Checklist for multiplex, real-time PCR

Before carrying out multiplex, real-time PCR with a QuantiTect® Multiplex PCR or RT-PCR Kit, please go through the checklist below. For detailed information, refer to the kit handbook or *Critical factors for success in real-time, multiplex PCR* or contact **QIAGEN Technical Service** (contact information on reverse side).

☑ **Design primers and probes**

Has a primer–probe set (e.g., TaqMan® Gene Expression Assay or published in literature) already been designed for each of your target genes?

- If yes, there is no need to design new primer–probe sets (however, it may be necessary to choose new reporter dyes; see next step).
- If no, use Primer Express® or other software to design primers and probes. Use BLAST® software (www.ncbi.nlm.nih.gov/BLAST) to check that the sequences are unique.

☑ **Choose reporter dyes and quenchers**

Refer to Table 1 (see reverse side) to choose the appropriate combination of reporter dyes for your probes. Check that the appropriate filters are installed on your real-time cycler. We recommend using a nonfluorescent quencher (e.g., Black Hole Quencher® dye) for all probes.

☑ **Reconstitute primers and probes**

Use TE to prepare a 100 µM stock solution of each lyophilized primer or probe. Check concentrations by spectrophotometry, and store frozen in small aliquots away from light.

☑ **Set up your real-time cycler**

Check whether your real-time cycler needs to be calibrated for each of the reporter dyes to be used in the multiplex assay. Be sure to activate the filter or detector for each reporter dye.

Be sure to adjust the analysis settings for each reporter dye channel in each run. Be aware that default analysis settings may not be optimal.

☑ **Evaluate the performance of the multiplex assay**

Check that each primer–probe set works in single PCR. Then compare the performance of the primer–probe sets in single PCR and multiplex PCR: using serial 10-fold dilutions of template, multiplex PCR should give C_T values comparable to those obtained in single PCR.

Table 1. Combinations of Reporter Dyes for Multiplex Assays*

Cycler	Reference dye	Dye 1†	Dye 2†	Dye 3†	Dye 4†
ABI PRISM® 7700	ROX	FAM	HEX, JOE, VIC®	—	—
ABI PRISM 7000 and 7900, Applied Biosystems® 7300	ROX	FAM	HEX, JOE, VIC	Bodipy® TMR, NED	—
Applied Biosystems 7500	ROX	FAM	HEX, JOE, VIC	Bodipy TMR, NED	Alexa Fluor® 647, Cy®5
iCycler iQ® and iQ5	Not required	FAM	HEX, JOE, TET, VIC	Texas Red®, ROX	Cy5
LightCycler® 2.0	Not required	FAM	HEX, JOE, VIC	Texas Red, ROX	Alexa Fluor 660, Bodipy 630/650, Pulsar® 650
Mx3000P™, Mx3005P™	Not required	FAM	HEX, JOE, VIC	Texas Red, ROX	Cy5
Rotor-Gene™ 3000	Not required	FAM	HEX, JOE, VIC	Texas Red, ROX	Cy5

* Visit www.qiagen.com/multiplex to view dye combinations for other cyclers.

† Preferably, select Dye 1 for the least abundant target, Dye 2 for the second least abundant target, and Dyes 3–4 for the most abundant targets.

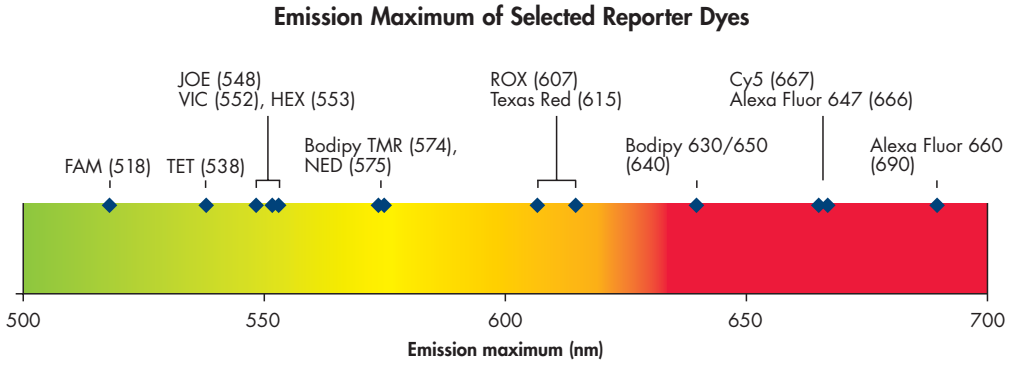

Figure 1 The emission maximum (nm) of selected reporter dyes are displayed in parentheses. Emission maximum may vary depending on buffer conditions. Other dyes with similar wavelengths may not be suitable for multiplex assays due to low fluorescence and/or stability.

See multiplex PCR application data from researchers at www.qiagen.com/multiplex .

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