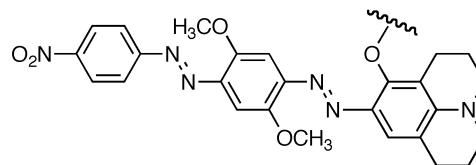


BlackBerry[®] Quencher 650 - A new dark quencher of fluorescence

- Excellent quencher of long-wavelength fluorescence in both FRET and contact modes.
- $\lambda_{\text{max}} \sim 650 \text{ nm}$; useful absorbance between 550 and 750 nm.
- Compatible with standard oligonucleotide synthesis chemistry.
- Internal, 3', or 5' installation.

While in the excited state, fluorophores are sensitive to their environment and may lose excitation energy by several processes besides emission of a fluorescence photon. Such *fluorescence quenching* can occur by collision or molecular motion (dynamic quenching), excited state reaction with other molecules (photobleaching), contact quenching (the formation of a nonfluorescent ground state complex, also known as static quenching), or energy transfer to another molecule (fluorescence resonance energy transfer, or FRET). Many nucleic acid fluorescence detection techniques use probes that bear both a fluorophore and a quencher, relying on FRET- or contact-mode quenching to diminish fluorescence until a hybridization event occurs. Upon hybridization, the fluorophore and quencher are separated in space, resulting in an increase in fluorescence. The efficiency of FRET from the fluorophore to the quencher (i.e., the magnitude of quenching) depends on the relative orientation of their transition dipoles, and the distance between them, and how well the absorption spectrum of the quencher overlaps the emission spectrum of the fluorophore. The efficiency of contact quenching depends on the ability of the fluorophore and quencher to form a ground-state complex, and correlates with the mutual affinity of the two species and the distance between them. Quenchers may themselves be fluorescent, emitting a photon at a longer wavelength than the acceptor fluorophore. More conveniently, *dark quenchers* can be used, which are non-fluorescent chromophores that, in FRET mode, can absorb energy from the excited state of the fluorophore, preventing emission of a fluorescence photon. The resultant excited state of a dark quencher relaxes to the ground state by radiationless decay (heat). In contact mode, the dark quencher forms a non-fluorescent ground-state complex with the fluorophore, masking its fluorescence until the complex is disrupted by a hybridization event.



The archetypal dark quencher is dabcyI, which is often installed at the 3'-terminus of an oligonucleotide probe and is useful for quenching relatively short wavelength fluorophores such as carboxyfluorescein (FAM). Its ability to quench longer wavelength fluorophores is limited by its absorption spectrum, which tails off in the range where these fluorophores emit. The advent of high-throughput multiplex assays involving the simultaneous use of fluorophores of different colors has fostered the development of dark quenchers that can quench fluorescence at longer wavelengths. A desirable characteristic of such quenchers is a long-wavelength absorption maximum. Unfortunately, the chemical stability of such compounds is often diminished. Extended π systems and/or the use of stronger donor-acceptor functional group pairings can lead to sensitivity to oligonucleotide synthesis reagents, e.g., oxidants such as iodine and deblocking agents such as ammonia and AMA.

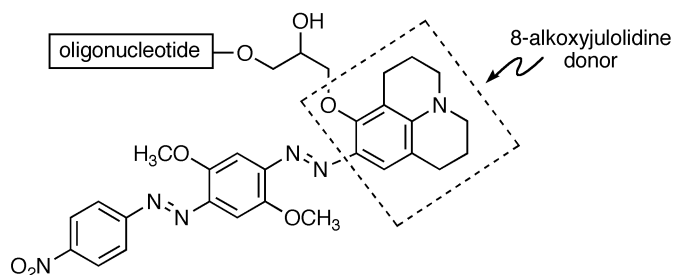


Figure 1. BlackBerry[®] Quencher 650 (BBQ-650[®]).

Berry & Associates has introduced the BlackBerry[®] Quencher 650 (BBQ-650[®]), a synthesis-stable dark quencher of long wavelength fluorescence (Figure 1). An 8-alkoxyjulolidine moiety was found to be a powerful π -electron donor, *affording a surprising bathochromic shift when compared to related compounds*. The absorption spectrum of a BBQ-650[®]-tagged oligonucleotide is shown in Figure 2. The broad absorbance centered around 650 nm effectively overlaps the emission maxima of popular long-wavelength fluorophores such as Cy3, TAMRA, Texas Red, ROX, Cy5, and Cy5.5, allowing efficient quenching.

