

# Single nucleotide polymorphism detection by combinatorial fluorescence energy transfer tags and biotinylated dideoxynucleotides

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## ABSTRACT

**Combinatorial fluorescence energy transfer (CFET) tags, constructed by exploiting energy transfer and combinatorial synthesis, allow multiple biological targets to be analyzed simultaneously. We here describe a multiplex single nucleotide polymorphism (SNP) assay based on single base extension (SBE) using CFET tags and biotinylated dideoxynucleotides (biotin-ddNTPs). A library of CFET-labeled oligonucleotide primers was mixed with biotin-ddNTPs, DNA polymerase and the DNA templates containing the SNPs in a single tube. The nucleotide at the 3'-end of each CFET-labeled oligonucleotide primer was complementary to a particular SNP in the template. Only the CFET-labeled primer that is fully complementary to the DNA template was extended by DNA polymerase with a biotin-ddNTP. We isolated the DNA extension fragments that carry a biotin at the 3'-end by capture with streptavidin-coated magnetic beads, while the unextended primers were eliminated. The biotinylated fluorescent DNA fragments were subsequently analyzed in a multicolor fluorescence electrophoresis system. The distinct fluorescence signature and electrophoretic mobility of each DNA extension product in the electropherogram coded the SNPs without the use of a sizing standard. We simultaneously distinguished six nucleotide variations in synthetic DNA templates and a PCR product from the retinoblastoma tumor suppressor gene. The use of CFET-labeled primers and biotin-ddNTPs coupled with the specificity of DNA polymerase in SBE offered a multiplex method for detecting SNPs.**

## INTRODUCTION

Single nucleotide polymorphisms (SNPs), the most common genetic variations found in the human genome, are important markers for identifying disease-associated loci and for pharmacogenetic studies (1,2). SNPs appear in the human genome with

an average density of once every 1000 bp (3). A variety of approaches have been used to detect SNPs, such as DNA sequencing (4), mass spectrometry (5), gene chips (6), single-stranded conformational polymorphism (7), denaturing capillary electrophoresis (8) and electrochemical detection (9).

Single base extension (SBE) is the main technique used for detecting SNPs (10). In this method, a primer is annealed to a DNA template and extended by DNA polymerase with a single dideoxynucleotide that is complementary to the nucleotide at the variable site. Primer extension with DNA polymerase discriminates single nucleotide differences at polymorphic sites with high accuracy. Multiplex fluorescence-based primer extension has been used in mutation analysis of mitochondrial DNA (11,12). We recently developed a high fidelity DNA sequencing method using solid phase capturable biotinylated dideoxynucleotide terminators by detection with fluorescence (13) and mass spectrometry (14), eliminating false terminations. We also reported an approach for developing a large number of combinatorial fluorescence energy transfer (CFET) tags by exploiting energy transfer (ET) and combinatorial synthesis to tune the fluorescence emission signatures for multiplex biological assays (15).

We here describe an approach that couples CFET tags and biotinylated dideoxynucleotides (biotin-ddNTPs) in SBE for multiplex SNP detection. The goal is to simultaneously detect CFET-labeled SBE fragments generated in a single tube, with the tag at the 5'-end of the fragments giving a unique fluorescence signature for each of the multiplex SNPs and a biotin at the 3'-end for solid phase capture, allowing only the extended DNA fragments to be isolated and detected. The nucleotide at the 3'-end of each CFET-labeled oligonucleotide primer was complementary to a particular SNP in the template. After SBE of the CFET-labeled primer, we isolated the primer extension fragments that carry a biotin at the 3'-end by capture with streptavidin-coated magnetic beads. The biotinylated DNA fragments are subsequently released and loaded onto a multicolor laser-induced fluorescence electrophoresis system to obtain accurate SNPs, while the unextended CFET-labeled primers are eliminated. The use of biotin-ddNTPs thus allows true SNPs to be isolated and identified. Using three different fluorescent dye molecules, we constructed eight CFET tags with unique fluorescence signatures. A 1',2'-dideoxynucleotide

