RT-PCR REACTION

Section of Cancer Genomics, Genetics Branch, NCI
National Institutes of Health

Reagents

cDNA (see cDNA Synthesis for RT-PCR Reaction Protocol)
Molecular Biology Grade Water
5µM Forward Primer
5µM Reverse Primer
Power SYBR GREEN PCR Master Mix [2x]
Applied Biosystems, Cat. 4367659

Materials and Equipment

RT-PCR instrument
Applied Biosystems Sequence Detection System 7000 (ABI Prism 7000)
MicroAmp Optical Tubes
Applied Biosystems, Cat. N801-0533

OR

Optical Tubes (8 tubes/strip)
Applied Biosystems, Cat. 4316567

OR

96-well Optical Reaction Plate with Barcode (code 128)
Applied Biosystems, Cat. 4306737
Optical Caps
Applied Biosystems, Cat. 4323032

Procedure

1. Program Sequence Detection System 7000 using Well Inspector.

2. Calculate volume of cDNA for 300 ng (~3 µL) for each sample.

3. Calculate the number of tubes/wells planned for each gene. On ice, make Master master mix for each gene by combining the following reagents. For each tube/well, the volumes required are (includes 15% extra for pipetting error):
   14.3 µl Power SYBR GREEN PCR Master Mix [2x]
   1.44 µl Forward Primer
   1.44 µL Reverse Primer
   8.05 µl water

4. Aliquot 22 µl Master master mix into tubes/wells on ice.

5. Add 300 ng cDNA (~3 µL) to each tube/well as appropriate and pipet up and down to mix.

6. Centrifuge tubes/plate for 1 min at 1200 rpm.

7. Load tubes/plate into Sequence Detection System 7000 and cap each tube/well with Optical Caps.

8. Set appropriate reaction conditions (number of cycles, temperatures, times, etc) and start reaction.