

TaqMan® multiplex real-time PCR—get more data out of your sample

- A complete multiplex real-time PCR solution for gene expression and genotyping applications
- New ABY® and JUN® dyes, QSY® quencher, and a multiplex master mix for optimal amplification performance
- Up to 4-plex reactions as sensitive as singleplex reactions to decrease the starting material required and minimize optimization processes

Obtaining the maximum amount of genetic information from an important but small amount of sample can be challenging. This is particularly true with FFPE samples or tumor biopsies that are used for translational research studies. Singleplex qPCR is frequently used for these clinical research samples, but this typically has a higher cost per sample than running in multiplex format. The additional time and materials required to set up multiple single-assay reactions could also significantly increase the cost of a complex project.

Multiplex qPCR, a strategy where more than one target in a sample is amplified and quantified in a single tube, can decrease the quantity

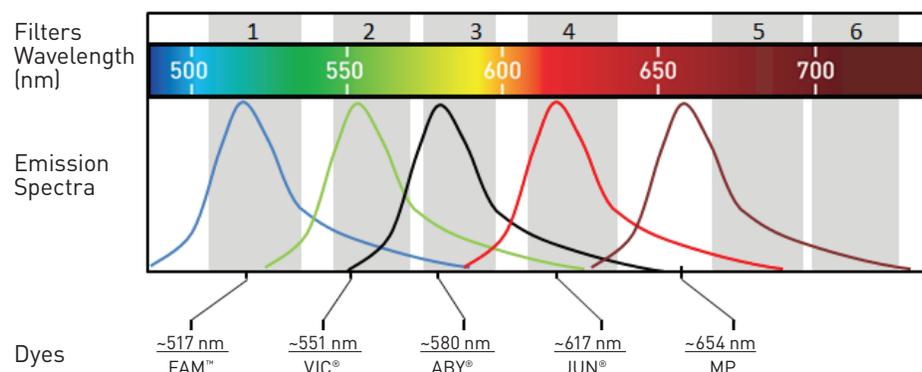


Figure 1. Fluorescence emission spectra of FAM™, VIC®, ABY®, and JUN® dyes used for multiplex real-time PCR. Grey zones represent the filters available on Applied Biosystems® real-time PCR systems: 1 through 6 for the QuantStudio® 7/12K Flex Real-Time PCR Systems; 1 through 5 for the QuantStudio® 6 Flex Real-Time PCR System, ViiA™ 7 Real-Time PCR System, and 7500/7500 Fast Real-Time PCR Systems. MP = MUSTANG PURPLE® dye.

of sample material and reagents required. A complete solution for multiplex qPCR is presented here, with components designed to work together for better data quality and less time for optimization. The solution consists of the following:

- TaqMan® probes using QSY® quencher, providing maximal PCR efficiency in a multiplex format. These new probes can be ordered with FAM™ and VIC® dyes and also with the ABY® and JUN® dyes, allowing amplification of up to 4 targets in a single reaction. These reporter dyes are optimized to work together

with minimal spectral overlap for optimal performance (Figure 1). In addition, the QSY® quencher is fully compatible with probes that have MGB quenchers.

- The new TaqMan® Multiplex Master Mix, which was developed to allow amplification of 4 targets simultaneously without competition between targets. This master mix contains MUSTANG PURPLE® dye, a new passive reference used for normalization instead of ROX® dye, allowing for measurement of JUN® dye in the channel previously used to measure ROX® dye.

- Off-the-shelf predesigned assays—an RNaseP assay using an ABY®-QSY® probe and a GAPDH assay using a JUN®-QSY® probe. Both assays are available in limited and non-limited primer concentrations.
- Calibration plates for ABY®, JUN®, and MUSTANG PURPLE® dyes are available in 96-well, 96-well Fast, and 384-well format.
- Additional services are provided through our custom services program—save time and let our TaqMan® Assay experts design your multiplex assays.

This multiplex solution is compatible with the QuantStudio® 6, 7, and 12K Flex Real-Time PCR Systems, as well as the ViiA™ 7 Real-Time PCR System and the 7500/7500 Fast Real-Time PCR systems.

Multiplex without compromise

The multiplex format enables cost savings and preservation of limited sample, but it's important to obtain the same sensitivity as in the singleplex format. Figure 2 demonstrates comparable results between reactions performed in individual tubes or in 4-plex reactions for a gene quantification experiment.

