Reverse Transcription (RT) Protocol with use of SYBR Green

Amount of RNA= 2 ug

Reaction Volume= 20ul

Sample Preparation:

1. Wear cloves and a lab coat. Handle Samples on ice

2. Remove the following reagents from the -20C and place on ice to allow them to thaw:

    - 5X Buffer
    - 0.1M DTT
    - 10mM dNTP
    - Oligo(dT)15 primer
    - M-MLV Reverse Transcriptase
    - RNAsin

3. For each RNA sample, calculate the volume needed for 0.5ug of RNA.

4. Then calculate the amount of DPEC H20 to bring the volume to 11.3 ul.

5. First add the calculated volume of DEPC H2O.

6. Add the appropriate amount of RNA for 0.5 ug of RNA

7. Cap tubes and heat at 70°C for 12 min, then cool on ice for 3

RT Master Mix (MM) preparation:

8. While samples are heating, prepare Reverse Transcription Master Mix (RT MM)

9. Calculate the volume of each RT reagent needed to add to a single tube. Make 10% more MM than is needed for pipetting error. Make sure that the reagents are thawed and mixed well prior to use.

Number of Samples + 10%	ul/Sample	Total required volume:	Reagent
	4		5X Buffer
	2		0.1 M DTT
	1		10mM dNTO
	1		Oligo(dT)15 primer
	0.5		M-MLV Reverse transcriptase
	0.2		RNAsin

10. Shake MM well and add 8.7ul to each RT tube for a final volume of 20ul.

RT cycle conditions:

11. Make sure that the caps are closed.

12. Set cycle conditions for 40°C for 1 hour, 95°C for 10 min.

13. Add 20ul of Biological water free H2O (now volume is 40 ul)

14. When completed, store RT vials in -20C.

Using SYBR Green

1. Thaw the frozen 2x iQ SYBR Green supermix (Orange cap), and the primers on ice.

2. Gently mix each tube to ensure thorough re-suspension of components before use.

3. Take 1.0ul from each of the stored cDNA and put in to a new, labeled tube

4. Briefly spin in centrifuge to collect samples at the bottom of the tubes.

5. Add each component to each reaction tube (alternatively, make up entire batch of supermix+H20 and an entire batch of combined primers, and add to each sample). Make up an extra 10% in case of error.

# Samples +10%	Reagent	Amount/Sample	Total Volume
	RNA-free Water	3.6 ul	
	SYBR Green Super Mix	5 ul	
	cDNA	1.0 ul	
	Forward Primer	0.2 ul	
	Reverse Primer	0.2 ul	

6. Quickly vortex each tube and centrifuge on low speed briefly

7. Load the reaction replicates into PCR tubes or microplates and seal the reaction vessels.

8. Briefly microcentrifuge at low speed to remove any air bubbles.

9. Samples can be stored at 4C or on ice until they are ready to be run in the PCR protocol.

10. Program the thermal-cycler with the recommended real-time PCR protocol with or without a melt curve step (Table 2 and Table 3).

11. Place the sealed reaction vessels in the thermal cycle block and start running PCR protocol. (NOTE: PCR products can be stored at -20C after the run)