Introducing GoTaq® qPCR Master Mix: The Bright Choice for Dye-Based qPCR

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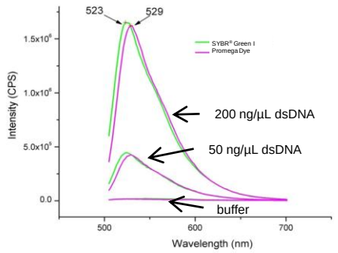
1. Abstract

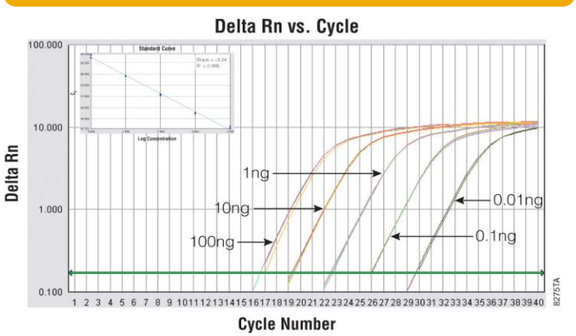
GoTaq® qPCR Master Mix introduces a new, proprietary dsDNA-binding dye that can be used at a higher concentration than SYBR® Green I because it is less inhibitory in an amplification reaction. The dye concentration in the master mix is optimized to produce significantly brighter fluorescence during qPCR than master mixes containing SYBR® Green I. Excitation and emission of the dye are similar to those of SYBR® Green I, so it is compatible with commonly available instrumentation platforms. GoTaq® qPCR Master Mix is a 2X master mix composed of an optimized buffer formulation complete with dNTPs and $MgCl_2$ and features GoTaq® Hot Start. The master mix comes premixed with a low level of CXR reference dye, which is identical to ROX™ reference dye. The master mix is compatible with existing experimental designs and requires addition of only DNA template, target-specific primers and water.

2. Spectral properties of GoTaq® qPCR dye and SYBR® Green I



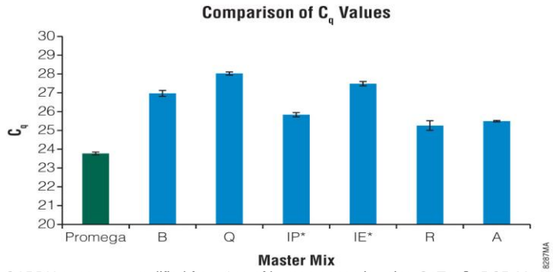
Fluorescence spectra of SYBR® Green I and the Promega proprietary dye in GoTaq® qPCR Master Mix. Fluorescence emission spectra were collected for both SYBR® Green I and the proprietary Promega in the absence and in the presence of different concentrations of dsDNA.

3. GoTaq® qPCR Master Mix provides high sensitivity in detecting a single copy gene



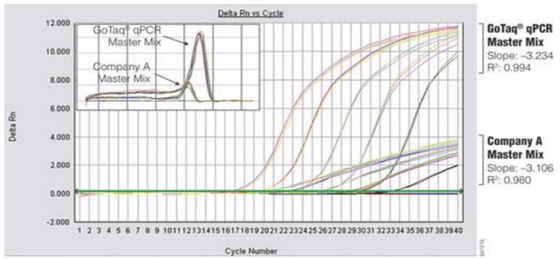
The GAPDH gene was amplified and detected from 10-fold serial dilutions (0.01ng to 100ng) of human genomic DNA. Inset shows the standard curve for the various dilutions (Slope=-3.2; R^2=0.995).

4. GoTaq® qPCR shows earlier C_q than leading competitors



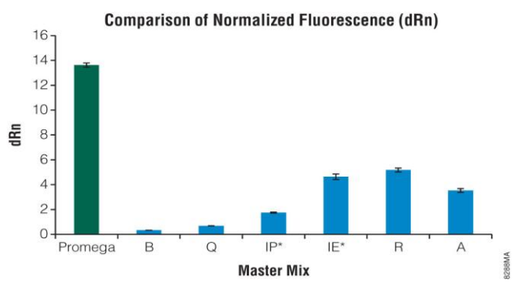
The GAPDH gene was amplified from 1ng of human genomic using GoTaq® qPCR Master Mix and six other commercially available dye-based master mixes using manufacturer protocols.
* Master Mixes were supplied without ROX reference dye. ROX was provided in the kit and added as recommended by the protocol.