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phosphate spacer is used to separate the donor-acceptor pair to tune the ET efficiency, producing unique fluorescence signatures in the CFET tags (15). The spacer also serves as a mobility tag to tune the mobility of CFET-labeled DNA for electrophoresis analysis. The previously reported multiplex SNP analysis methods using fluorescence primers require the use of a sizing standard in gel electrophoresis (16–19). Our method using CFET-labeled oligonucleotide primers and biotin-ddNTPs does not need a sizing standard because each of the SNPs is coded by a unique fluorescence signature and mobility in the electropherogram. We simultaneously detected six nucleotide variations in synthetic DNA templates and three nucleotide variations in a PCR product from the retinoblastoma tumor suppressor (*RBI*) gene. The use of CFET tag-labeled primer and biotin-ddNTPs coupled with the specificity of DNA polymerase for SBE provides a multiplex approach to conduct high throughput analyses of SNPs.

## MATERIALS AND METHODS

### Synthesis of CFET tags and oligonucleotides

The CFET tags were prepared by phosphoramidite chemistry in a DNA synthesizer and solution coupling synthesis. 6-Carboxyfluorescein (FAM) and *N,N,N',N'*-tetramethyl-6-carboxyrhodamine (TAM) were incorporated into the oligonucleotides with fluorescein-dT and TAMRA-dT phosphoramidites (Glen Research, Sterling, VA), respectively. A modified thymidine having an amino linker (amino modifier C6-dT; Glen Research) was incorporated into the oligonucleotide for coupling with Alexa 594 *N*-hydroxysuccinimidyl (NHS) ester (Molecular Probes, Eugene, OR). The 1',2'-dideoxyribose phosphate spacers were incorporated into the CFET tags with dSpacer phosphoramidite (Glen Research), which provided high coupling yield. To synthesize the CFET tag containing Alexa 594, 10–12 nmol FAM-labeled oligonucleotides containing an amino linker in 40  $\mu$ l of 0.25 M  $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$  buffer pH 9.0, were incubated for 10 h at room temperature with an ~27-fold excess of the Alexa 594 NHS ester in 12  $\mu$ l of anhydrous dimethylsulfoxide. Unreacted dye was removed by size exclusion chromatography on a PD-10 column (Amersham Pharmacia Biotech, Piscataway, NJ). The CFET tags were then purified by gel electrophoresis and desalted with an oligonucleotide purification cartridge.

### PCR protocol for DNA template preparation

DNA template was obtained from human genomic DNA from a patient blood sample. A 278 nt sequence between nucleotide 156 615 and 156 892 of the *RBI* gene was amplified by PCR in 20  $\mu$ l reactions that contained the following components: 4 pmol forward and reverse primers (5'-ATG CTA CTT AAC AGC ATT ATA ATT AG-3' and 5'-ATC AGT TAA CAA GTA AGT AGG GAG-3'), 1 $\times$  PCR buffer (50 mM KCl, 1.5 mM  $\text{MgCl}_2$ , 10 mM Tris-HCl), 0.2 mM dNTPs, 600 pg genomic DNA and 1 U *Taq* DNA polymerase (Amersham Pharmacia Biotech). The PCR conditions were 30 cycles of 95°C for 20 s, 57°C for 20 s, 72°C for 2 min. At the end of the PCR reaction, 20  $\mu$ l of an enzymatic mixture containing 5 U shrimp alkaline phosphatase (SAP), 4  $\mu$ l of 10 $\times$  SAP buffer, 6 U *Escherichia coli* exonuclease I and 10  $\mu$ l of water were added to the PCR reaction to degrade the excess primers and

dNTPs. The reaction mixture was incubated at 37°C for 90 min before the enzymes were heat inactivated at 72°C for 30 min. The region of the PCR product sequence containing the SNPs (underlined) was: 5'-CAC AGT GTA TCG GCT AGC CTA TCT CCG GCT AAA TAC ACT TTG TGA ACG CCT TCT GTC TGA GCA CCC AGA ATT-3'. These SNPs were confirmed by DNA sequencing using energy transfer terminator chemistry and a MegaBACE 1000 capillary electrophoresis DNA sequencer (Amersham Pharmacia Biotech).