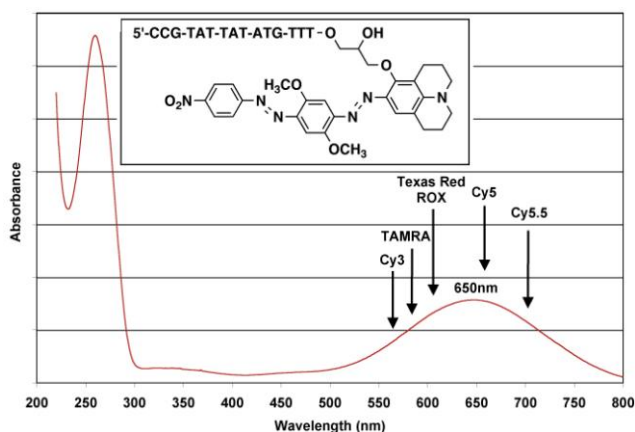


Figure 2. Absorption spectrum of a 15-mer bearing a 3' BlackBerry® Quencher 650.

We offer BlackBerry Quenchers that may be installed at the 3' terminus, internally, or at the 5' position (See Ordering Information Table). Standard phosphoramidites and synthesis protocols may be employed with these reagents except that BBQ-650®-dT CEP (BL 1010) should be dissolved in 4:1 dichloromethane-acetonitrile and coupled for 15 min. *We recommend mild nucleobase deprotection with ammonium hydroxide or AMA to avoid degradation of the quencher (ammonium hydroxide, 10 min. 65 °C).* For quantification, the following extinction coefficients may be useful, which were determined using a simple BBQ chromophore (i.e., no oligonucleotide): At 598 nm in methanol, $\epsilon = 40,667 \text{ M}^{-1}\text{cm}^{-1}$; at 260 nm in methanol, $\epsilon = 15,077 \text{ M}^{-1}\text{cm}^{-1}$.

To evaluate BlackBerry® Quencher 650 in *contact quenching mode*, molecular beacon probes bearing various 5'-fluorophores (FAM, Cy3, Texas Red, Cy5, Cy5.5) were synthesized using 3'-BBQ-650® CPG. Signal-to-background ratios upon binding to fully-complementary target were excellent, e.g., >90 with Cy5 and >88 with Cy5.5. Melting temperatures showed that BBQ-650® had a particularly high affinity for Cy dyes; labeled stem hybrids melted 11-14 °C higher than non-labeled stem hybrids. The probes were successful in typing C to T transitions at positions 627 and 630 of the human chemokine receptor 5 gene,¹ and produced excellent results in real-time PCR studies.

BlackBerry® Quencher 650 was also found to be an *excellent FRET-mode quencher*. Pairs of complementary strands were designed that would bring a 5'-Cy5.5 fluorophore to within 5 or 10 base pairs (20-40 Å) of a 3'-BBQ-650® upon hybridization,² where quenching efficiencies of ≥ 98.3 and $\geq 98.9\%$, respectively, were observed. Melting temperatures of these hybrids were unchanged from non-labeled hybrids, showing that these quenching efficiencies were due to FRET quenching and not contact quenching.

Acknowledgement: We thank Dr. Salvatore A. E. Marras for helpful discussions and for probe studies involving BlackBerry® quenchers.

References

- (1) Marras, S. A. E.; Kramer, F. R.; Tyagi, S. *Methods in Molecular Biology* **2003**, *212*, 111-128.
- (2) Marras, S. A. E.; Kramer, F. R.; Tyagi, S. *Nucleic Acids Res.* **2002**, *30*, e122.

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BlackBerry® Quencher Products - Ordering Information

Item	Catalog No.	Size/pack	Price (USD)
3'-BBQ-650 [®] CPG	BL 2010	200 nmol columns (pkg of 4)*	\$75.00
		1 µmol columns (pkg of 4)*	\$210.00
		100 mg	\$135.00
		1 g	\$1050.00
3'-BBQ-650 [®] CPG II	BL 2020	200 nmol columns (pkg of 4)*	\$75.00
		1 µmol columns (pkg of 4)*	\$210.00
		100 mg	\$135.00
		1 g	\$1050.00
3'-BBQ-650 [®] CPG III	BL 2030	200 nmol columns (pkg of 4)*	\$80.00
		1 µmol columns (pkg of 4)*	\$230.00
		100 mg	\$147.00
		1 g	\$1140.00
Fmoc-Lysine(BBQ-650 [®])-OH	BL 2040	50 mg	\$95.00
		100 mg	\$710.00
BBQ-650 [®] -dT CEP	BL 1010	50 µmol	\$150.00
		100 µmol	\$275.00
		0.25 g	\$650.00
5'-BBQ-650 [®] CEP	BL 1020	50 µmol	\$160.00
		100 µmol	\$280.00
		0.25 g	\$675.00
BBQ-650 [®] (DMT) CEP	BL 1030	100 µmol	\$390.00
		0.25 g	\$695.00
BBQ-650 [®] NHS ester	BL 3010	5 mg	\$95.00
		25 mg	\$375.00

*Add "-E" to part number for Expedite columns, "-A" for ABI-style crimp columns

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