

RT-PCR First Strand Synthesis

Annealing reaction:

RNA	0.5-1 μ g	DNase treated
Random Hexamer	1 μ L	Invitrogen #48190011
Oligo dT primer	1 μ L	Operon primers 18-mer (0.5 μ g/ μ L)
ddH ₂ O	to 10 μ L	

RT Mix:

5X cDNA synthesis buffer	4 μ L	*supplied with thermoscript
0.1M DTT	1 μ L	*supplied with thermoscript
RNase OUT	1 μ L	Invitrogen #10777019
10mM dNTPs	2 μ L	Applied Biosystems #44162 (core)
Thermoscript	1 μ L	Invitrogen #12236022
ddH ₂ O	1 μ L	
Total	10 μ L	

To anneal primers, first incubate the annealing reaction at 65°C for 5min.

Add 10 μ L of RT Mix to each annealing reaction.

Incubate at 55°C for 1hr.

Inactivate RT at 85°C for 5min.