TaqMan QSY and QSY2 probes

Multiplex better with the newest additions to the TaqMan portfolio

Product overview

Assay developers are under constant pressure to save time and reduce costs, while also assuring accuracy and delivering reproducible results. This can be accomplished by maximizing the number of targets per sample by multiplexing. With new Applied Biosystems™ TaqMan™ QSY™2 probes, users can now multiplex up to six targets in a single real-time PCR (qPCR) reaction with higher sensitivity, greater dynamic range, and better assay performance retention when scaling from 1-plex to 6-plex. These probes will enable users to speed up optimization of their multiplex assays and leverage the full capabilities of their qPCR instruments with higher multiplexing. Equipped with the cyanine 5 and cyanine 5.5 long-wavelength dyes, the TaqMan QSY2-quenched probes enable lower background and greater signal-to-noise ratios while detecting targets in the far-red spectrum. This wavelength coverage complements that of the Applied Biosystems™ TaqMan™ QSY™ probes that are available with Applied Biosystems™ FAM™, VIC™, ABY™, and JUN™ reporter dyes. TaqMan QSY and QSY2 probes may be configured and ordered online via the Applied Biosystems™ Custom TaqMan™ Probes tool.

Head-to-head performance—TaqMan QSY and QSY2 probes vs. IDT probes

Two 6-plex combinations of TaqMan QSY and QSY2 probes were run against Iowa Black™ single-quenched probe, and Zen™/Iowa Black™ and TAO™/Iowa Black™ double-quenched probes (Integrated DNA Technologies). All other variables were controlled. Double-quenched probes were selected from Integrated DNA Technologies (IDT™) products where available. Each probe was also run in a single-plex assay on the same plate.

The results from testing show that TaqMan QSY and QSY2 probes are more sensitive, have greater dynamic range, and better retain single-plex performance when scaling to a 6-plex assay, than their IDT counterparts (Figures 1–5).

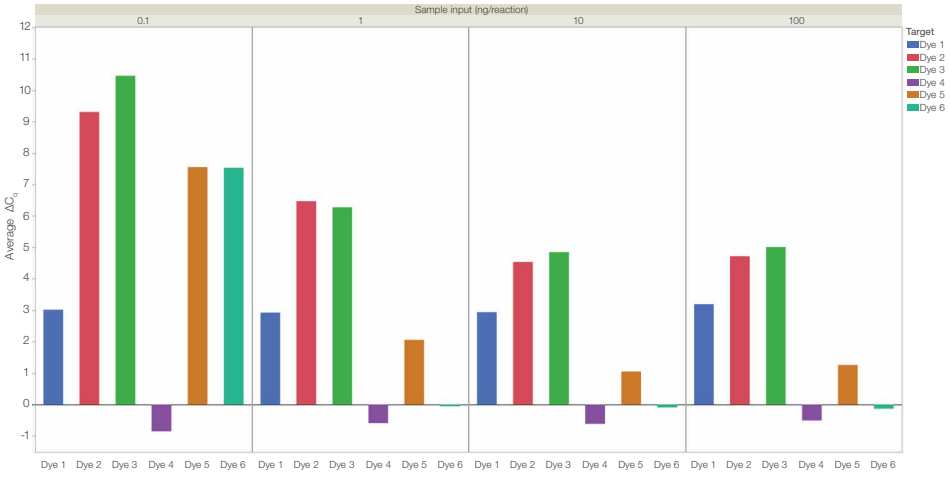

Figure 1. Average ΔC_q between TaqMan and IDT probes in six-plex assays. TaqMan probes demonstrate higher sensitivity and earlier detection across multiple sample inputs, as indicated by earlier average C_q values and positive average ΔC_q values. Average ΔC_q = (average C_q values using IDT probes)–(average C_q values using TaqMan probes).

Sensitivity

Sensitivity is the ability to detect targets at low concentrations.

When comparing two probes targeting a controlled input, an earlier C_q indicates greater sensitivity. Figure 1 shows the average ΔC_q between the IDT and TaqMan probes.