### SBE assay using CFET tags and biotin-ddNTPs

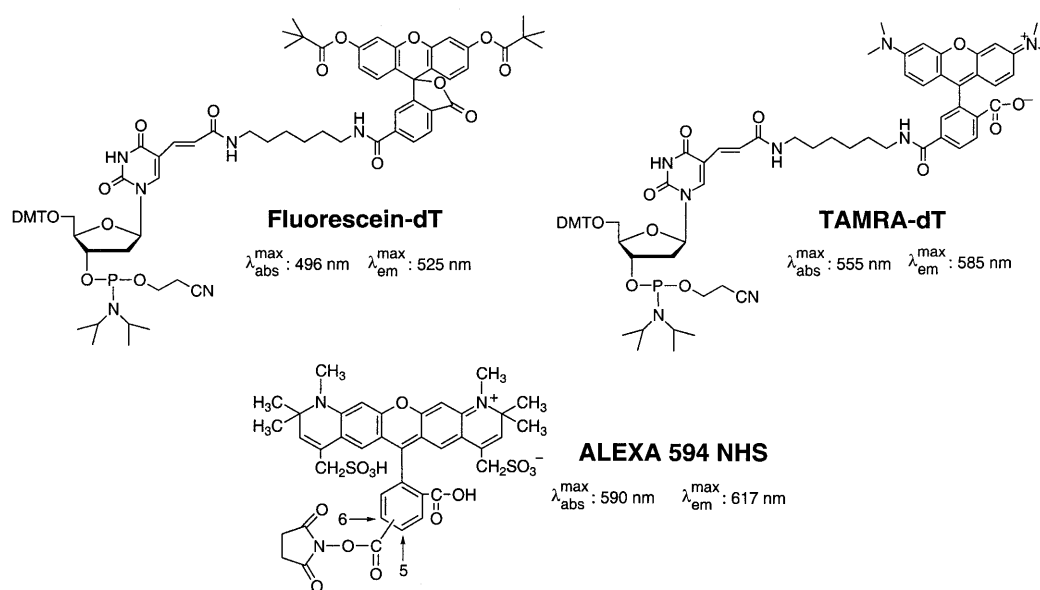
For individual single base extension, 10  $\mu$ l of SBE solution contained 0.5–2 pmol CFET-labeled oligonucleotide primer, 1–4 pmol DNA template, 2 pmol each biotinylated dideoxynucleoside triphosphate [biotin-11-dd(A,C,G,U)TP] (NEN Life Science, Boston, MA), 2  $\mu$ l of Thermo Sequenase reaction buffer (260 mM Tris-HCl pH 9.5, 65 mM  $\text{MgCl}_2$ ) and 1 U Thermo Sequenase in its dilution buffer (Amersham Pharmacia Biotech). Extension was carried out at 54°C for 30 s. For multiplex SBEs, the contents of the reaction mixture were the same as those for individual SBEs described above except that 0.4–5 pmol CFET-labeled primers and 2–4 pmol DNA templates were used. For SBE on the PCR product, 0.5–2.8 pmol CFET-labeled primer and 0.68 pmol DNA template were used. The extension was performed at 94°C for 30 s and 54°C for 30 s.

