Quantitative Real-time PCR

Material

SYBR® Premix Ex Taq™ (Tli RNaseH Plus)　(TaKaRa(Code No. RR420A)

Procedure

1. Prepare the PCR mixture shown below.

Reagent	Volume	Final conc.
SYBR Premix Ex Taq (Tli RNaseH Plus) (2X)	10 μl	1X
PCR Forward Primer (10 μM)	0.4 μl	0.2 μM
PCR Reverse Primer (10 μM)	0.4 μl	0.2 μM
Template (< 100 ng)	2 μl	
dH2O (sterile distilled water)	7.2 μl	
Total	20 μl	

2. Start the reaction using LightCycler 480 System
   1) Denature:
      95℃ 30 sec. (Ramp rate: 4.4℃/sec.)
      1 cycle
   2) PCR :
      95℃ 5 sec. (Ramp rate: 4.4℃/sec.)
      60℃ 30 sec. (Ramp rate: 2.2℃/sec.)
      40 cycles
   3) Melting
      95℃ 5 sec. (Ramp rate: 4.4℃/sec.)
      60℃ 1 min. (Ramp rate: 2.2℃/sec.)
      95℃ (Ramp rate: 0.11℃/sec.)
      1 cycle
   4) Cooling
      50℃ 30 sec. (Ramp rate: 2.2℃/sec.)
      1 cycle

3. After the reaction is complete, check the amplification and melting curves and plot a standard curve if absolute quantification will be performed.