BioFeedback

The Biofeedback section accommodates letters to the editor, addenda, errata and other comments on previously published articles.

Novel Energy Transfer Fluorescence Labeling Cassette

The recent article by Vladimir Didenko (3) reviews the design approaches and applications of various DNA-based probes that use fluorescence resonance energy transfer (FRET). It is exciting to see the increasing applications and benefits of this energy transfer (ET) labeling technology. Although comprehensive, this excellent review did not mention the latest energy transfer cassette reagent, probably because it is such a recent development (1,2,4). We would like to complete this review by briefly mentioning this advancement, which is important because it can make fluorescent ET labeling technology a practical option for all users of fluorescently labeled oligonucleotides.

We recently developed a convenient and simple synthetic method for ET labeling any primer or oligonucleotide of interest with an ET cassette (1). Figure 1A presents the structure of such an ET cassette-labeled primer. The synthesis and photophysical properties of these conjugates have been discussed in detail elsewhere (1). Briefly, the cassette consists of a sugar-phosphate spacer with a 6-FAM (6-carboxyfluorescein) donor at the 3'-end, an acceptor dye linked to a modified T-base at the 5'-end of the spacer, and a mixed disulfide group for coupling to the 5'-end of a thiol-modified primer or other target of interest through a disulfide exchange reaction (1). The coupling reaction is a two-step process (Figure 1B) that consists of primer deprotection in the presence of dithiothreitol (DTT), followed by conjugation with the reactive ET cassette (1,2). The acceptor-dye emission intensities of ET cassette-labeled primers produced in this manner are comparable to commercially available ET primers

Figure 1. ET cassette structure and labeling reaction. (A) Schematic representation of an ET cassette-labeled primer. R = 1',2' dideoxyribose monomer. (B) Schematic of the two-step quantitative ET cassette-primer conjugation (1,2,4).

(1), whose use has been limited in the past to standard sequencing applications. These ET cassettes are versatile and can be optimized for a particular use since the number of sugar phosphate monomers in the spacer can be fine-tuned to adjust electrophoretic migration and to alter the FRET efficiency, optimizing acceptor emission intensity or minimizing spectral cross talk (1,2).

DNA primers labeled with four-color ET cassettes (6-FAM donor and R110, R6G, TAMRA and ROX acceptor dyes; see Reference 1) have been constructed and used to demonstrate sequencing as well as multiplex genotyping with single-base-pair resolution (1,2,4). This labeling technology is particularly suited to large-scale genotyping, sequencing, and hybridization array assays (1–4). Further, it should be possible to develop cassettes with different dyes or dye/quencher combinations. When fully implemented, ET cassette technology has the potential to make FRET labeling, a very versatile and facile tool, available to a wide variety of users.

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Response to the Letter to the Editor by Igor L. Medintz, Lorenzo Berti, and Richard A. Mathies

I am gratified by the positive response to my recent review article (1), especially when it comes from the scientific group that made very significant contributions to the field of fluorescence resonance energy transfer probes. The papers mentioned in the letter are important and were not cited in the review because they were published after the final version of the review had already been submitted. This fast appearance of the new and interesting papers is just another sign of the rapid progress in the field of energy transfer probes.

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Early Shape-Shifting FRET Probe

It was brought to my attention that in my recent review of DNA probes using fluorescence resonance energy transfer (FRET) (2) I did not mention an early UniFluor probe (1). I will briefly discuss it here to correct this omission and give a better historical perspective of the homogenous FRET assays for hybridization detection.

Homogenous assays are performed in a closed-tube format and do not require additional washing steps to get rid of the unbound probe. The first homogenous assays employing FRET for hybridization detection appeared in the 1980s (3,5). However the FRET probes used in them consisted of two labeled oligonucleotide species. Positioning of both donor and acceptor fluorophores on the same DNA strand is more ad-

vantageous and ensures efficient energy transfer within the probe.

The UniFluor probe was the first single-molecule oligonucleotide probe designed to detect specific nucleic acid sequences with the help of FRET. The probe changes conformation when in contact with specific target sequences. This induces separation of fluorophore tags with cessation of FRET and results in the fluorescence increase. This mechanism is in general similar to that employed in molecular beacons, introduced by Tyagi and Kramer in 1996 (6). In this regard, the UniFluor can be viewed as their early precursor. However, the long dumbbell-shaped probe formed by two imperfect hairpins (1) is topologically different from molecular beacons. In addition, the "specificity sequence", which should hybridize to the target, is placed in the stem of the dumbbell (molecular beacons carry a single-stranded hybridization sequence in the loop). To maintain the stem, the probe also contains a complementary sequence, which is almost identical to the target it is supposed to detect. This design lessens the sensitivity of the probe and is disadvantageous in a multiplex simultaneous detection of various targets, which is one of the most attractive features of molecular beacons.

Shortly after the paper (1) was published, it was found that a linear oligonucleotide labeled with donor and acceptor fluorophores at its 5' and 3' ends makes a much simpler FRET probe (4). This resulted in the development of the more versatile and efficient TaqMan probes. Together with molecular beacons, these practical and convenient systems completely overshadowed the early UniFluor probe, which can still be viewed as a first attempt to employ the probe conformation change to signal-specific sequence detection.

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Corrigendum

The caption describing the cover of the January 2002 issue of *BioTechniques* was incorrect. We regret this error. The correct caption follows.

Laser confocal microscope images of fluorescent and DIC channels of hnRNPA1-dsRed1 and eGFP-NLS- β -gal in a bikaryon with the green and DIC channels (right panels) and the red and DIC channels (left panels) merged. Pre-bleach (top), 0' post-bleach (middle), and 30' post-bleach (bottom) panels are indicated. Fluorescence recovery after photobleaching (FRAP) can be seen for hnRNPA1-dsRed1 by comparing the right (acceptor) nucleus in the left center and bottom panels. No recovery is seen for the eGFP-NLS- β -gal protein (right center and bottom panels). See article by Howell and Truant, p. 80.