A positive average ΔC_q value indicates a later C_q for the IDT probe and earlier C_q for the TaqMan probe—and therefore greater sensitivity of the TaqMan probe.

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70% (17/24) of the time, TaqMan probes have an earlier average C_q than IDT probes when run in 6-plex. On average, the C_q values of TaqMan probes are 2.7 cycles earlier than those of IDT probes when run in a 6-plex assay.

Dynamic range

A greater dynamic range means a larger window in which your samples can be detected **accurately and precisely**. In execution, this means an assay is more sensitive and accurate when detecting lower sample inputs, and on the high end, more tolerant of sample saturation. Figure 2 demonstrates that greater

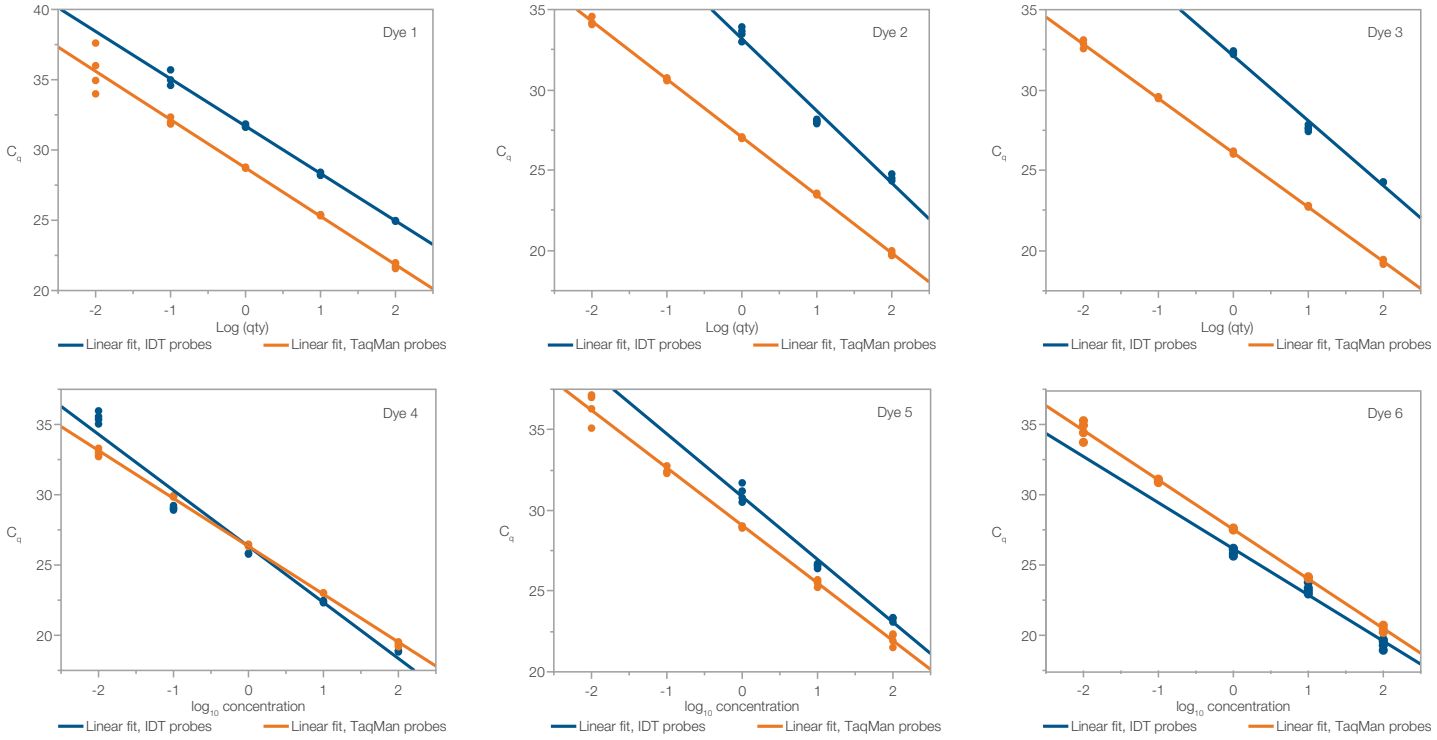

Figure 2. Dynamic range of TaqMan and IDT probes across four orders of magnitude of template concentration.

or equivalent dynamic range was achieved with TaqMan probes than with IDT probes, for all probes tested in the 6-plex assay.

