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Real-time quantitative polymerase chain reaction (RT-qPCR)

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ABSTRACT

Real-time quantitative polymerase chain reaction (RT-qPCR) helps determine the expression level of a certain gene by amplifying DNA according to the target mRNA template. This protocol describes the procedure of conducting RT-qPCR using Promega GoTaq® qPCR Master Mix A6001.

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MATERIALS TEXT

8-Tube Strip 0.1 ml, sterile, aerosol-resistant pipette tips, nuclease-free pipettors, cDNA template and qPCR primers (reference gene and target gene), GoTaq® qPCR Master Mix.

1 Thaw the GoTaq® Master Mix and Nuclease-Free Water. Thaw the cDNA templates and primer. GoTaq(R) qPCR Master Mix, 40 reactions **Promega Catalog #A6000**

Do not thaw the GoTaq® Master Mix at temperatures above room temperature.

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2 Vortex the GoTaq® Master Mix for 3–5 seconds to mix. Vortex at low speed to avoid aeration.

3 Determine the number of reactions to be set up, including negative control reactions. Add 1 or 2 reactions to this number to compensate for pipetting error.

While this approach does require using a small amount of extra reagent, it ensures that you will have enough reaction mix for all samples.

4 Prepare the reaction mix (minus DNA template) by combining the GoTaq® qPCR Master Mix, PCR primers and Nuclease-Free Water as described below. The DNA template is added in Step 6. Vortex briefly to mix.

Component	Volume	Final Concentration
GoTaq® qPCR Master Mix (2X)	10µl	1X
Forward Primer (20X)	_____µl	200nM–1µM
Reverse Primer (20X)	_____µl	200nM–1µM
Supplemental CXR Reference Dye (if required)	0.2µl per reaction	300nM
Nuclease-Free Water	to a final volume of 20µl	

The component of reaction mix. Calculate the volume of primer according to the concentration of primer used.

5 Add the DNA template (or water for the no-template control reactions) to the appropriate wells of the reaction plate.

6 Seal the tubes or optical plate, and centrifuge briefly to collect the contents of the wells at the bottom. The samples are ready for thermalcycling.

Protect the samples from extra light exposure or elevated temperatures.

7 Thermalcycle in qPCR equipment. Realtime qPCR is performed with an initial denaturation of 3 min at 95°C, followed by 40 cycles of 20 s at 95°C, 20 s at 60°C, and 20s at 72°C.

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