qRT-PCR

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Abstract

qRT- PCR for each gene was carried out using a thermal cycler (Bio-Rad, Hercules, CA, USA) and amplification conditions were 40 cycles of 30s at 95°C, 3 s at 95°C, and 30 s at 60°C.

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Protocol

Step 1.

1. Gene specific primers are retrieved from Primer Premier 6.0 and Beacon designer 7.8 Software□These primers are ordered from the Bioneer (TaKaRa,Japan).

The reagent composition	Volume□20 ul□
SYBR Premix Ex Tap	10.0 ul
Forward Primer (10 uM)	0.4 ul
Reverse Primer (10 uM)	0.4ul
cDNA	2ul
DEPC H_2O	to 20ul
1.95℃□30sec□ 2. 95℃□3sec→60℃□30sec□40 cycles	

Step 2.

After PCR is finished, remove the tubes from the machine. The PCR specificity is examined by 3% agarose gel using 5 ml from each reaction.

Step 3.

Put the tubes back in SDS 7000 and perform dissociation curve analysis with the saved copy of the setup file.

Step 4.

Analyze the real-time PCR result with the SDS 7000 software. Check to see if there is any bimodal dissociation curve or abnormal amplification plot.