Scaling to a 6-plex assay

Multiplexing can be challenging, and probe performance can change when scaled to a higher-plex assay. Deviations in performance can lengthen the design process for multiplexed assays.

Figure 3 shows that 67% (4/6) of the time, TaqMan probes have smaller differences in C_q values between 6-plex and single-plex assays than IDT probes. Therefore, TaqMan QSY and QSY2 probes have more consistent and predictable performance when scaling to a 6-plex assay.

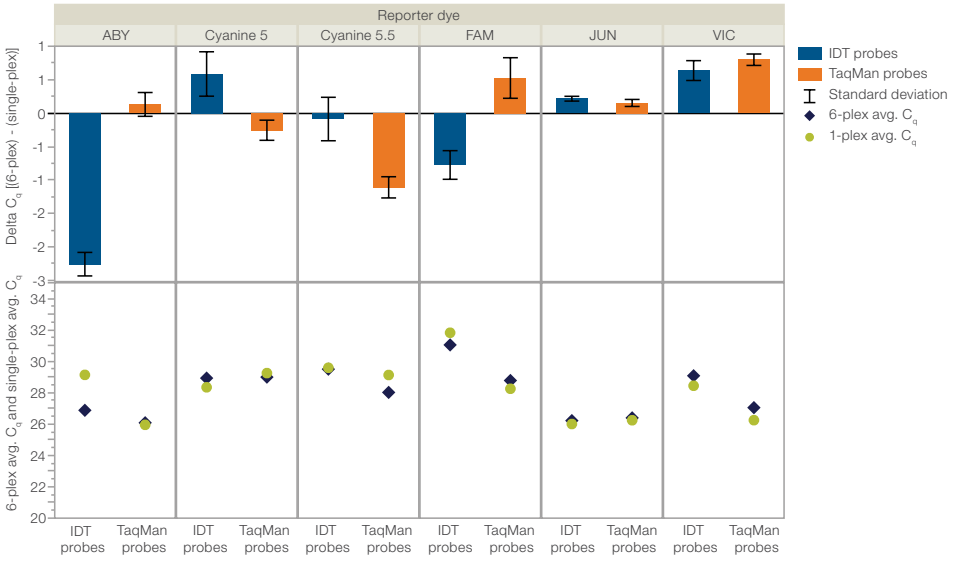

Figure 3. Mean ΔC_q and average C_q values for TaqMan and IDT probes in 6-plex versus singplex assay. TaqMan probes demonstrate smaller differences in performance in 6-plex compared to single-plex assays, as indicated by smaller mean ΔC_q values compared to those of IDT probes.

Figure 4 shows amplification plots for the PCR assays performed with cyanine 5.5–labeled probes.

