ApplicationForum

Multiplex Amplification Targets for Gene Expression Analysis in a 1-step Probe-based RT-qPCR System

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Overview

The ability to multiplex amplification targets for probe-based gene expression analysis of samples can save researchers time and money. Promega has developed an optimized set of reagents for 1-step probe-based chemistry detection, the GoTaq® Probe 1-Step RT-qPCR System, which we will show can be used for multiplex gene expression analysis with RNA samples. This master mix is also resistant to many of the common PCR inhibitors that challenge researchers.

Benefits of Multiplexing:

- Saves money, time, and reagents
- Uses less sample and Master Mix
- Maximizes instrumentation time

Methods and Discussions

Gene expression analysis of RNaseP, GAPDH, HPRT, and B2M was performed with human total colon RNA (Clontech Laboratories) using the GoTaq® Probe 1-Step RT-qPCR System. An Applied Biosystems® 7500 FAST Real Time PCR System was used for amplification. Serial dilutions of RNA were run in single or 4-plex (Figure 1A-D). Cq values and amplification efficiencies were comparable for reactions in multiplex and singleplex reactions over a wide range of RNA concentrations. These results demonstrate successful multiplexing with four targets in a single reaction.

Conclusion

The GoTaq® Probe 1-Step RT-qPCR System can be used in qPCR studies that require multiple genes of interest to be analyzed in the same reaction well. We demonstrated that the GoTaq® Probe 1-Step RT-qPCR System can be successfully used for multiplex reactions with four targets.

The benefits of this system include:

- Sensitive detection for earlier quantitation of low- and high-copy number targets.
- Resistance to a wide range of PCR inhibitors.
- Room temperature setups, suitable for automation and high-throughput detection.
- Compatible with both fast and standard qPCR cycling methods
- Backed by Promega's PCR Satisfaction Guarantee

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Figure 1. Expression of the B2M (a), HPRT (b), GAPDH (c) and RNaseP (d) genes analyzed with a serial dilution of human total colon RNA in 4-plex or single reaction. Bars and error represent average $\pm$ standard deviation of N=4. PCR efficiencies are: 98.81% for B2M singleplex and 92.42% for B2M 4-plex, 98.22% for HPRT singleplex and 103.79% for HPRT 4-plex, 106.22% for RNaseP singleplex and 107.39% for RNaseP 4-plex, and 97.90% for GAPDH singleplex and 91.58% for GAPDH 4-plex.