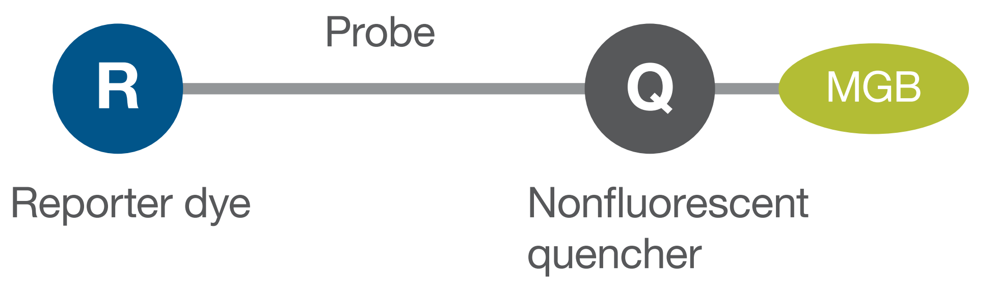
ThermoFisher SCIENTIFIC

Comparative testing of TaqMan probes in singleplex and multiplex assays

Discover how Applied Biosystems™ TaqMan™ probes can help you save time and reduce costs by enabling high-quality data and advanced multiplexing. Here we highlight results of head-to-head testing between TaqMan probes from Thermo Fisher Scientific and their counterparts from IDT in singleplex and multiplex qPCR assays. With the outstanding performance of TaqMan probes, you can transform the way you approach assay development.

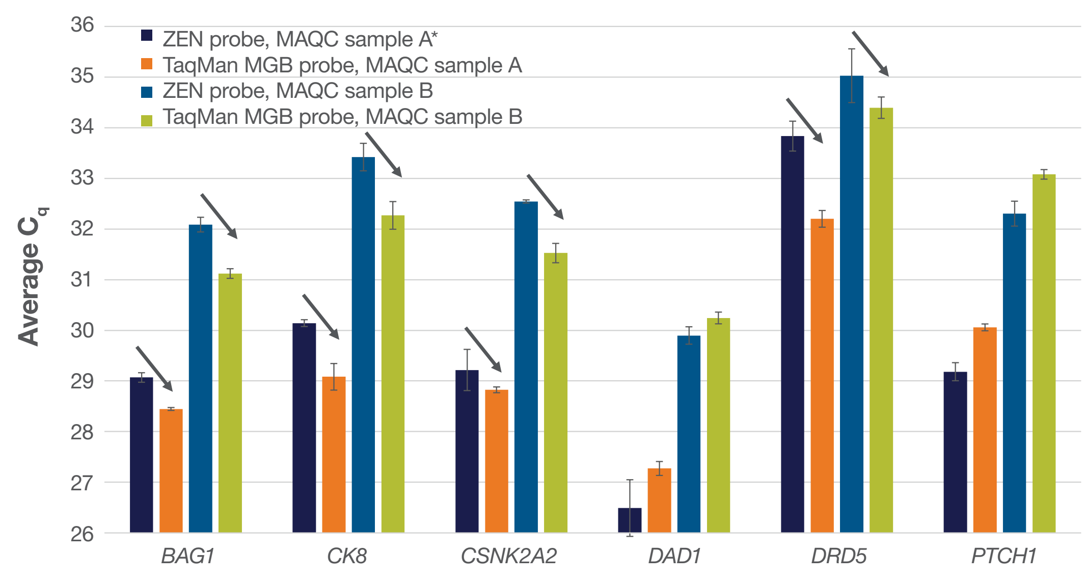
TaqMan MGB probes



Key features

- Applied Biosystems™ TaqMan™ MGB probes incorporate a minor groove binder (MGB) moiety, which stabilizes probe–target hybrids
- The MGB increases the melting temperature of the probe, allowing for shorter probes with higher specificity compared to longer designs from IDT
- Over 20 million predesigned Applied Biosystems™ TaqMan™ Assays incorporate MGB probes for enhanced specificity and sensitivity