### Solid phase purification and laser-induced fluorescence detection

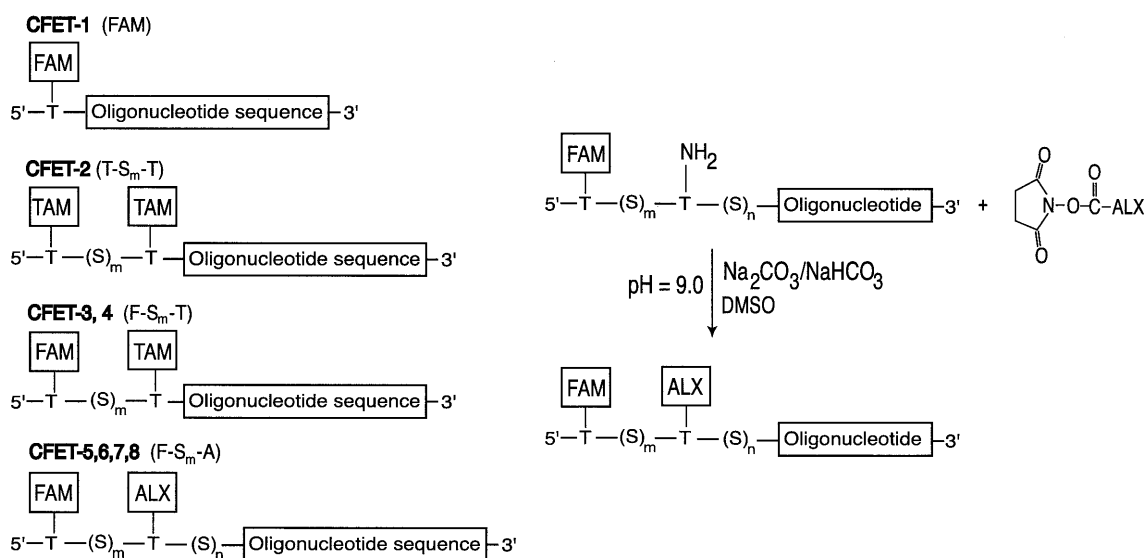
Fifty microliters of streptavidin-coated magnetic beads (Dynabeads M-280 Streptavidin; Dynal, Oslo, Norway) were washed with 2  $\times$  50  $\mu$ l of binding and washing (B&W) buffer (10 mM Tris-HCl, 1 mM EDTA, 2.0 M NaCl, pH 7.5) and then resuspended in 10  $\mu$ l of B&W buffer. The primer extension product mixture was then combined with the streptavidin-coated magnetic beads at 25°C for 0.5 h with occasional mixing. The supernatant was removed while the magnetic beads were immobilized with a magnet. The beads were then washed with 3  $\times$  50  $\mu$ l of B&W buffer. The DNA template was denatured from the extended primer by treatment with 50  $\mu$ l of fresh 0.1 M NaOH at 25°C for 5 min. The reaction mixture was then washed with 2  $\times$  50  $\mu$ l of water. The extended primers were released from the magnetic beads in 98% formamide containing 10 mM EDTA at 94°C for 5 min. An aliquot of 0.3–0.8  $\mu$ l of the supernatant was loaded onto a sequencing gel (5% Long Ranger, 6 M urea) for electrophoresis and fluorescence signature detection on an ABI 377 DNA sequencer (Applied Biosystems, Foster City, CA) with an argon laser for excitation (488/514 nm). Electrophoresis was conducted at 1000 V with 1 $\times$  TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.0).

## RESULTS AND DISCUSSION

We used the following three fluorescent dyes to construct the CFET tags for labeling the oligonucleotide primers: 6-carboxyfluorescein (FAM or F), *N,N,N',N'*-tetramethyl-6-carboxyrhodamine (TAM or T) and Alexa 594 (ALX or A). Figure 1 shows the chemical structures of the dye derivatives used for labeling the oligonucleotides. FAM was used as the donor, while TAM or ALX was selected as the acceptor in



**Figure 1.** Chemical structures of the dye derivatives, fluorescein-dT, TAMRA-dT and ALEXA 594 NHS ester, used to label the oligonucleotides.

