TaqMan multiplex real-time PCR

Get more data out of your sample

- A complete multiplex real-time PCR (qPCR) solution for gene expression and genotyping applications
- Applied Biosystems™ 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, decreases the starting material required, and minimizes optimization processes

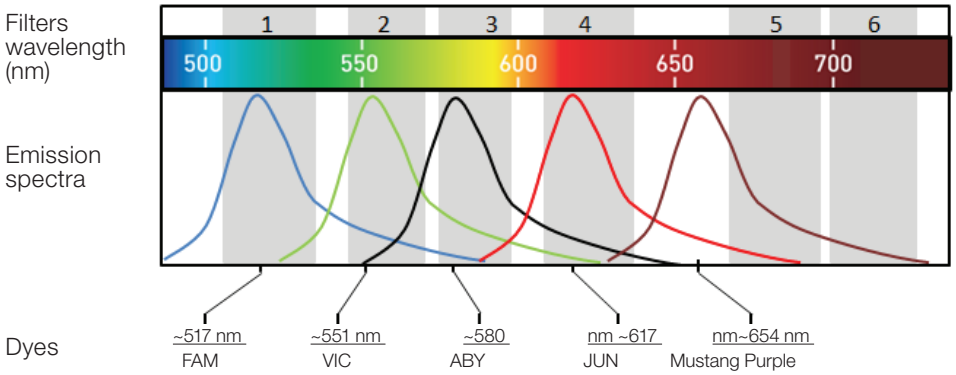

Figure 1. Fluorescence emission spectra of FAM, VIC, ABY, and JUN dyes used for multiplex real-time PCR. Grey zones represent the filters available on Applied Biosystems™ real-time PCR systems: 1 through 6 for the QuantStudio™ 7 or 12K Flex Real-Time PCR Systems; 1 through 5 for the QuantStudio™ 6 Flex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

Obtaining the maximum amount of genetic information from an important but small amount of sample can be challenging. This is particularly true with formalin-fixed, paraffin-embedded (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 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:

- Applied Biosystems™ TaqMan® probes using QSY quencher, providing maximal PCR efficiency in a multiplex format. These probes can be ordered with Applied Biosystems™ 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 improved performance (Figure 1). In addition, the QSY quencher is fully compatible with probes that have minor-groove binder (MGB) quenchers.
- The Applied Biosystems™ TaqMan® Multiplex Master Mix was developed to allow amplification of 4 targets simultaneously, without competition between targets. This master mix contains the Applied Biosystems™ Mustang Purple™ dye, a passive reference used for normalization instead of the Applied Biosystems™ ROX™ dye, allowing for measurement of JUN dye in the channel previously used to measure ROX dye.

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- Off-the-shelf, predesigned assays—an RNase P assay using an ABY-QSY probe and a GAPDH assay using a JUN-QSY probe. Both assays are available in limited and nonlimited primer concentrations.
- Calibration plates for ABY, JUN, and Mustang Purple dyes, available in 96-well, 96-well Fast, and 384-well formats.
- Additional services provided through our custom services program—save time and let our Applied Biosystems™ TaqMan® Assay experts design your multiplex assays.

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

Multiplexing 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.

