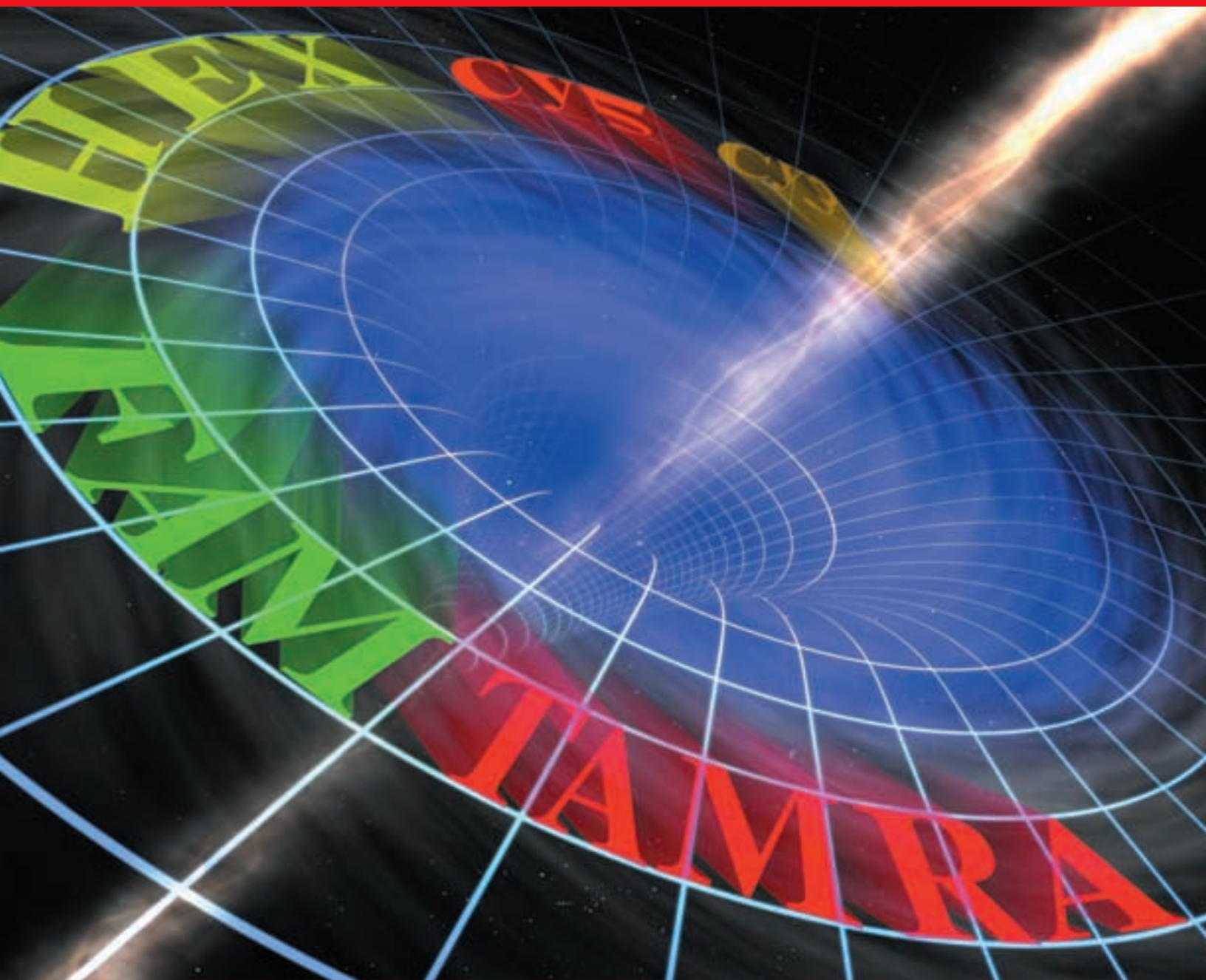


Black Hole Quencher™ Dyes

The Inescapable SolutionSM for DNA Hybridization Probes



A New Class of High-Efficiency Dark Quenchers

- Real-time, quantitative PCR
- SNP discovery, detection & scoring
- Allelic discrimination
- Spectral genotyping
- *in situ* hybridization
- Single-tube multiplexing

They're Here!

Black Hole Quenchers

- A NEW class of high-efficiency dark quenchers
- Optimized for FRET — by design
- Dyes that really go the distance
- DABCYL eclipsed by BHQ™ dyes
- TRUE dark quenchers — NO native fluorescence
- Access visible into near-IR spectrum for reporting — 480 to 730 nm
- Enable wider choice of reporter dyes for multiplexing
- Wide variety of probe formulations

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.