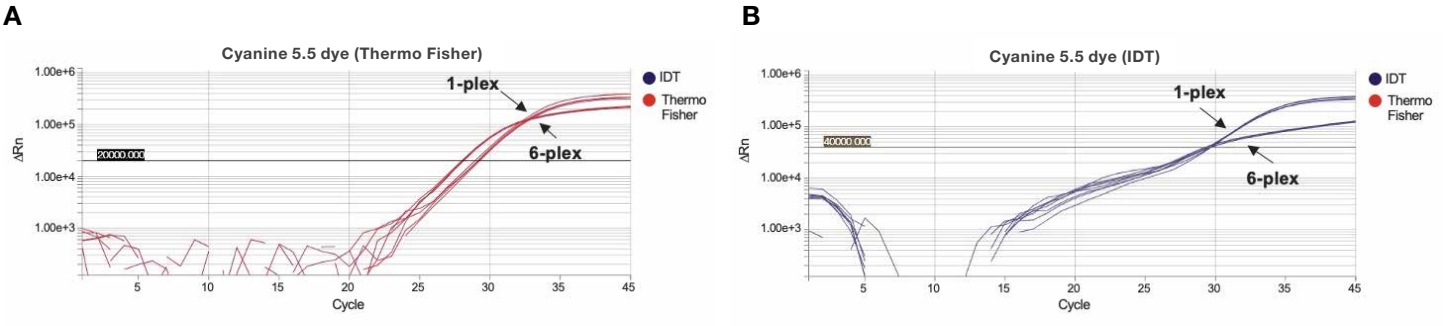

Figure 4. Amplification plots from PCR assays performed with cyanine 5.5–labeled (A) TaqMan probes and (B) IDT probes. Note the similar shapes of the 6-plex and 1-plex amplification curves for the Thermo Fisher product compared to the different shapes of the amplification curves for the IDT product.

PCR efficiency

PCR efficiency is a specificity indicator for an assay, where 100% represents the exact doubling of template per cycle. Efficiency between 90% and 110% helps ensure more accurate template quantitation and data interpretation.

For all 6 probes tested in a 6-plex assay, TaqMan probes had better PCR efficiency than IDT probes.

Figure 5 demonstrates that 83% (5/6) of TaqMan probes showed PCR efficiency between 90% and 110% when run in a 6-plex assay, but only 33% (2/6) of IDT probes passed that same metric when run in a 6-plex assay.

Data from Figures 1–5 were compiled from two independent sets of 6-plex assays using TaqMan and IDT probes. Double-quenched equivalent IDT probes were utilized where available. Six-plex assays were tested with five concentrations of 10-fold serial dilutions of the template, plus non-template controls. All probes were tested with the same master mix and analyzed on the Applied Biosystems™ QuantStudio™ 7 Pro PCR instrument. To learn more about multiplexing, visit our multiplex optimization guide.

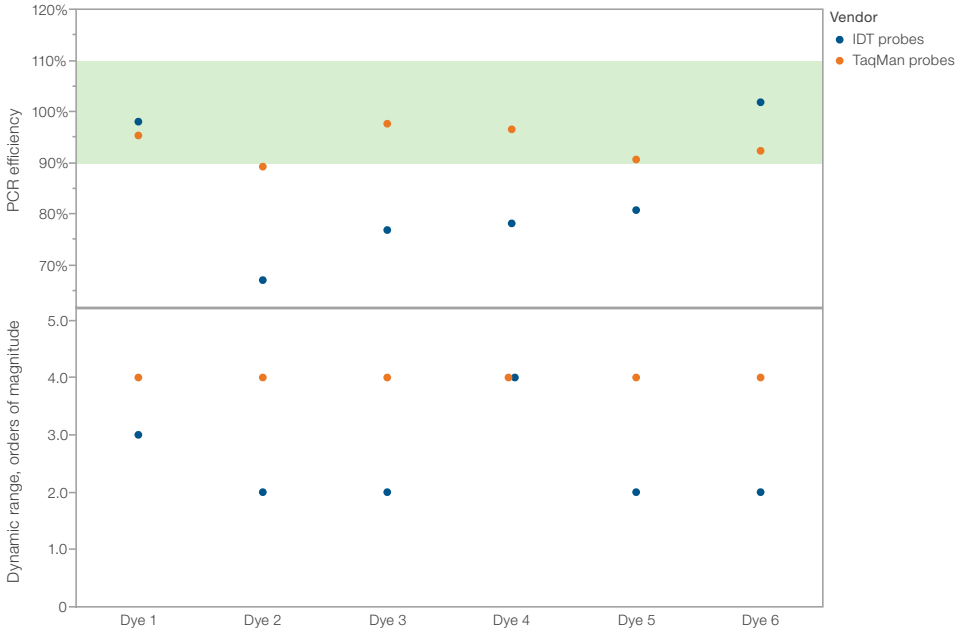

Figure 5. PCR efficiency and dynamic range of TaqMan vs. IDT probes in 6-plex assays.

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