Sensitivity of probes in a panel of singleplex qPCR assays

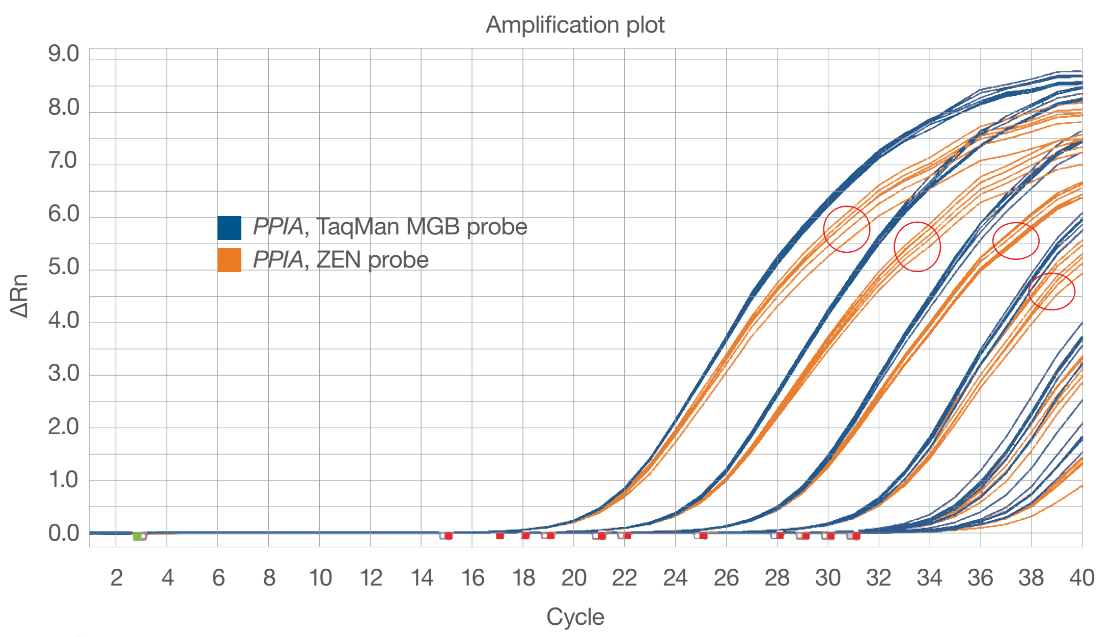


* MicroArray Quality Control (MAQC) samples A and B have known expression differences based on previous sequencing and microarray studies.

Key takeaways

- TaqMan MGB probes were more sensitive than ZEN™ probes from IDT, for 4 out of 6 targets tested, as demonstrated by lower C_q values (arrows)
- The sensitive performance of TaqMan Assays is crucial when evaluating low-abundance targets, such as rare transcripts

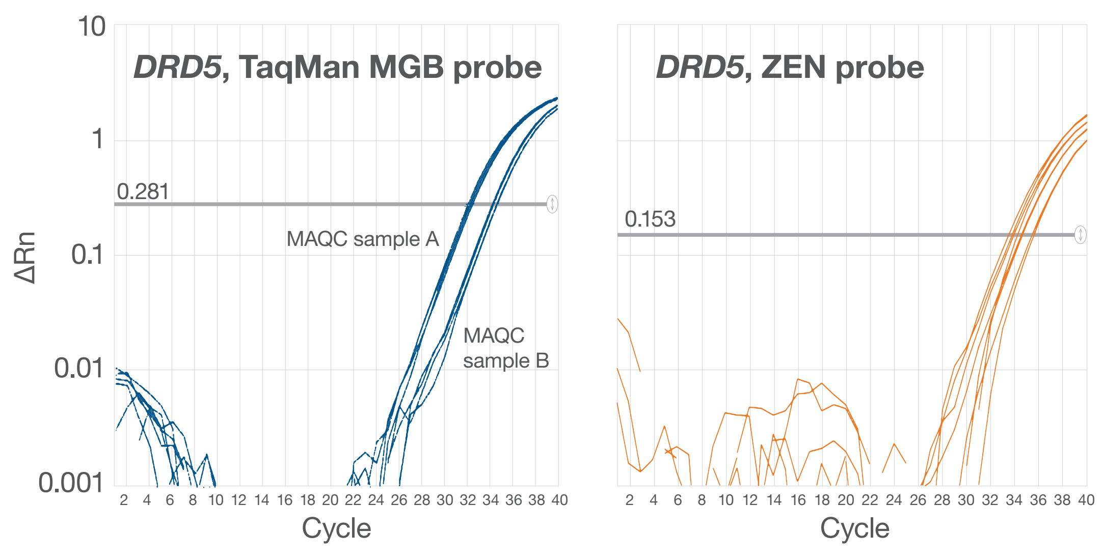
Consistency of probes in singleplex qPCR assays



