# Quantitative Real-Time PCR

## Materials

First-strand cDNA
RNase-free water
2× SYBR Green Pro Taq HS Premix (ROX Plus) (Accurate Biology)
Forward primer (10 μM)
Reverse primer (10 μM)

## Procedure

### 1. cDNA Dilution

Prepare a dilution tube by adding 32 μL RNase-free water.
Transfer 8 μL of cDNA into the dilution tube.
Mix thoroughly to obtain a 1:5 diluted cDNA template (final volume: 40 μL).

### 2. qPCR Reaction Setup

Prepare the following 20 μL qPCR reaction mixture for each sample.

| **Reagent** | **Volume** |
|---|---|
| 2× SYBR Green Pro Taq HS Premix (ROX Plus) | 10 μL |
| Diluted cDNA template | 9 μL |
| Forward Primer (10 μM) | 0.4 μL |
| Reverse Primer (10 μM) | 0.4 μL |
| RNase-free water | 0.2 μL |
| Total | 20 μL |

Mix gently by pipetting and load the reactions into a real-time PCR instrument.

### 3. qPCR Cycling Conditions

Run the qPCR using the following two-step amplification program.

| Step | Temperature | Time | Cycles |
|---|---|---|---|
| Initial denaturation | 95°C | 30 sec | 1 |
| Denaturation | 95°C | 5 sec | 40 |
| Annealing/Extension | 60°C | 30 sec | |
| Melt curve analysis | Dissociation stage | — | — |

After amplification, perform melt curve (dissociation) analysis to verify the specificity of the PCR products.