

Reverse transcription (RT)

Materials required

RT-PCR kit (Gibco)	Primers (300 nM each)
PCR thermocycler	Microfuge
Microfuge	0.5 ml thin walled PCR tubes

Thermocycler – Caution
 Thermocycler can reach temperatures of 95 °C.

RT set-up

1. To a sterile 500 µl Eppendorf tube add in the following order:

	<u>µl</u>
(1) Total RNA (0.5 µg)	x
(2) dNTPs (10 mM)	1
(3) oligo (dT) ₁₂₋₁₈ (0.5 µg/ml)	1
(4) DEPC treated H ₂ O	to 10

2. Incubate 65 °C for 5 min.

3. Cool slowly to room temperature .

4. Prepare the following in a *separate* tube

	<u>µl</u>
(1) 10 x RT-PCR Buffer	2
(2) MgCl ₂ (25mM)	4
(3) 0.1 M DTT	2
(4) RNase OUT (RNase inhibitor)	1

5. Add this mixture to the RNA/dNTP/oligo mixture from step 1.

6. Incubate at 42 °C for 2 min.

7. Add 1 µl (50 units) SuperscriptII polymerase.

8. Incubate at 42 °C for 50 min.

9. Heat at 70 °C for 15 min (to inactivate SuperscriptII polymerase), then chill on ice.

10. Pulse-spin

11. Add 1µl RNaseH and incubate at 37°C for 20 min.

Polymerase chain reaction (PCR)

Materials required

RT-PCR kit (Gibco)	Primers (300 nM each)
Microfuge	PCR thermocycler
0.5 ml thin walled PCR tubes	

Thermocycler – Caution
 Thermocycler can reach temperatures of 95 °C.

1. To a sterile 0.5 ml thin walled PCR tube add in the following order:

	<u>μl</u>
(1) 25 mM MgCl ₂	3
(2) 10 x RT-PCR Buffer (minus MgCl ₂)	5
(2) RT reaction (i.e. cDNA)	2
(3) 5' or forward primer (300 nM)	1
(4) 3' or reverse primer (300 nM)	1
(5) dNTP mix (final concentration 800 μM)	1
(6) Taq polymerase (1 unit)	0.4
(7) H ₂ O to a final volume of 50μl	36.6

2. Place your sample into a pre-programmed thermal cycler, the running time is approximately 80 min and 30 sec as described below.

PCR Cycle conditions (pre-programmed for this protocol)

First denature:	94°C for 3 min	1 cycle
Then programme amplification cycle:	94°C for 30 sec	
	60°C for 30 sec	45 cycles
	72°C for 30 sec	
End with an extension:	72°C for 5 min	1 cycle
<i>Total running time</i>	<i>80 min and 30 sec</i>	