Key takeaways

- A TaqMan MGB probe targeting *PPIA* displayed lower variability among technical replicates than the corresponding ZEN probe (circled)
- TaqMan Assays, which are designed using our proprietary bioinformatics design pipeline, enable highly consistent results

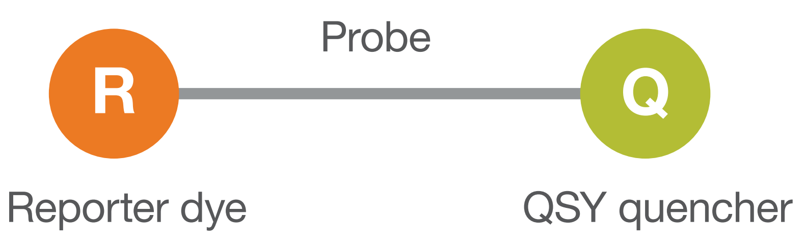
Discrimination between template levels



Key takeaways

- In a test of two samples containing different levels of *DRD5* template, the TaqMan MGB probe produced tighter amplification curves within replicates of each sample
- Less variability among technical replicates enables more reliable discrimination between samples

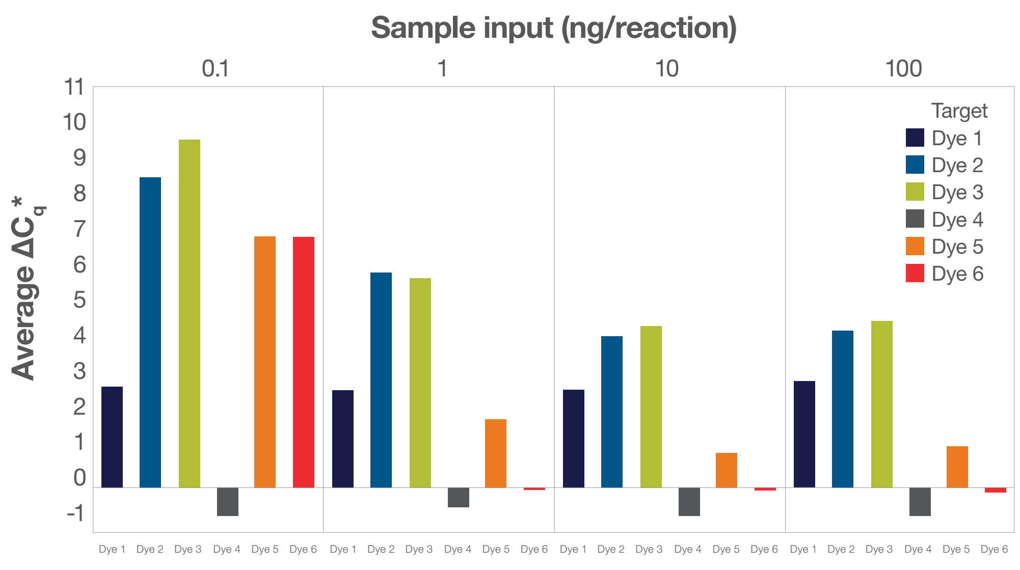