Improved probe performance

Introduction of ABY® and JUN® reporter dyes and MUSTANG PURPLE® passive reference dye allows for optimal 4-color multiplex assays when used with our FAM™ and VIC® reporter dyes. Please note that ABY® and JUN® reporter dyes are available only with QSY® quencher, while FAM™ and VIC® dyes are available with either MGB or QSY® quencher. A comparison with a set of competitor dyes shows that our combination of dyes provides an earlier C_t for the majority of assays (Figure 3).

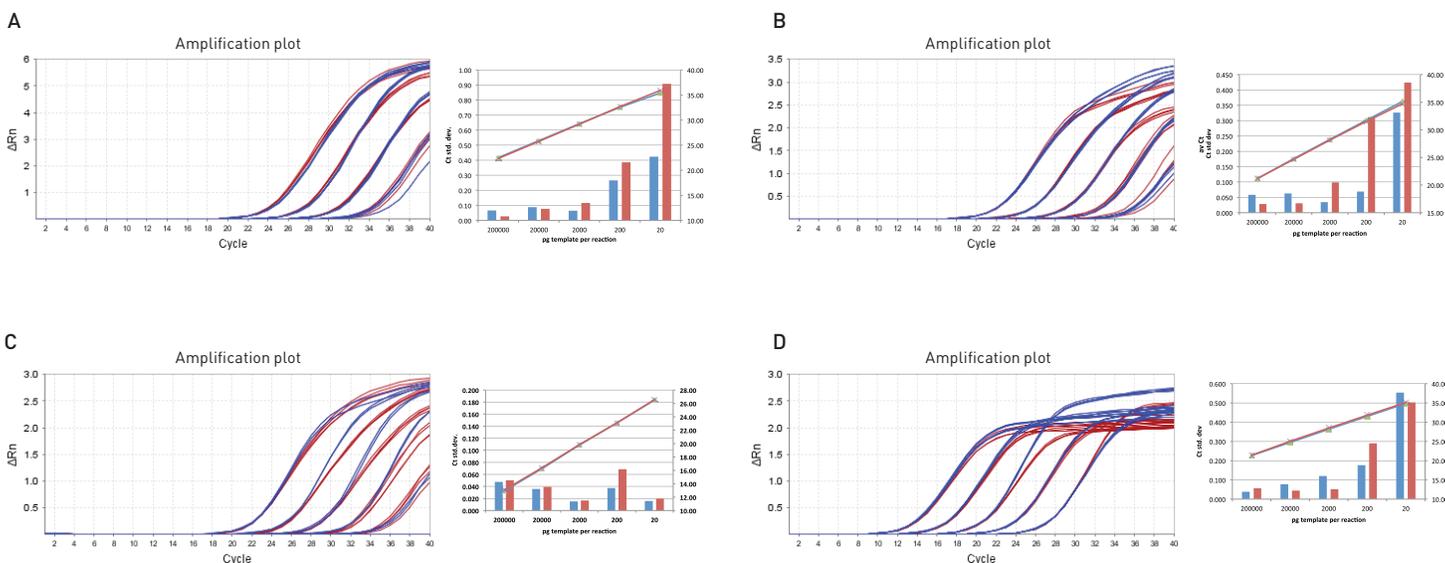
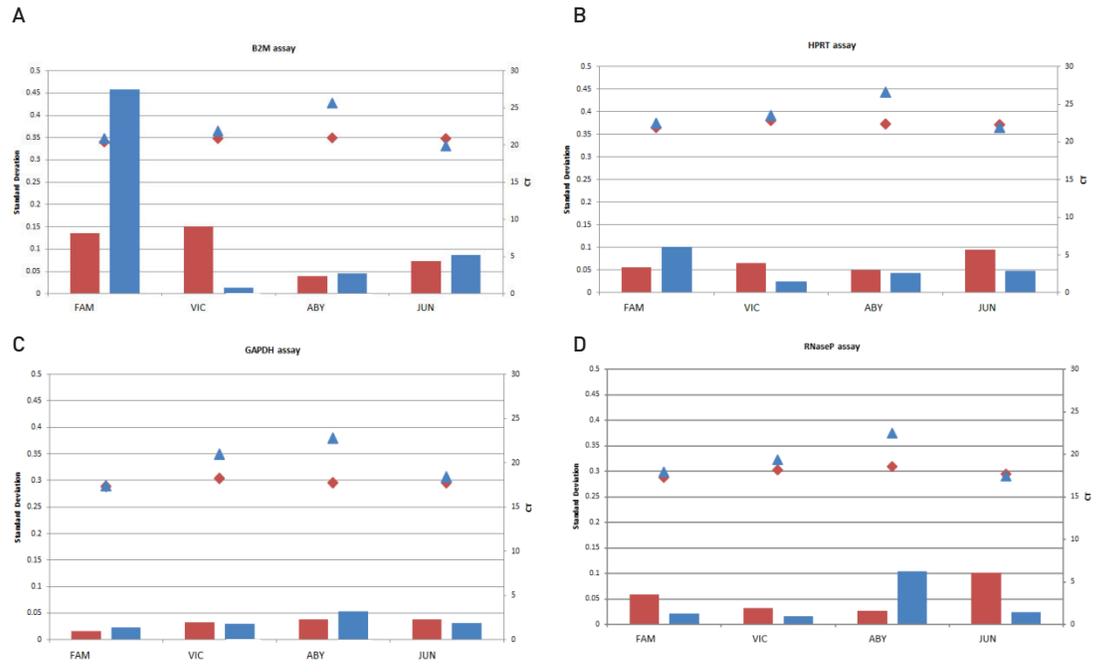