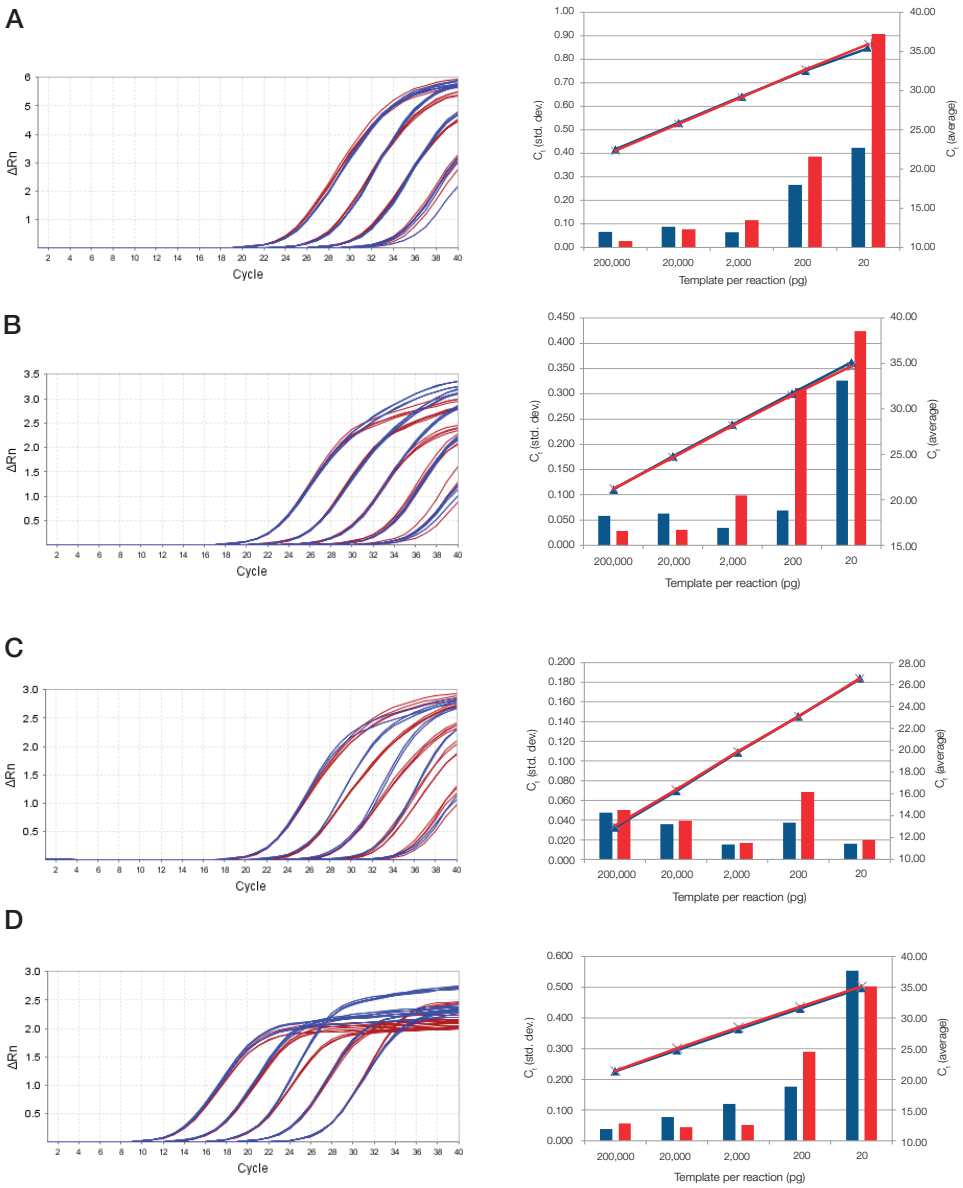

Figure 2. Comparison of singleplex and multiplex gene expression assays. (A) EGFR assay, FAM dye; **(B)** BRCA1 assay, VIC dye; **(C)** ESR1 assay, JUN dye; **(D)** RNase P assay, ABY dye. Amplification was performed on the QuantStudio 7 Real-Time PCR System using TaqMan Multiplex Master Mix. The figure shows amplification plots (left) and linear curves (right) 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. Average C_t value (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 EGFR singleplex and 96.39% for EGFR 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 RNase P singleplex and 98.1% for RNase P 4-plex.

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 dyes from another supplier shows that our combination of dyes provides an earlier C_t for the majority of assays (Figure 3).

Optimized multiplex master mix

In multiplex PCR, it's important to have a robust master mix that allows for 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 master mix from another supplier 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).