TaqMan QSY and QSY2 probes



Key features

- Designed for multiplexing, Applied Biosystems™ TaqMan™ QSY™ and QSY2™ probes enable assay developers to maximize the number of targets per sample
- QSY probes are for multiplexing up to 4 targets with ABY™, JUN™, FAM™, and VIC™ reporter dyes
- QSY2 probes are for 5th- and 6th-target multiplexing with cyanine 5 and cyanine 5.5 reporter dyes, which provide excellent signal-to-noise ratios for detecting targets in the far-red spectrum

Sensitivity of probes in multiplex assays

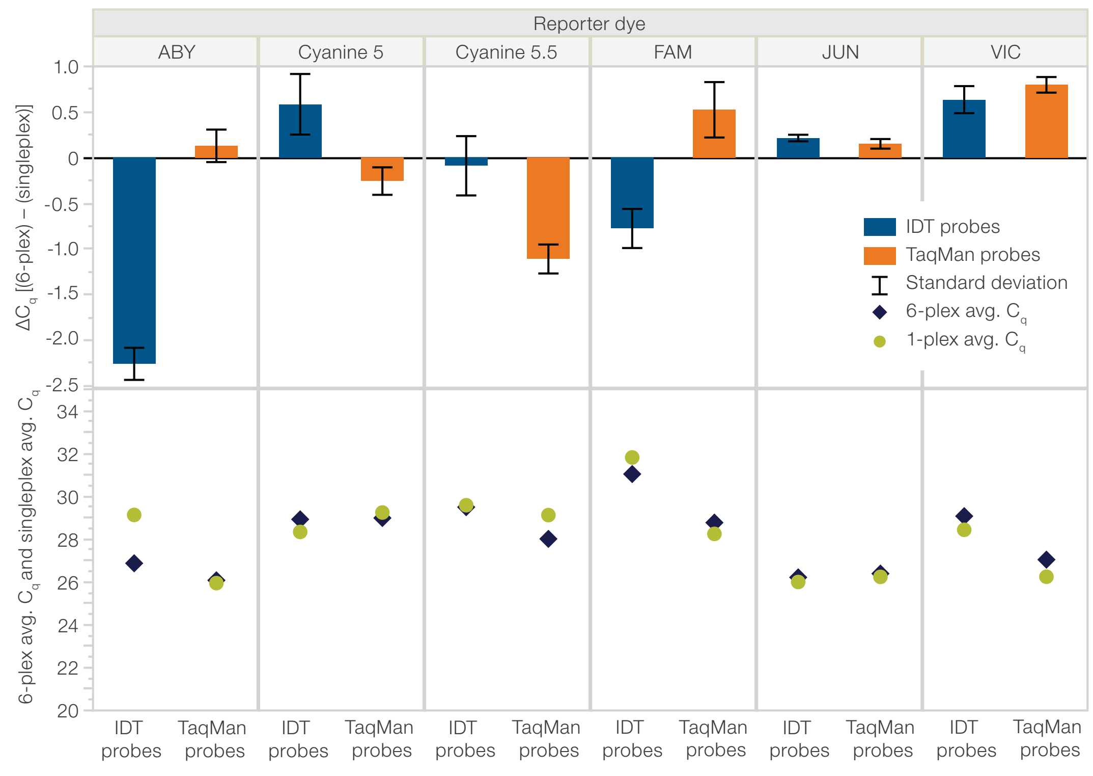


* Average ΔC_q = (average C_q using IDT probes) – (average C_q using TaqMan probes)

