25°C

3. Label 2 microfuge tubes for each sample – one for cDNA synthesis and one for a no-RT control.

4. Prepare a 100ng/ul solution of random primers.
Add 2ul of stock solution (3ug/ul) to 58 uL of RNase free water.

5. Briefly spin down primers or dNTPs before using.
If using IEC centrifuge, spin for <4 seconds. Otherwise spin at 3,000-4,000 rpm for 15-30 s.

6. For each sample, add the following to the microfuge tube:
   a. RNase-free water – calculate this to fill to 13uL
   b. RNA – calculate to desired amt.
   c. 2 ul random primers at 100ng/ul (200ng)
   d. 1 ul dNTPs at 10mM each (1mM)

7. Heat to 65°C for 5 min and incubate on ice for at least 1 min. Reset Heatblock to 50°C.

8. Centrifuge tubes very briefly to spin down contents
no more than 4 seconds at 13,000rpm)

9. Add the following reagents to the tube:
If you have 10 or more tubes, you may want to mix the appropriate volume of 5x buffer, DTT, and RNaseOUT and add 6ul of this mix to each tube. Aliquot the Superscript III separately because it will not go into the noRT controls.

   a. 4ul 5x First-Strand Buffer (1X)
   b. 1ul 0.1M DTT (5mM)
   c. 1uL RNaseOUT (40units)
   d. 1ul SuperScript III RT (200 units) – **DO NOT ADD TO NO-RT CONTROLS**

Note: RNase OUT and Superscript III RT are enzymes and should never be at room temp or even for long periods on ice. Remove from freezer immediately before using and put away immediately after using.

10. Incubate tube at 25 °C for 5 min.

11. Remove tubes from heatblock 2 and reset to 70°C. Incubate tubes at 50°C for 30-60 min – This may be increased to 55°C for templates with high secondary structure.

12. Inactivate the reaction by heating at 70°C for 15 minutes