# qRT-PCR

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## Abstract

Gene specific primers are retrieved from Primer Premier 6.0 and National Center for Biotechnology Information Software，Fluorescent dye are SYBR® Premix Ex Taq™ II (Tli RNaseH Plus)(TaKaRa,China).

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## Protocol

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**Step 1.**

| The reagent composition | Volume（20 ul） |
|---|---|
| SYBR Premix Ex Tap | 5.0 ul |
| Forward Primer (10 uM) | 0.5 ul |
| Reverse Primer (10 uM) | 0.5 ul |
| cDNA | 1.0 ul |
| dd$H_2O$ | to 10ul |
| 1. 95℃（30sec） 2. 95℃（5sec→51℃-60℃（30sec（40 cycles） 3. 72℃（45sec. | |

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**Step 2.**

After PCR is finished, remove the tubes from the machine. The PCR specificity is examined by 3% agarose gel using 8 ml from each reaction.

**Step 3.**

Put out the tubes from Real time PCR instrument(Bio-Rad，USA)and perform dissociation curve analysis with the saved copy of the setup file.

**Step 4.**

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

**Step 5.**

Exported the results to Excel software, and the expression values are calculated according to the 2-ΔΔCT method.

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**Step 7.**