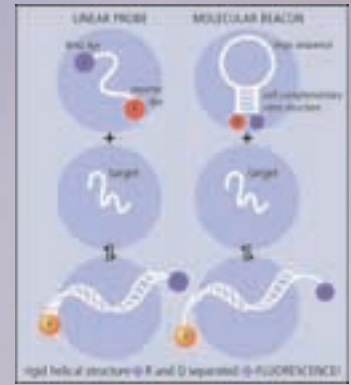
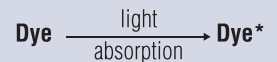


Figure 1 Hybridization event turns on fluorescence. BHQ dyes are compatible with linear probes and beacons on any real-time Q-PCR platform or end-point fluorescent plate reader.

Optimized for FRET— by design!

Hybridization probes are designed to take advantage of quenching by fluorescence resonance energy transfer (FRET) to detect and report binding to target molecules.

1st step: The dye molecule absorbs light and generates the dye excited state.



2nd step: In the absence of quenching—fluorescence!



2nd step when FRET occurs: The dye excited state transfers energy to the quencher ground state generating quencher excited state.



3rd step: The quencher returns to the ground state.

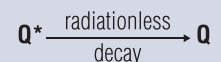


Figure 2 The FRET Process.

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.

In order for efficient FRET quenching to take place: a) the fluorophore and quencher molecules must be close to each other (approx. 10 - 100 Å) and, b) the absorption spectrum of the quencher must overlap with the emission spectrum of the fluorophore.

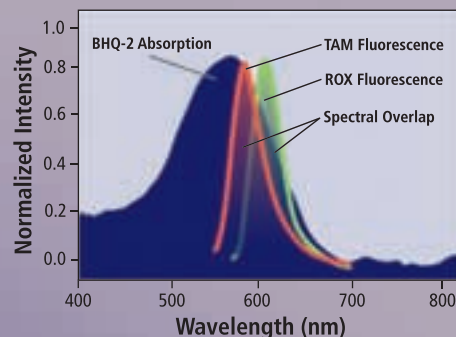
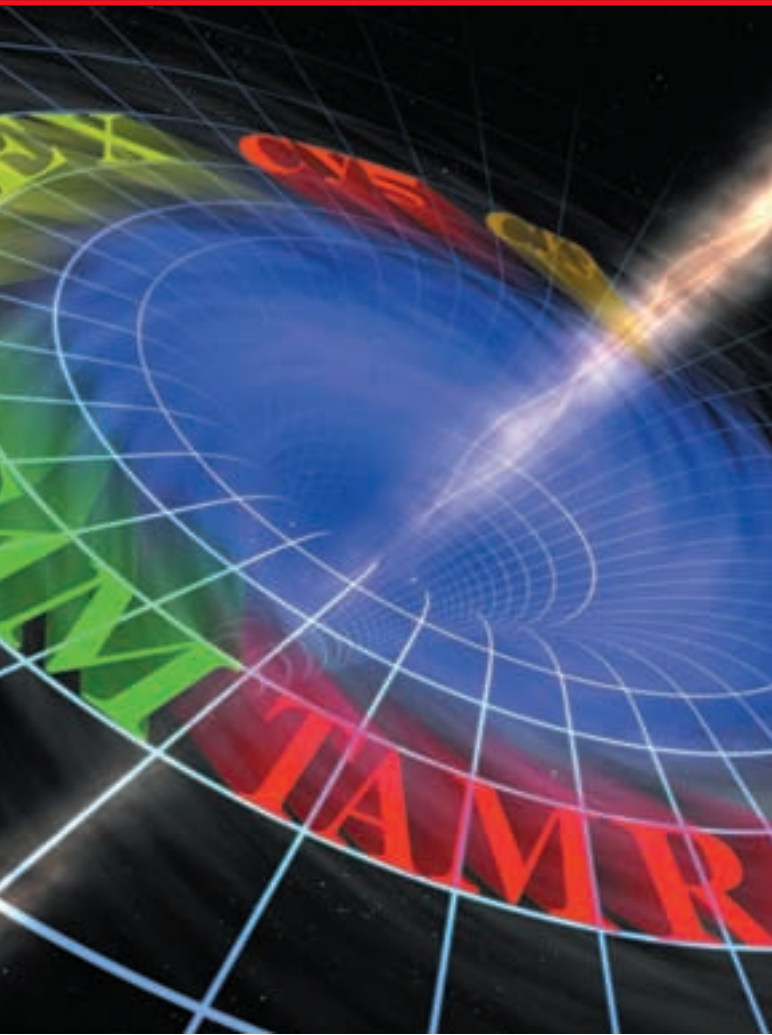


Figure 3 Spectral overlap of BHQ-2 dye with TAMRA and ROX.