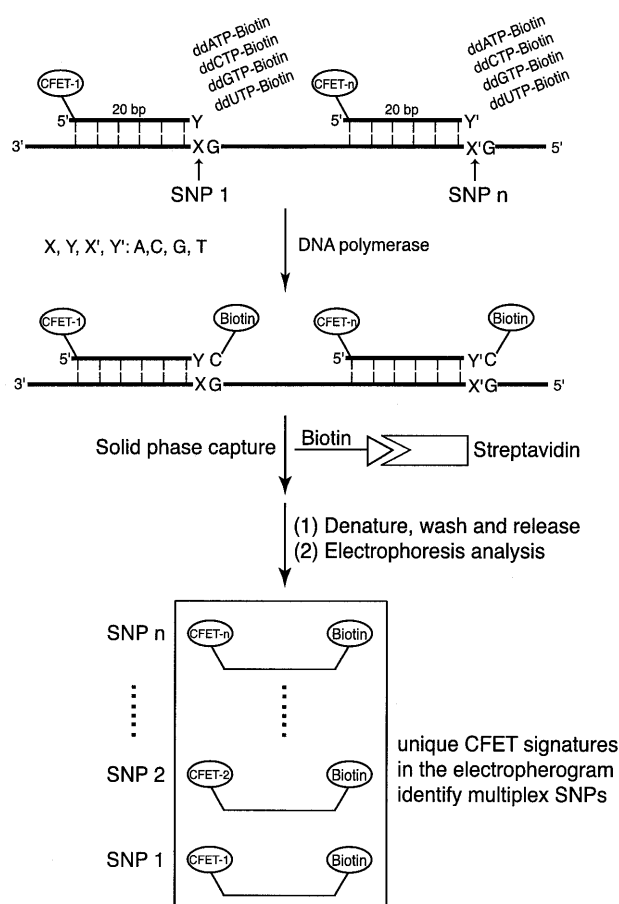


**Figure 2.** (Left) CFET tag-labeled oligonucleotides. The CFET tags are constructed from three chromophores FAM (F), TAM (T) and ALEXA 594 (A). The number of 1',2'-dideoxyribose phosphate spacers (S) between the chromophores is indicated in the CFET tag designation: FAM (CFET-1); T-4-T (CFET-2,  $m = 4$ ); F-9-T (CFET-3,  $m = 9$ ); F-13-T (CFET-4,  $m = 13$ ); F-5-A (CFET-5,  $m = 5$ ,  $n = 13$ ); F-7-A (CFET-6,  $m = 7$ ,  $n = 10$ ); F-10-A (CFET-7,  $m = 10$ ,  $n = 11$ ); F-13-A (CFET-8,  $m = 13$ ,  $n = 8$ ). Spacers ( $S_{m,n}$ ) are used to separate the chromophores to tune the ET efficiency and as mobility tags for electrophoresis analysis. The CFET-labeled oligonucleotides (20 bp) are designed to be complementary to the DNA template containing multiple SNPs. (Right) A synthetic scheme for the preparation of a CFET tag containing FAM and ALEXA.

constructing the CFET tags. Since FAM ( $\lambda_{em}^{max} = 525$  nm), TAM ( $\lambda_{em}^{max} = 585$  nm) and ALX ( $\lambda_{em}^{max} = 617$  nm) have distinct maximum fluorescence emission wavelengths, the CFET tags can be distinguished by a multicolor fluorescence detection system, such as the ABI 377 DNA sequencer, without any modification of its detector. ALX was used because it has higher fluorescence quantum yield and photostability than other fluorophores with similar emission maxima (20). A spacer (S) constructed from 1',2'-dideoxyribose phosphates (21) was used to separate the donor and acceptor

chromophores to tune the ET efficiency and also as a mobility tag to tune the electrophoretic mobility of the SBE fragments.

