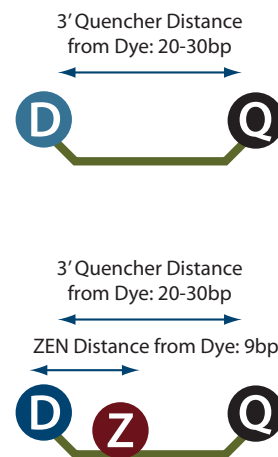


ZEN™ Internal Quencher Double-Quenched Probes™

IDT has developed a new internal ZEN quencher that enables the production of double-quenched probes with less background and more signal!

- **Reduce Background Fluorescence** - The internal ZEN placement decreases the length between the dyes and quenchers to only 9 bp and significantly reduces background fluorescence.
- **Improve Sensitivity** - Using double-quenched probes increases the end point signal and reduces C_q values.
- **Improve Precision** - Double-quenched probes increase precision by reducing background noise.
- **Maximum Quenching for Longer Probes** - Even probes as long as 40 bp stay quenched due to the proximity of ZEN to the reporter dye.



The double-quenched probe (5'FAM/ZEN/3'IBFQ) has both a lower background and higher signal than traditional dye-quencher combinations. The fluorescent background (Rn) of the double-quenched probe is nearly 4 times lower than traditional dual-labeled probes (Figure 1a) and the delta Rn signal is increased by approximately 30% when using 5'FAM/ZEN/3'IBFQ (Figure 1b).

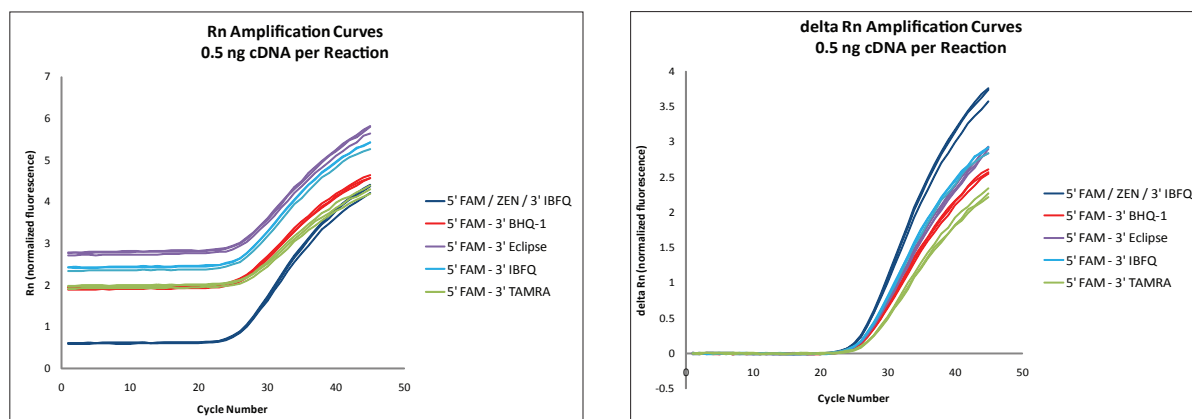


Figure 1a and 1b. 5'FAM probes with five different quenchers: ZEN - Iowa Black® FQ (ZEN/IBFQ), Black Hole Quencher® (BHQ), Eclipse®, Iowa Black FQ (IBFQ), and TAMRA. Each probe was synthesized to target the ACTB locus and all were run in triplicate with the same primer products and 0.5ng of cDNA. All reactions were run with the Applied Biosystems TaqMan® Gene Expression Master Mix under standard cycling conditions on the Applied Biosystems 7900HT.

Improve qPCR Sensitivity and Precision

The 5' FAM/ZEN/3' IBFQ probes exhibit a lower average C_q and increased C_q precision than traditional dual-labeled probes at all cDNA concentrations tested. Thus, use of the double-quenched probe can allow researchers to experience both increased sensitivity and precision in their qPCR experiments.