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



Dyes that really go the distance

FRET quenching efficiency depends on $1/r^6$ where r is the dye-quencher distance. In solution, unhybridized FRET probes are poised to exist as random coils allowing the reporter and quencher dyes to remain in close proximity favoring FRET quenching. Upon hybridization to a complementary target, the probe is stretched out of its random coil configuration. Thus, the reporter and quencher are separated and increased fluorescence results.

The efficiency of FRET is given by:

$$E = R_0^6 / (R_0^6 + r^6) \quad \text{where } R_0 = \text{Förster distance}$$

$$r = \text{donor-quencher distance}$$

$$R_0^6 \propto Q_d \text{ and } J \quad \text{where } Q_d = \text{donor fluorescence efficiency}$$

$$J = \text{overlap integral}$$

DABCYL eclipsed by BHQ dyes

Quenching efficiency increases as spectral overlap J of the dye emission and quencher absorption profile increases. Historically, DABCYL has been routinely used as a general-purpose dark quencher for many commonly used fluorophores including FAM, TET, and JOE. As shown in Figure 4, however, DABCYL's absorption maximum of 474 nm places it below the maxima of the shown fluorophores, limiting its efficiency to quench via FRET. The BHQ-1 dye (with an absorption maximum of 534 nm) is above DABCYL and directly superimposable with emission maxima of FAM, TET and JOE, providing a significant increase in FRET quenching efficiency.

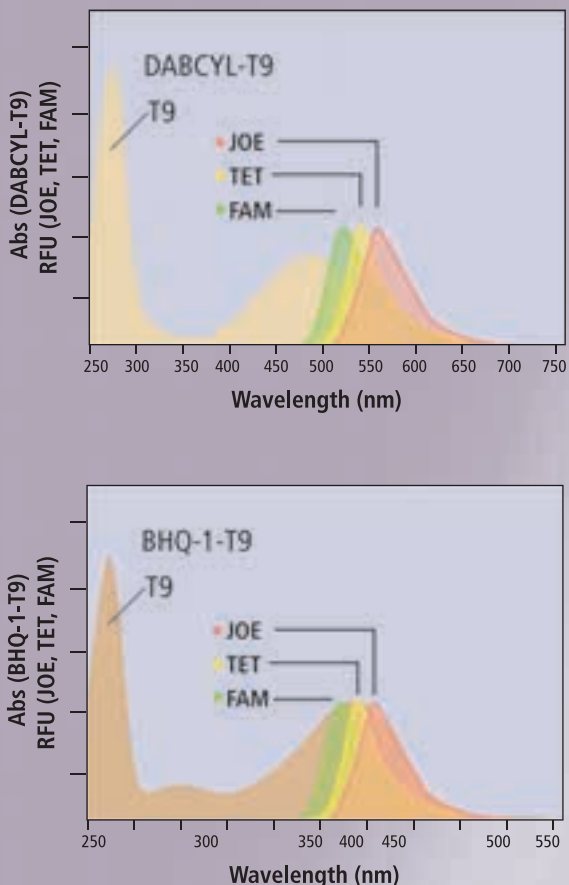


Figure 4 Absorption spectra of DABCYL-T9 and BHQ-1-T9 with the emission spectra of FAM, TET and JOE. DABCYL and BHQ-1 dye were normalized at the poly-T absorbance (i.e. 260 nm) to better demonstrate the larger extinction coefficient of the BHQ-1 dye. RFU=relative fluorescence units.

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 5, each of the BHQ dye probes have much larger signal-to-noise ratios when compared to the corresponding DABCYL and TAMRA probes.

