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Real-Time qPCR: Considerations for Comparing Reagent Performance

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Guidelines for a Successful qPCR Master Mix Comparison

Real-time quantitative PCR (qPCR) is a powerful tool to detect and quantify nucleic acids. By incorporating fluorescently labeled probes or fluorescent double-stranded DNA (dsDNA)-binding dyes into the PCR, product formation can be monitored following each PCR cycle. Promega GoTaq® qPCR Master Mixes for dye-based or probe-based product detection are optimized for fast and reproducible qPCR assays. In this guide, we outline some of the most important considerations for comparing either GoTaq® qPCR Master Mix with BRYT® Dye or GoTaq® Probe qPCR Master Mix to work with your specific qPCR assay.

Testing previously optimized qPCR assays with a new qPCR master mix requires careful experimental design to look at several factors of reagent performance. We have developed guidelines to provide a means to quickly compare qPCR Master Mix performance within two plates and two instrument runs. Analysis of the amplification data will allow you to assess key qPCR performance aspects of specificity, repeatability, linearity, sensitivity and efficiency.

What to Expect

- **Performance of qPCR reagents must be compared over a range of template concentrations.** A single-point comparison of C_q with different qPCR reagents can be misleading, as an earlier C_q does not necessarily reflect a better assay. Determining the C_q values over a range of template concentrations will allow you to compare assay specificity, repeatability, linearity, sensitivity, and efficiency for the qPCR reagents being tested.
- **Add passive reference dye as appropriate for your real-time instrument.** Some instruments require passive reference dye for signal normalization. Promega GoTaq® products use CXR, which can be directly substituted for ROX™ passive reference dye. GoTaq® qPCR Master Mix contains a concentration of CXR appropriate for no- or low-ROX™ instruments. GoTaq® Probe qPCR Master Mix does not contain CXR, but it is provided with the kit.
- **Melt temperatures of PCR product(s) may shift.** PCR product melting temperature is influenced by the sequence and length of the PCR product, as well as the salts and additives present in the master mix. Different PCR master mixes may result in different melt temperatures for the same product.
- **Optimum primer annealing temperatures may be different in different master mixes.** As with product melt temperatures, the salts and additives in PCR master mixes may influence primer annealing temperatures. For assays already in use, the existing conditions are typically a good starting point, but low amplification efficiency may indicate a need for optimization of thermal cycling conditions.
- **Each qPCR master mix should be compared on individual plates and in separate instrument runs.** Running the experiments on separate plates allows for the run parameters to be adjusted according to the required differences in the thermal cycling protocols (e.g., hot-start activation time). Additionally, it ensures that the data with each qPCR master mix are collected independently to avoid any influence of the other qPCR master mix on signal gain adjustments applied plate-wide by the instrument software.
- **Baseline and threshold settings are affected by both the assay and the master mix.** When comparing two master mixes, ensure that the software analyzes each assay independently (by indicating unique target names, or by omitting other assays). Do not apply a manual threshold when testing new master mixes.

- **The shape of the amplification curves beyond the threshold cycle (C_q) does not reflect assay performance.** As amplification product accumulates in the later cycles, there may be other activities in the reaction that will affect the rate of amplification and thus affect the overall shape of the amplification curve. However, these differences late in amplification do not affect the quantitation in the earlier amplification cycles.

Serial dilutions of template DNA prepared in nuclease-free water

Master Mix A

Stock
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
NTC
Serial Dilutions of DNA Template

Primer Set 1 | Primer Set 2 | Primer Set 3 | Primer Set 4

Amplification with Master Mix A: Use current standard conditions.

GoTaq® qPCR Master Mix

Stock
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
NTC
Serial Dilutions of DNA Template

Primer Set 1 | Primer Set 2 | Primer Set 3 | Primer Set 4

Amplification with GoTaq® qPCR Master Mix: Use your standard cycling protocol, but with GoTaq® standard hot-start activation of 2 minutes at 95°C.

Assay Setup: Separate plates for each qPCR master mix to be tested; 20µl reactions.

Amplification

Quality Control: Confirm baseline and threshold settings for amplification data and amplification specificity.

Standard Curve Analysis:
· Repeatability
· Linearity
· Sensitivity
· Efficiency

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Figure 1. Outline of steps to perform a qPCR reagent comparison.

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