Key takeaways

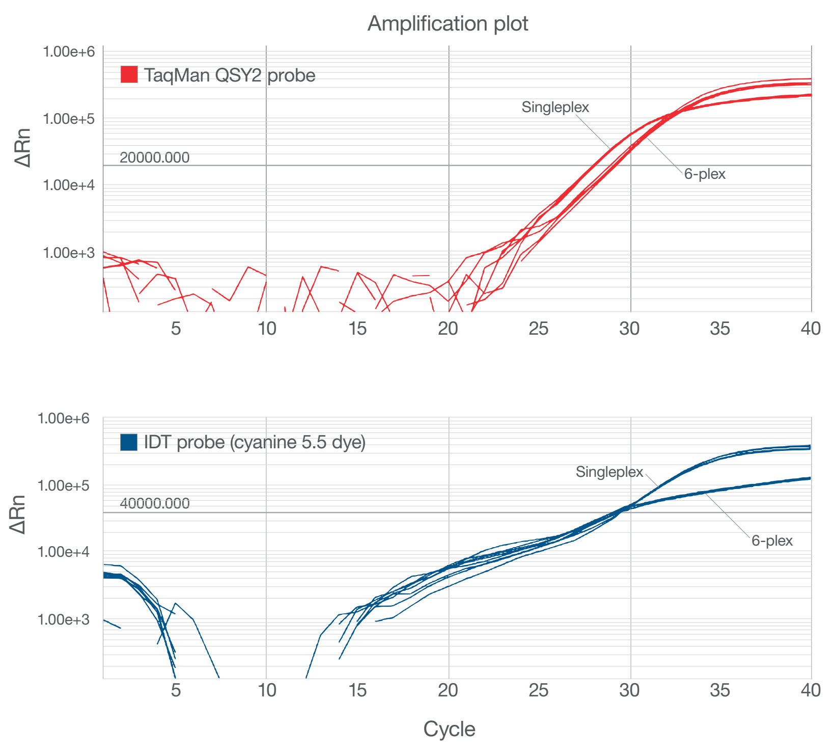
- In this 6-plex assay, a positive average ΔC_q indicates a lower average C_q for the TaqMan probe, and thus greater sensitivity compared to its counterpart from IDT
- TaqMan QSY and QSY2 probes showed a positive average ΔC_q 70% of the time (17 out of 24 assays) using different sample inputs
- Enhanced sensitivity allows TaqMan Assays incorporating QSY probes to detect targets at lower concentrations—even when multiplexing