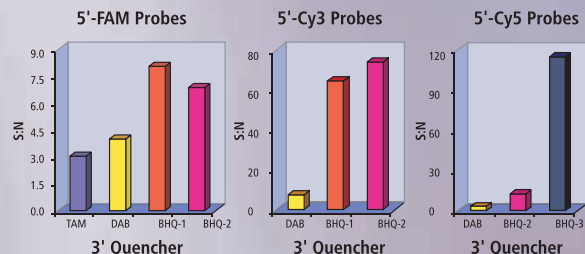


Figure 5 Signal-to-noise (S:N) ratios were calculated by dividing the fluorescence signal of a 25-mer in the presence of a five-fold excess of an exactly complementary target sequence by the fluorescence intensity of the probe alone. Each probe was formulated with a 5' reporter group (FAM, Cy3, Cy5) 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 6, 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.

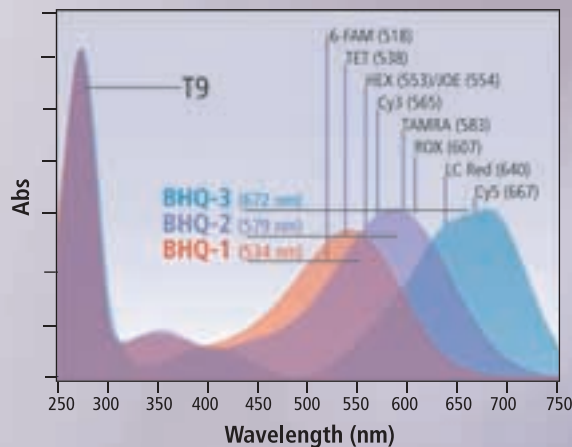


Figure 6 Absorption spectra of the three BHQ dyes (conjugated to T-9 and normalized to the poly-T absorbance of 260 nm) with the emission maxima of many commonly used reporter groups indicated.

Enable wider choice of reporter dyes for multiplexing

As detection instrumentation has become more sophisticated, the ability to multiplex several fluorophores in a single reaction tube has gained much interest. One of the major drawbacks in any FRET-based multiplexing assay is cross-talk between the fluorophores, which hampers the ability to distinguish between dyes. 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 7, 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.

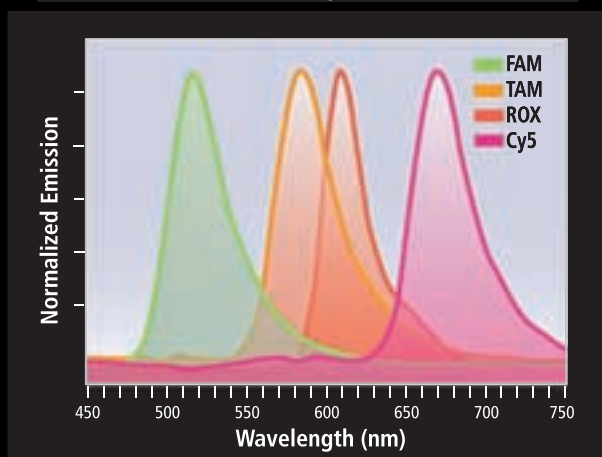
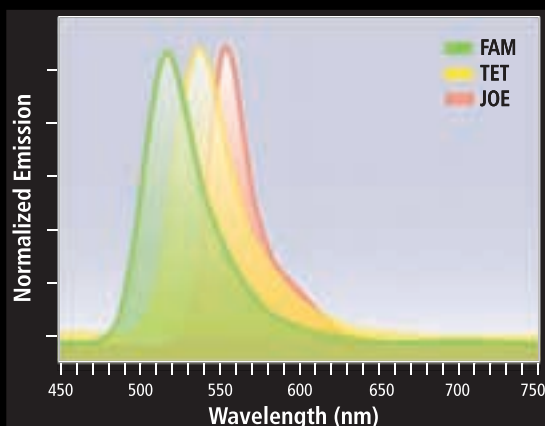


Figure 7 The unique characteristics of the BHQ class of quenchers permits flexibility in the choice of spectrally well-resolved fluorophores enabling single-tube multiplexing with little or no cross-talk.

Wide variety of probe formulations

Biosearch can incorporate BHQ dyes into almost any probe formulation: from linear DNA probes to molecular beacons. 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 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.

BHQ Dye Absorption Maxima and Quenching Range

Quencher	Abs _{max}	Quenching Range (nm)
BHQ-1	534	480 – 580
BHQ-2	579	550 – 650
BHQ-3	672	620 – 730

BHQ Dye / Reporter Combinations

Quencher	Suggested Fluorophores
BHQ-1	FAM, TET, JOE, HEX, Oregon Green®
BHQ-2	TAMRA, ROX, Cy3, Cy3.5, CAL Red™, Red 640
BHQ-3	Cy5, Cy5.5

References

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