

Real-time PCR & RT reaction

RT reaction:

1. 65°C for 5 min to open RNA structure

	<u>1X</u>
5X B	5
0.1 M DTT	2.5
10 mM dNTP	5
SSII	1
Random primer	1
RNA (1 ug)	
H ₂ O	
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Total	25

2. RNA+Primer+H₂O heat at 70°C for 10 min then sit on 4°C

3. Run RT reaction

25°C	10 min
42°C	70 min
72°C	10 min
4°C	forever

4. Dilute RT reaction to 20X-100X

Real-time PCR (SYBR Green) reaction (ABI part#: 4309155, Sybr Green Master Mix)

1. Prepare primer for PCR (100 pmole → 1 pmole → 5ul/well → dry)

2X SYBG Master Mix	5
ddH ₂ O	4
cDNA	1
Total	10

2. PCR condition:

95°C	10 min
95°C	15 sec
60°C	1 min

Run step 2 for 39 times

Melting curve

50-95°C read every 0.2°C, hold 3 sec