Schematics and the nomenclature of the CFET tags are shown in Figure 2 (left): CFET-1 consists of a single dye (FAM) covalently linked to an oligonucleotide primer, while CFET-2 (T- $S_4$ -T) is doubly labeled with TAM; CFET-3 and CFET-4 (F- $S_m$ -T) and CFET-5, CFET-6, CFET-7 and CFET-8 (F- $S_m$ -A) consist of two dyes (FAM and TAM, or FAM and ALX), where  $S_m$  indicates the number of dideoxyribose spacers between each donor-acceptor pair. The spacers ( $S_{m,n}$ )



**Figure 3.** SBEs for multiplex SNP detection using CFET-labeled primers and biotin-ddNTPs. DNA template containing polymorphic sites is incubated with CFET-labeled primers, biotin-ddNTPs and DNA polymerase. The nucleotide at the 3'-end of each CFET-labeled primer is complementary to a particular SNP in the template. Only the fully complementary CFET-labeled primer was extended by DNA polymerase with a biotin-ddNTP. After solid phase capture and isolation of the biotinylated DNA extension fragments the SBE products are analyzed for their fluorescence signatures, each of which codes for a unique SNP.

were also used as mobility tags in the electrophoretic analysis. A synthetic scheme for coupling ALX to the oligonucleotide is shown in Figure 2 (right) (see Materials and Methods for details).

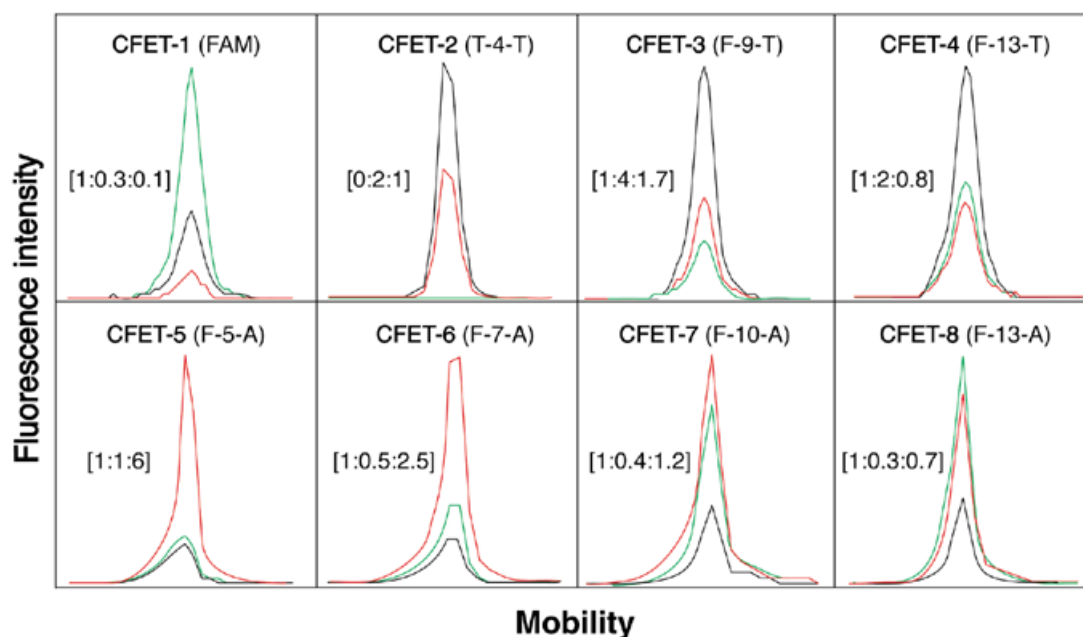
The multiplex SNP detection approach using CFET-labeled oligonucleotide primers and biotin-ddNTPs is shown in Figure 3. Upon hybridization to the DNA templates, each of the CFET-labeled primers that has a perfect match to the SNP site will incorporate a biotin-ddNTP at its 3'-end in the presence of DNA polymerase. Under our experimental conditions, no extension reaction occurred when there was a mismatch between the 3'-end of the CFET-labeled primers (Fig. 3, Y and Y') and the nucleotides at the polymorphic sites (Fig. 3, X and X') in the target DNA template. After SBE, the CFET-labeled primer extension products were immobilized on streptavidin-coated magnetic beads and the other components were washed away. The extension products were cleaved from the magnetic beads by denaturing the biotin-streptavidin interaction with formamide and analyzed with a fluorescence DNA sequencer. The CFET-labeled extension products were unambiguously