Figure 2. Comparison of singleplex and multiplex gene expression assays. (A) EGRF assay, FAM™ dye; (B) BRCA1 assay, VIC® dye; (C) ESR1 assay, JUN® dye; (D) RNaseP assay, ABY® dye. Amplification was performed on the QuantStudio® 7 Real-Time PCR System using TaqMan® Multiplex Master Mix. The amplification plot shows linear curves for 4 assays amplified in singleplex (blue) and 4-plex reactions (red) in a dilution series from 20,000 pg to 2 pg of reference colon cDNA per 10 μL reaction. In all four amplification plots, blue represents 4-plex reactions and red represents singleplex reactions. Average C_t (lines) and average standard deviation (bars) for the dilution series are represented in their respective graphs and show the concordance between singleplex and 4-plex reactions. PCR efficiencies are: 96.09% for EGRF singleplex and 96.39% for EGRF 4-plex; 93.56% for BRCA1 singleplex and 94.93% for BRCA1 4-plex; 97.13% for ESR1 singleplex and 95.81% for ESR1 4-plex; 96.91% for RNaseP singleplex and 98.1% for RNaseP 4-plex.

Figure 3. Comparison of our new dye combination with a competitor's dye combination offering. Probes for (A) B2M, (B) HPRT, (C) GAPDH, and (D) RNaseP gene expression assays were synthesized with FAM[™], VIC[®], ABY[®], and JUN[®] dyes with QSY[®] quencher (red bars and diamonds) and with another commercially available dye combination (blue bars and triangles). All possible gene-dye combinations were tested. Reactions were prepared with TaqMan[®] Multiplex Master Mix using 900 nM of primer, 250 nM of probe, and 10 ng of cDNA. Amplification was performed on the QuantStudio[®] 7 Real-Time PCR System using TaqMan[®] Multiplex Master Mix. Bars represent average standard deviation. Triangles and diamonds represent average C_t value.



Optimized multiplex master mix

In multiplex PCR, it's important to have a robust master mix that allows amplification of each target in a highly competitive environment. Our new master mix composition was developed to provide optimal multiplex performance for each target in the reaction. A comparison of our master mix and a competitor's

master mix in a 4-plex reaction shows an earlier C_t for 3 of the targets amplified with our new master mix and a lower standard deviation for most of the dilution points, demonstrating the excellent performance of our solution (Figure 4).