5. GoTaq® qPCR shows significantly higher fluorescence and earlier C_q at all template levels



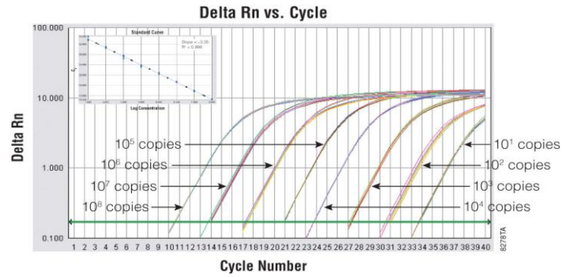
Performance comparison of GoTaq® qPCR Master Mix and Company A's master mix. GAPDH was amplified from tenfold serial dilutions (0.01–100 ng) of human genomic DNA. Inset shows comparison of dissociation profiles for both master mixes. GoTaq® qPCR shows significantly higher fluorescence and earlier C_q at all template levels.

6. GoTaq® qPCR shows brighter fluorescent signal than leading competitors.



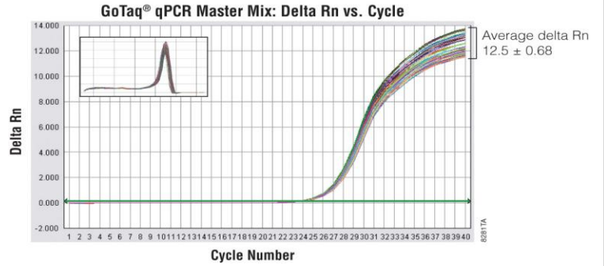
GAPDH was amplified from 1ng of human gDNA using GoTaq® qPCR Master Mix and six other commercially available dye-based master mixes using manufacturer protocols.
*Master Mixes IP and IE were supplied without ROX reference dye. ROX was provided in the kit and added to the master mix as recommended by the protocol.

7. Excellent amplification efficiency and accurate quantification over a broad dynamic range



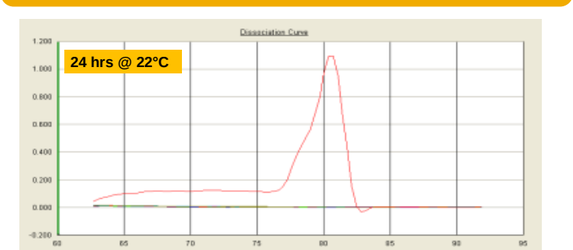
Amplification of kanamycin from plasmid DNA over 8 logs orders. Inset blue squares represent 10-fold dilutions of plasmid DNA from 10 copies to 1 x 10^8 copies. Black crosses show accurate quantification of 10-fold dilutions from 30 to 30 x 10^7 copies.

8. Exceptional reproducibility across a 96-well plate



GoTaq® qPCR shows very tight distribution. Variability of normalized fluorescence intensity (dRn) of GoTaq qPCR Master Mix and Company A. GAPDH was amplified from 1ng of human gDNA, n=96.

9. GoTaq® qPCR is stable after 24 hours at room temperature



The reaction is stable after 24 hours at room temperature making the product ideal for automated instruments. Human dystrophin gene was amplified with GoTaq® qPCR Master Mix after 24 hours at room temperature. Expected specific melt curve peak is at 81°C.

10. GoTaq® qPCR Master Mix is compatible with FAST cycling parameters

GAPDH amplified from 0.01 – 10 ng human gDNA with GoTaq® qPCR Master Mix and Company A Fast master mix, using the ABI 7500 FAST default cycling parameters: *Activation*: 95°C for 20 sec. 40 cycles: *Denaturation*: 95°C for 3 sec *Anneal/Extend*: 60°C for 30 sec. Data shows GoTaq® qPCR Master Mix is compatible with FAST cycling parameters as is.

11. Instrument Compatibility

Use GoTaq ® qPCR Master Mix directly.

Applied Biosystems 7500 and 7500 Fast Real Time PCR Systems
Stratagene Mx3005P® Quantitative PCR Systems
Roche LightCycler® 480
BioRad Chromo4™ Real-Time Detector
Eppendorf Mastercycler® ep *realplex*[4] and *realplex*[4] S*

Require addition of 100X CXR. Reference Dye to 1X per reaction.

Applied Biosystems 7000 Sequence Detection System
Applied Biosystems 7300 Real-Time PCR System
Applied Biosystems 7700 Sequence Detection System
Applied Biosystems 7900HT Real-Time PCR System
Applied Biosystems StepOne® and StepOne® Plus Real-Time PCR Systems

May require addition of fluorescein as reference dye.**

MyiQ™ System
iQ™5 Real-Time PCR Detection System

*Users must use clear-well plates with these instruments.
**Users should run a test reaction using their experimental target to determine if it is necessary to add fluorescein to the master mix.

12. Summary

- Early C_q and detection of low copy targets.
- Enhanced stability for automated setup.
- Direct substitute for SYBR® Green I products.
- The robust, reliable performance of GoTaq® Hot Start.
- The Promega PCR Performance Guarantee.

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