**Table 1.** Oligonucleotide sequences labeled by the eight CFET tags and the portion of DNA template sequences containing SNPs identified by the CFET tags (SNPs in bold)

Primer/template	Sequence
CFET-1 labeled primer	5'-GAG-ATA-GGC-TAG-CCG-ATA-CA-3'
CFET-2 labeled primer	5'-GAG-ATA-GGC-TAG-CCG-ATA-CT-3'
CFET-3 labeled primer	5'-TCA-CAA-AGT-GTA-TTT-AGC-CG-3'
CFET-4 labeled primer	5'-TCA-CAA-AGT-GTA-TTT-AGC-CA-3'
CFET-5,6 labeled primer	5'-CAG-ATG-ATA-TGT-TCT-AAT-TC-3'
CFET-7,8 labeled primer	5'-CAG-ATG-ATA-TGT-TCT-AAT-TA-3'
Template for CFET-1 primer	5'~ <b>GTG</b> -TAT-CCG-CTA-GCC-TAT-CTC~3'
Template for CFET-2 primer	5'~ <b>GAG</b> -TAT-CCG-CTA-GCC-TAT-CTC~3'
Template for CFET-3 primer	5'~ <b>CGG</b> -CTA-AAT-ACA-CTT-TGT-GA~3'
Template for CFET-4 primer	5'~ <b>TGG</b> -CTA-AAT-ACA-CTT-TGT-GA~3'
Template for CFET-5 primer	5'~ <b>AGA</b> -ATT-AGA-ACA-TAT-CAT-CTG~3'
Template for CFET-7 primer	5'~ <b>ATA</b> -ATT-AGA-ACA-TAT-CAT-CTG~3'

detected due to their distinct mobilities and fluorescence signatures in the electropherogram. In the case of heterozygous genotypes, we used two CFET tags with different fluorescence signatures and electrophoretic mobilities to label the oligonucleotides corresponding to each allele. The CFET-labeled oligonucleotides, biotin-ddNTPs and DNA polymerase thus formed a high fidelity SNP identification system in which the nucleotide at the 3'-end of the oligonucleotide dictated its extension by incorporating a specific biotin-ddNTP, and the unique fluorescence signature in the electropherogram identified each of the corresponding SNPs.

Eight unique fluorescence signatures of the CFET tags described in Figure 2 (see Table 1 for the oligonucleotide sequences) are shown in Figure 4. Excited by an argon laser at 488/514 nm and detected by an ABI 377 sequencer, the fluorescence emission signatures were distinct as a result of different fluorescence signal intensities measured in the three detection channels from each CFET tag (FAM channel, 530–541 nm, green; TAM channel, 581–591 nm, black; ALX channel, 610–620 nm, red). For clarity, the unique fluorescence emission signature of each CFET tag is denoted by a digital ratio (*DR*) of the signal intensity from each of the three detection channels ( $DR = \text{green:black:red}$ ; the FAM signal is set to 1). In the FAM-labeled oligonucleotide (CFET-1), the chromophore was efficiently excited by the argon laser at 488/514 nm in the ABI 377 sequencer, displaying a dominant FAM emission (green) and a *DR* of 1:0.3:0.1. To achieve higher fluorescence emission intensity, we coupled two TAM molecules in T-4-T (CFET-2) in order to compensate for the fact that TAM cannot be excited efficiently by the argon laser. Accordingly, CFET-2 produced a unique fluorescence signature with a *DR* of 0:2:1 with a dominant TAM emission (black). Since FAM is not present in CFET-2, there was no signal detected from the FAM channel and, thus, the green signal is 0 in the signature of T-4-T. Coupling TAM to the oligonucleotide primer as an acceptor for FAM with separation distances of 9 and 13 dideoxyribose spacers produced F-9-T (CFET-3) and F-13-T (CFET-4). The ET efficiency decreased as the number of dideoxyribose spacers increased from 9 to 13, as indicated by the different fluorescence emission ratios in F-9-T (CFET-3) ( $DR = 1:4:1.7$ ) and F-13-T (CFET-4) ( $DR = 1:2:0.8$ ). A set of CFET tags with four distinct fluorescence signatures was thus created by fluorescently labeling the oligonucleotides with two individual dyes (FAM and TAM). Four additional CFET tags with unique fluorescence signatures were generated using FAM as the