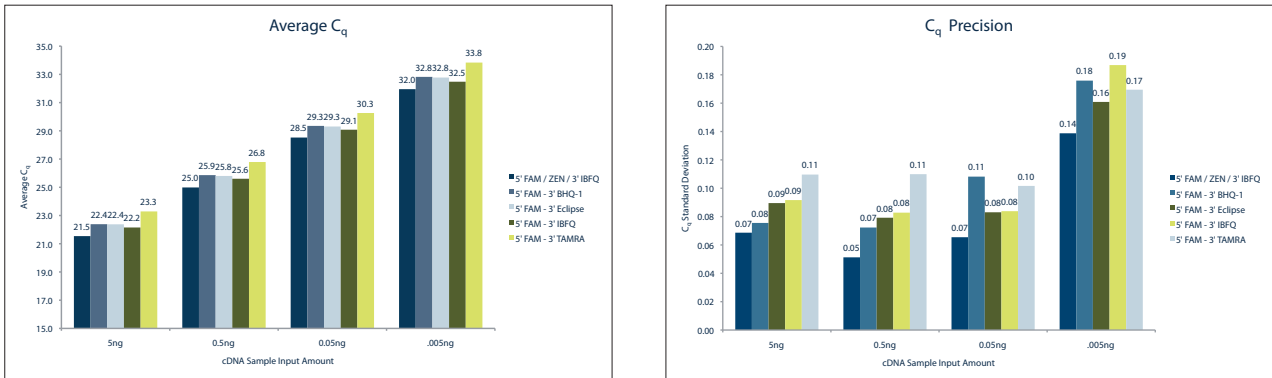


Figure 2a and 2b. 5' FAM probes with five different quenchers: DQP – ZEN and lowa Black FQ (ZEN/IBFQ), Black Hole Quencher (BHQ), Eclipse, lowa Black FQ (IBFQ), and TAMRA were individually synthesized a total of ten times for each quencher type for a total of 50 unique primer-probe assays. Each reaction used the same primer and probe sequences targeted to a region of the ACTB locus and all were run in triplicate with the same primer products and the indicated amount of cDNA. Each standard curve was the result of a 5-log, 10-fold cDNA dilution starting at 5ng per reaction. All reactions were run with the Applied Biosystems TaqMan® Gene Expression Master Mix under standard cycling conditions on the Applied Biosystems 7900HT.

Low Background Even With Long Probes

When tested with increasing probe lengths, the double-quenched probes (5' FAM/ZEN/3' IBFQ) showed consistently low levels of background fluorescence. Although most traditional probes do not remain well quenched over 30 bp, the double quenched probes maintained consistently low background. Even at 40 bp the double-quenched probe was almost four times darker than the BHQ-quenched probe. This ability to have effective, longer probes will allow design even in AT-rich targets.

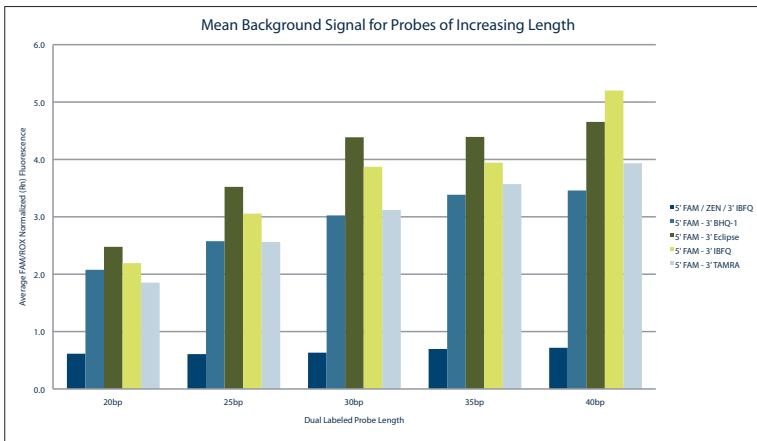


Figure 3. Probes with 5 different quenchers: DQP – ZEN and lowa Black FQ (ZEN/IBFQ), Black Hole Quencher (BHQ), Eclipse, lowa Black FQ (IBFQ) and TAMRA and 5 different base pair lengths (40, 35, 30, 25, and 20) were tested for a total of 25 different probe types. Six replicates of each probe type were mixed with 10ng/μL cDNA and the Applied Biosystems TaqMan® Gene Expression Master Mix and then cycled under standard cycling conditions on the Applied Biosystems 7900HT. Mean background (Rn) measurements were calculated to determine the average background fluorescent signal.

How to Order

Visit the IDT website at www.idtdna.com to order double-quenched probes and other PrimeTime® products.

TaqMan® is a registered trademark of Roche Molecular Systems that is licensed exclusively to Applied Biosystems Inc. for use in certain non-diagnostics fields. Black Hole Quencher® (BHQ) is a registered trademark of Biosearch Technologies, Inc. PrimeTime® and lowa Black® (IBFQ) are registered trademarks of Integrated DNA Technologies, Inc. Eclipse® is a registered trademark of Epoch Biosciences.