BIOLUMINOR

Black Hole Quencher Dyes

—The Inescapable Solution for DNA Hybridization Probes

A NEW class of high efficiency Dark quenchers

Custom-synthesized hybridization probes incorporate spectrally paired fluorophores and quenchers, each covalently linked to minimize interference with probe-target hybridization. The BHQ dyes are a new class of high-efficiency dark quenchers that prevent fluorescence until a hybridization event occurs. These powerful and highly specific dyes enable the identification and quantification of a variety of biomolecules.

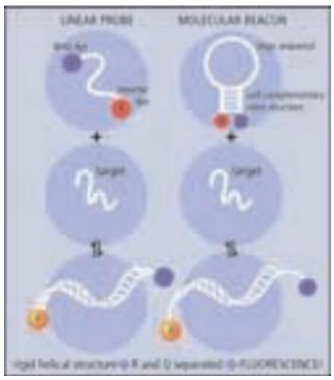


Figure **1** Hybridization event turns on fluorescence. BHQ dyes are compatible with linear probes and beacons on any real-time qPCR platform or end-point fluorescent plate reader.

Optimized for FRET— by design

FRET is a highly distance-dependent interaction between a reporter dye in an excited state and a quencher in its ground state. Energy is transferred from one molecule (the fluorophore) to the other (the quencher) without the emission of a photon.

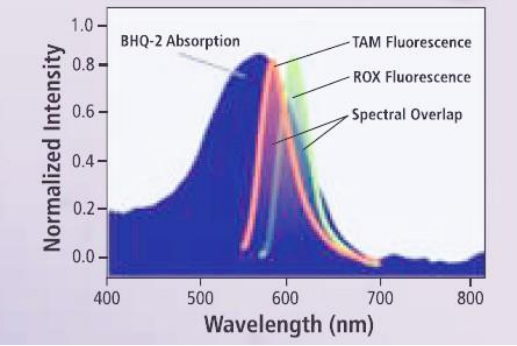


Figure 2 Spectral overlap of BHQ-2 dye with TAMRA and ROX.

Each member of the family of BHQ dyes was conceived and designed to maximize spectral overlap, thus increasing the efficiency of quenching.

True dark quenchers—NO native fluorescence

The two most commonly used quenchers, DABCYL and TAMRA, both limit the ultimate sensitivity and flexibility of FRET assays. DABCYL has an inadequate absorption footprint that overlaps very poorly with fluorophores emitting above 480 nm. TAMRA is not a dark quencher and contributes to an overall increase in background because of its own native fluorescence. As shown in Figure 3, each of the BHQ dye probes have much larger signal-to-noise ratios when compared to the corresponding DABCYL and TAMRA probes.

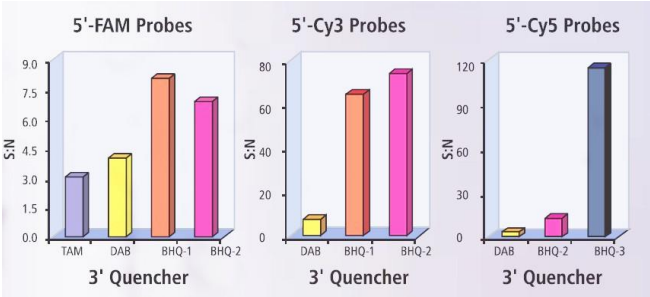


Figure 3 Signal-to-noise (S:N) ratios were calculated by dividing the fluorescence signal of a 25-mer in the presence of a five-fold excess of an exactly complementary target sequence by the fluorescence intensity of the probe alone. Each probe was formulated with a 5’ reporter group (FAM, Cy3, Cy5) and a quencher (TAMRA, DABCYL, BHQ-1, BHQ-2 or BHQ-3).

Access the visible spectrum and near-IR for reporting — 480 to 730 nm

The BHQ family of quenchers was developed to provide excellent spectral overlap over the entire

range of commonly used reporter dyes. As shown in Figure 4, the BHQ dyes cover the spectrum from 480 nm into the near IR making it possible to utilize reporter dyes that emit anywhere in this range.

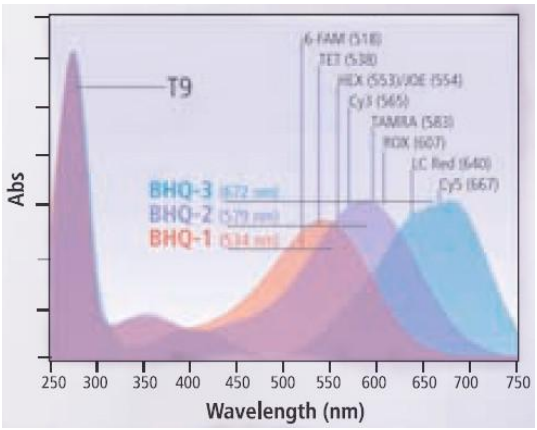
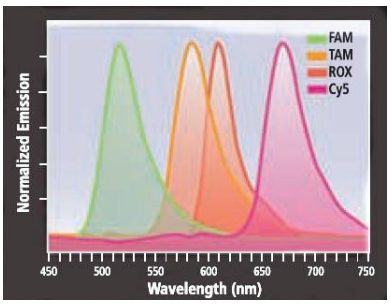


Figure 4 Absorbtion spectra of the three BHQ dyes (conjugated to T-9 and normalized to the poly-T absorbance of 260 nm) with the emission maxima of many commonly used reporter groups indicated.

Enable wider choice of reporter dyes for multiplexing

Historically, this drawback has resulted from an absence of quenchers able to effectively quench throughout the visible and near-IR spectra. As shown in Figure 5, the BHQ family readily permits single-tube multiplexing due to the increased variety of reporter dyes that can be effectively quenched with little or no cross-talk between reporters. The broader spectral coverage of the BHQ dyes provides the scientist with a larger pool of distinct, spectrally resolved reporter dyes which simplifies the design, implementation and interpretation of multiplexed hybridization probe assays.



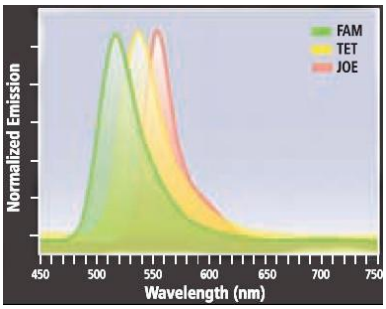


Figure 5 The unique characteristics of the BHQ class of quenchers permits flexibility in the choice of spectrally well-resolved fluorophores enabling single-tube multiplexing with little or no cross-talk.

Wide variety of probe formulations

BHQ dyes function as efficient dark quenchers over the entire visible spectrum and into the near-IR, re-emitting their energy as heat rather than light. Probes made with BHQ dyes exhibit extremely low background fluorescence, enabling enhanced detection sensitivity.

In addition, we have developed functionalized BHQ dyes for internal modifications. We would be pleased to work with you to design BHQ probes specific for your application!

BHQ Dye Absorption Maxima and Quenching Range

Quencher	Abs_{max}	Quenching Range (nm)
BHQ-1	534	480-580
BHQ-2	579	550-650

BHQ Dye / Reporter Combinations

Quencher	Suggested Fluorophres
BHQ-1	FAM, TET, JOE, HEX, Oregon Green®
BHQ-2	TAMRA, ROX, Cy3, Cy3.5, CAL Red™, Red 640

References

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