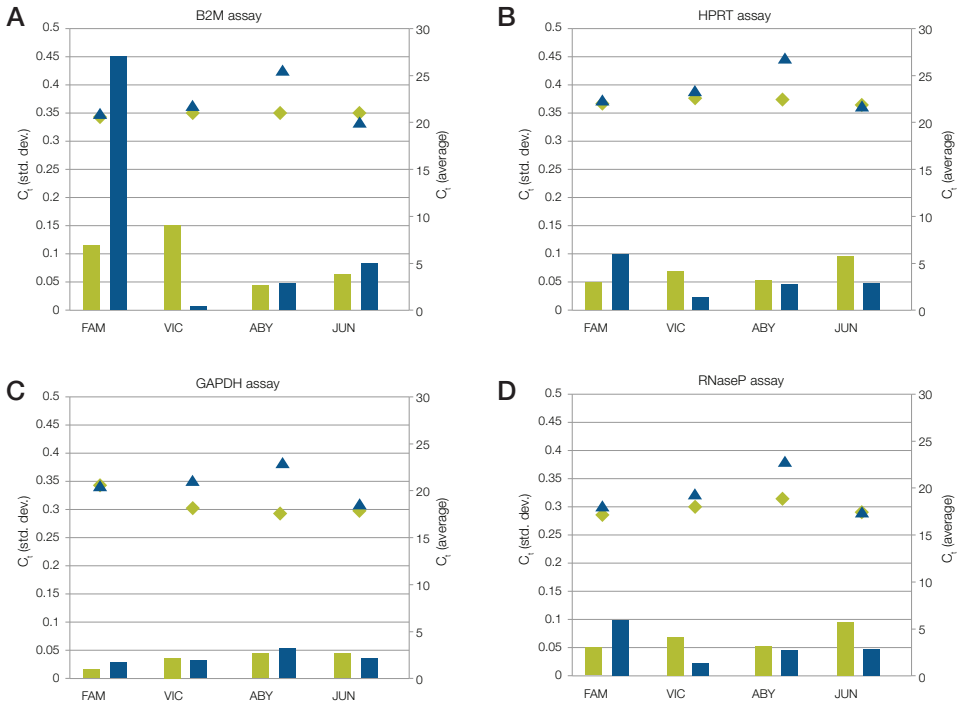

Figure 3. Comparison of our new dye combination with a dye combination from another supplier. Probes for **(A)** B2M, **(B)** HPRT, **(C)** GAPDH, and **(D)** RNase P gene expression assays were synthesized with FAM, VIC, ABY, and JUN dyes with QSY quencher (green bars and diamonds) and with another 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 values.

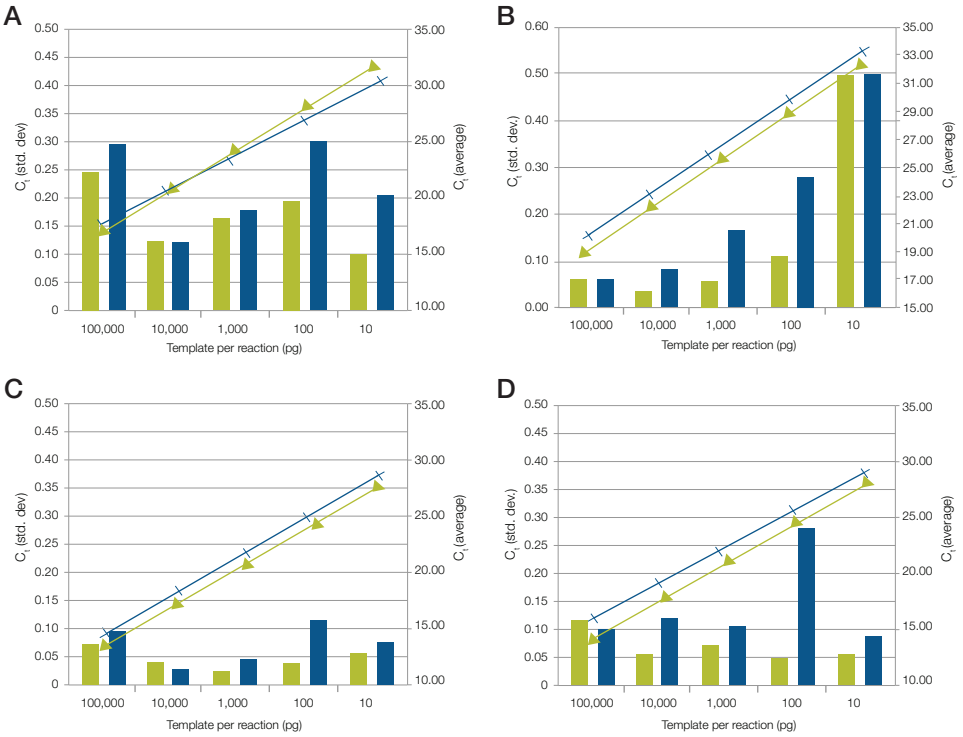

Figure 4. Comparison of TaqMan Multiplex Master Mix with another commercially available master mix. (A) B2M assay, FAM dye; **(B)** RNase P assay, VIC dye; **(C)** GAPDH assay, ABY dye; **(D)** HPRT assay, JUN dye. All assays used QSY quencher. The graph shows average standard deviation (bars) and average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

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Optimized to minimize time-to-results

Developing a multiplex PCR assay requires time to correctly design the assay and optimize the reaction. Using our complete solution, for which all components were developed to work together, helps increase your chances of success and limits your development time. A new multiplex PCR user guide was developed to guide you through the development and optimization process [1], and our custom services will allow you to delegate assay design to our experienced team to minimize your efforts.

References

1. Multiplex PCR User Guide. Available at thermofisher.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 **thermofisher.com/multiplexqpcr** for more information.

Find out more at **thermofisher.com/multiplexqpcr**

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