How to Prepare Reverse Transcriptase PCR (RT-PCR) cDNA Synthesis

(Lan Zhou 11/02/00, updated by Yang Bi 02/24/08, commented by TCH)

NOTE:

A) Yang has recently compared the RT enzyme from NEB (**Cat# M0253S**) vs. Invitrogen's Superscript II, and found no significant difference between those enzymes. Thus, the **NEB RT enzyme is the recommended choice** in our lab.

B) After numerous pre-testing runs by former/current lab members, we decide that the RT enzyme in each reaction can be reduced to as low as **0.25ul** (the original suggested volume was 1.25ul).

C) These conditions work well for most regular RT-PCR cDNAs prepared for real-time PCR assays, especially if your RNA is isolated from cultured cells. However, if you are concerned about the yield and quality of the RNA samples (especially from tissues), you may consider using more RT enzyme and/or using Superscript II. If you are not sure, check a couple microliters of your RNA samples on RNA gel.

1. Turn on two heating blocks: one at 70^{o}C and one at 37^{o}C.
2. Make Hexamer mix:

	1 X
Hexamer(random primer,1ug/ul)	4 ul
SSB (0.5ug/ul) (**optional**)	2 ul
RNase-free H2O	3 ul
Total volume	9 ul

3. Make Hexamer mix-RNA:

Hexamer mix	9 ul
Total RNA (10ug) or mRNA (0.5ug)	x ul
RNase-free H2O	xx ul
Total volume	25 ul

4. Incubate @ 70^{o}C for 3-5 minutes.
5. Prepare RT mix:

5 X First strand buffer	10 ul
0.1M DTT	4 ul
10mM dNTPs	2 ul
RNasin	0.4 ul
Total volume	16.4 ul

6. Prepare +/- RT Reactions:

	+RT tube	**-RT tube** (optional)
Hexamer mix-RNA	12.0 ul	12.0 ul
RT mix	7.8 ul	7.8 ul
RT enzyme (NEB)	0.25 ul	0.0ul
RNase-free H2O	0.0 ul	0.25 ul
Total volume	20ul	20ul

7. The cycling program of RT-PCR:

   37^{o}C X 60 minutes
   95^{o}C X 1 minute (for killing RT; RT interferes with Taq)
   4 ^{o}C Hold or kept at -20°C or -80°C freezers

8. Add 80ul ddwater to get 100ul original cDNA, and then take 10ul out, add 40ul ddwater to dilute into 5 times (**total volume = 100ul**), and then use the diluted cDNA for real-time PCR. **It's a good practice to aliquot the initial 100ul into multiple aliquots. Keep the aliquots at -80°C**.

9. For most real-time PCR reactions, the cDNA mix needed to be further diluted 5 to 50 times, depending on the transcript abundance of the gene of your interest.