**Removal of DNA contamination from RNA using DNase I**

(DNase = Ambion cat no. AM2222 or AM2224, RNAseOUT = Invitrogen cat no. 10777-019)

1. Mix together in a 1.5μl tube:

RNA (up to10 ug) 1-43 μl

10 × DNase I Buffer 5 μl

DNase I (Ambion) 1 μl

RNase out 1 μl

Nuclease-free water 0-49 μl

TOTAL 50 μl

2. Incubate at 37º C for 30 minutes.

3. Adjust the volume to 100 μl with nuclease-free water.

4. Perform a cleanup using Qiagen RNeasy kit (follow protocol in Qiagen manual).

5. Check RNA quantity on the Nanodrop and then dry 3 ug in speed vac for RT.

**SuperScript III First-Strand Synthesis System for RT-PCR**

(Invitrogen, cat. no. 18080-044)

Mix-1 (each sample)

RNA (3 ug DNase treated) 8 μl

Oligo (dT)20  (50 µM) 1 μl

dNTPs (10 mM) 1 μl

1. Incubate Mix-1 at 65º C for 5 min.

2. Place tubes on ice *immediately* for at least 1 min.

3. Quick spin to collect contents at bottom of tube.

Mix-2 *(add reagents in the order listed)*

1 rxn

5X FS Buffer 4 μl x \_\_\_\_\_\_ = \_\_\_\_\_\_\_\_

50 mM MgCl2 2 μl x \_\_\_\_\_\_ = \_\_\_\_\_\_\_\_

0.1 M DTT 2 μl x \_\_\_\_\_\_ = \_\_\_\_\_\_\_\_

RNase OUT 1 μl x \_\_\_\_\_\_ = \_\_\_\_\_\_\_\_

SuperScript III 1 μl x \_\_\_\_\_\_ = \_\_\_\_\_\_\_\_

Total: 10 μl

4. Add Mix-2 to Mix-1, mix by pipeting, and incubate at 50º C for 1 hour.

5. Terminate the reaction by incubation at 70º C for 15 min.

6. Add 1 μl of RNase H to remove RNA and incubate at 37 ºC for 20 min.

7 Add 1 ul of 0.5M EDTA to stop the reaction.

8. Use the Qiagen Qiaquick PCR Purification kit to do a cleanup of the sample(s), following the protocol in the Qiagen manual.

9. Measure cDNA on the Nanodrop and dilute to 30 ng/ul of each sample. Use 1-2 ul for PCR.