ABGENT CUSTOM SERVICES:

RT-PCR Protocol

RT reaction

1. Before performing the RT reaction, heat 5 ug of total RNA in a 10uL volume at 65 ℃ for 5 ~ 10 minutes, then quench on ice.
2. Set up the following components in a 1.5 mL Eppendorf tube:

10.0 uL	heat denatured RNA
3.0 uL	10 × PCR buffer
2.5 uL	10 mM dNTPs
6.0 uL	25 mM $MgCl_2$
1.0 uL	random primers (1.8 mg/mL)
0.5 uL	SuperScript II reverse transcriptase
17.0 uL	water

Warning: The use of DEPC-treated water for the RT reaction is unavailable, as DEPC will inhibit the RT and PCR reactions!!

3. Leave the samples at 25 ℃ for 10 minutes then incubate at 42 ℃ for 1 hour.
4. Denature the cDNA at 95 ℃ and place on ice.

PCR reaction

1. Set up the following components in a 0.5mL PCR tube:

6.0 uL	cDNA product
1.5 uL	10 × PCR buffer
0.2 uL	Taq polymerase
0.5 uL	primer 1 (1.0 mg/mL)
0.5 uL	primer 2 (1.0 mg/mL)
10.3 uL	water

2. Perform PCR with 30 cycles. Each cycle includes denaturation (30 seconds at 95 ℃); annealing (45 seconds at 60 ℃); and extension (60 seconds at 72 ℃).

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