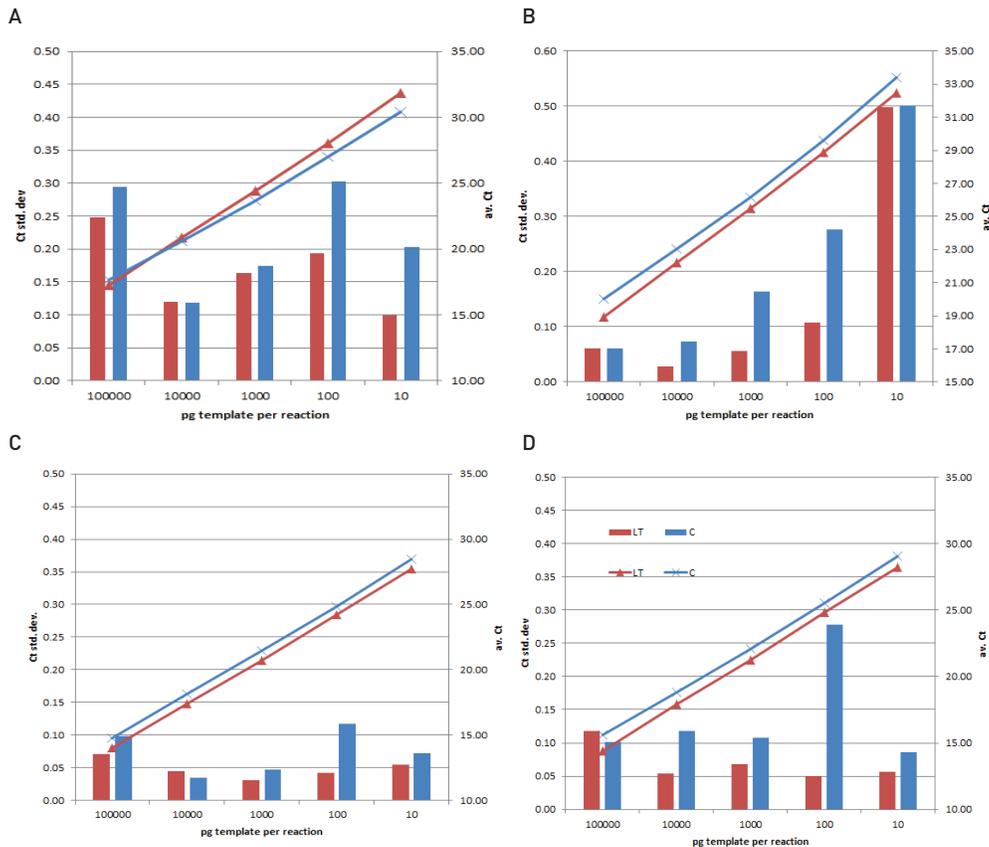


Figure 4. Comparison of TaqMan[®] Multiplex Master Mix with another commercially available master mix. (A) B2M assay, FAM[™] dye; (B) RNaseP assay, VIC[®] dye; (C) GAPDH assay, ABY[®] dye; (D) HPRT assay, JUN[®] dye. All assays used QSY[®] quencher. The graph shows average standard deviation (bars) and average C_t (cross and triangle) for 4-plex reactions using a dilution series from 100 ng to 10 pg of cDNA per 10 μ L reaction. All amplifications were performed on the ViiA[™] 7 Real-Time PCR System using the cycling conditions recommended for each master mix. Red represents TaqMan[®] Multiplex Master Mix, and blue represents 4-plex reactions with another commercially available master mix.

Optimized to minimize time-to-results

Developing a multiplex PCR assay requires time to correctly design the assay and optimize the reaction. By using our complete solution, for which all components were developed to work together, you increase your chances of success

and limit your development time. A new multiplex PCR user guide [1] was developed to guide you through the development and optimization process, and our custom services will allow you to delegate assay design to our expert team to minimize your effort.

References

1. Multiplex PCR User Guide. Available at lifetechnologies.com/multiplexqpcr
2. TaqMan® multiplex qPCR: Accurate, sensitive, and as efficient as traditional singleplex qPCR. Application note available at lifetechnologies.com/multiplexqpcr

Ordering information

Product	Cat. No.
TaqMan® QSY® Probes	
TaqMan® QSY® Probe, 6,000 pmol	4482777
TaqMan® QSY® Probe, 20,000 pmol	4482778
TaqMan® QSY® Probe, 50,000 pmol	4482779
Control kits	
TaqMan® GAPDH Assay, JUN®-QSY® 20X	4485712
TaqMan® GAPDH Assay, JUN®-QSY® PL 20X	4485713
TaqMan® RNaseP Assay, ABY®-QSY® 20X	4485714
TaqMan® RNaseP Assay, ABY®-QSY® PL 20X	4485715
Multiplex master mixes	
TaqMan® Multiplex Master Mix, 1 mL	4461881
TaqMan® Multiplex Master Mix, 5 mL	4461882
TaqMan® Multiplex Master Mix, 50 mL	4486295
Other formats are available at lifetechnologies.com/multiplexqpcr	
Calibration plates	
96-Well Calibration Plate, MUSTANG PURPLE® dye	4461599
96-Well Calibration Plate, JUN® dye	A24737
96-Well Calibration Plate, ABY® dye	A24738

Calibration plates are also available for 96-well Fast and 384-well plate formats. Visit lifetechnologies.com/multiplexqpcr for more information.

For detailed recommendations that include user manuals and product literature, please visit lifetechnologies.com/multiplexqpcr



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