Sugden lab.

Standard Real-time PCR protocol

Products

- AmpliTaq Gold PCR Master Mix (4327059):
Applied Biosystems 2500 units/50 ml

Contains:
AmpliTaq Gold DNA Polymerase, 250 U (0.05 U/ml)
GeneAmp PCR Gold Buffer 30 mM Tris/Hcl, pH 8.05, 100 mM KCl
dNTP, 400 mM each
$MgCl_2$, 5 mM
Stabilizers (??)
This product is aliquotted into Eppendorf tubes and stored at 4C until use. Multiple freeze-thaw cycles are not recommended, but the reagent can be frozen and thawed up to 5 cycles before losing activity.

- Rox Reference Dye (12223-012):
Invitrogen 500 µl

Normalizes non-PCR related fluctuations in fluorescence
Provides a stable baseline for multiplex quantitative PCR and RT-PCR
Uses existing ROX spectral settings eliminating the need to recalibrate the thermal cycler
Is sold separately for use with any quantitative PCR or RT-PCR system

- Primers and Probes obtained from MWG:

http://www.mwg-biotech.com/html/subsidiaries/subsidiary.php?SUBID=3

Probes and primers- order lyophilized 0.05 µM and HPSF purified then resuspended at a concentration of 100 pmol/µl

Real-time reaction mix (final concentrations):

1x	2 x AmpliTaq Gold
0.5 µM	5' primer
0.5 µM	3' primer
0.2 µM	probe
0.4 µl	Rox reference dye
20 µl	Final Volume (including sample and dH20)

Only barrier tips and reagents set aside specifically for real-time PCR are used. 2 – 4 columns of a 96-well plate at a time are filled, capped, and foiled to protect light-sensitive reagents like Rox dye and probes. Once the mix has been added to the entire plate, the plate is spun to ensure liquid is at the bottom of the well and run on the AB7700. Plates can be stored at 4C up to one night before running them. I keep primers, cDNA, and probes on ice but load the 96-well plate at room temperature. The polymerase is activated only at 95C.

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Reaction Conditions

Step Activity

	50 C 2 min
Polymerase activation	95 C 10 min
Denaturation	95 C 15 seconds
Anneal and extend	60 C 1 min

These steps are typically repeated for 40 cycles.

Sample Number	45						
2 x AmpliTaq Gold Mix	450 µl						
10 mM 5' primer	45 µl						
10 mM 3' primer	45 µl						
10 mM probe	18 µl						
Rox	18 µl						
dH20	280 µl						
Sample	45						