Consistency between multiplex and singleplex qPCR assays



Key takeaways

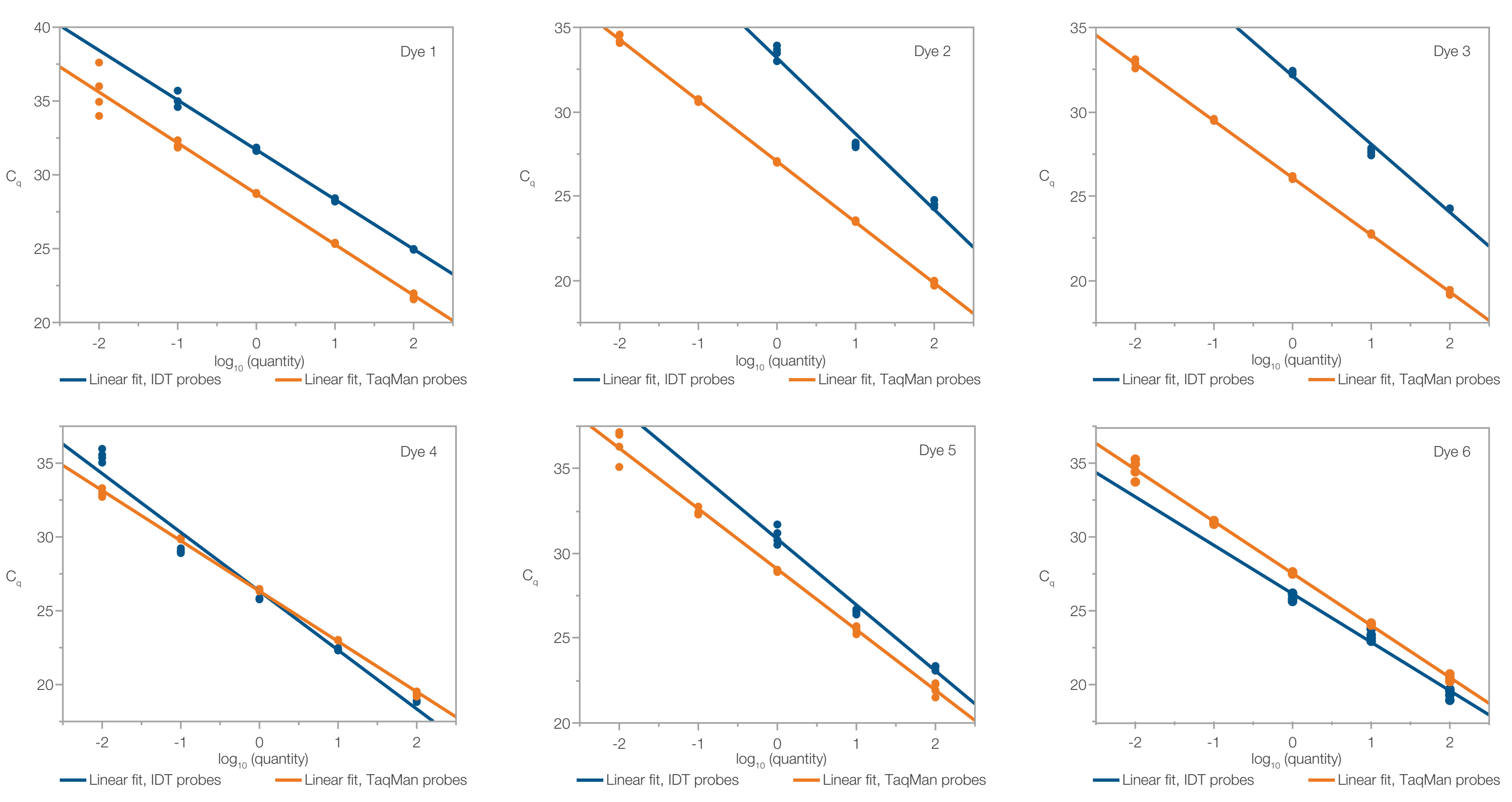
- In this experiment, ΔC_q values (top of figure) result from subtraction of singleplex C_q from 6-plex C_q (both shown at bottom of figure)
- TaqMan QSY and QSY2 probes had smaller ΔC_q values 67% (4/6) of the time, indicating greater consistency between 6-plex and singleplex assays



Key takeaways

- TaqMan QSY and QSY2 probes also produced more similar data when run in singleplex and 6-plex formats, as illustrated by this example showing tighter overlay of amplification curves
- Scaling to a 6-plex assay can be challenging, but more consistent and predictable performance allows for easier assay development

Dynamic range in multiplex qPCR assays



Key takeaways

- TaqMan QSY and QSY2 probes demonstrated equal or greater dynamic range compared to IDT probes in a 6-plex assay
- The wide dynamic range of TaqMan Assays allows for accurate and precise detection of targets within a larger sample window

Experience the difference with TaqMan Assays

TaqMan probes consistently outperformed their IDT counterparts in sensitivity, consistency, and scalability of qPCR assays. These results are part of a track record of excellent performance—TaqMan Assays have been cited in over 296,000 scientific publications. With TaqMan Assays, you can confidently push the boundaries of what's possible in assay development.

For more details on the experiments performed, please see our **TaqMan QSY and QSY2 flyer**. To order QSY probes for multiplexing, please visit our **product page**.

Finding the right TaqMan Assays

- Easily search our comprehensive library of over 20 million predesigned TaqMan Assays using our **TaqMan Assay Search Wizard**
- Assays are designed for popular applications like gene expression analysis, SNP genotyping, microRNA analysis, and copy number detection
- Or, design your own TaqMan Assay using the online tools available at our **Assay Design Hub**

Learn more at **thermofisher.com/taqman**

applied biosystems