**Figure 4.** The eight unique fluorescence signatures of CFET tags generated by a fluorescence DNA sequencer (ABI 377). FAM channel (530–541 nm), green; TAM channel (581–591 nm), black; ALEXA channel (610–620 nm), red. The digital ratio denoting the fluorescence signature for each CFET tag from the three channels [green:black:red] is shown in brackets. The fluorescence signatures in the electropherogram were obtained using the eight CFET-labeled oligonucleotides described in Figure 2.

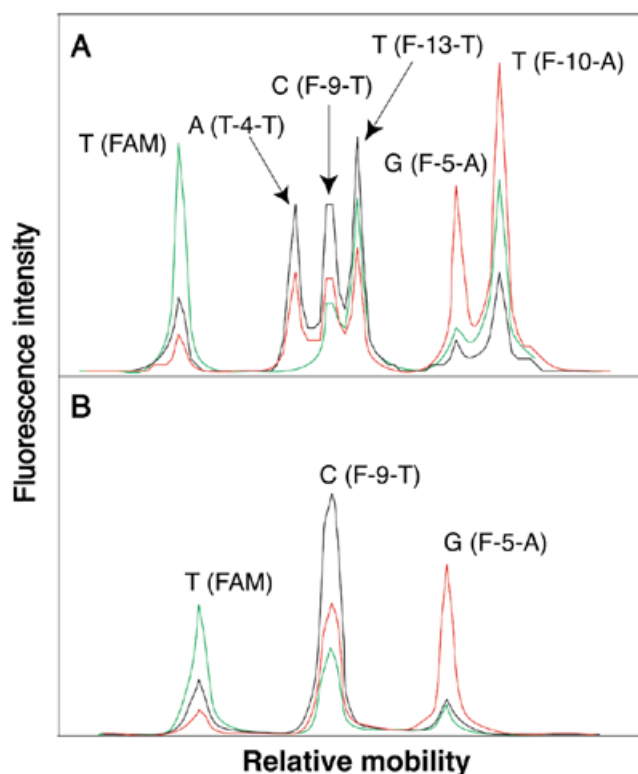
donor and ALX as the acceptor: the fluorescence signatures of F-5-A (CFET-5), F-7-A (CFET-6) and F-10-A (CFET-7) displayed considerable ALX emission (red) that was produced through ET from FAM to ALX after excitation at 488/514 nm. On the other hand, the fluorescence signature of F-13-A (CFET-8) had a higher FAM than ALX signal. Again, consistent with the ET principle, a greater separation distance between the donor and acceptor lowered the ET efficiency, as indicated by the different *DR* values in CFET-5 (*DR* = 1:1:6), CFET-6 (*DR* = 1:0.5:2.5), CFET-7 (*DR* = 1:0.4:1.2) and CFET-8 (*DR* = 1:0.3:0.7), respectively.

We used CFET-labeled oligonucleotide primers and biotin-ddNTPs in SBE for multiplex SNP detection. The reactions were carried out simultaneously in a single tube with six different CFET-labeled oligonucleotide primers directed to six nucleotide variations in synthetic templates mimicking exon 20 of the *RBI* gene. Mutations affecting both alleles of the *RBI* gene are a prerequisite for the development of retinoblastoma, which is a malignant tumor of the eye (22). Table 1 shows the sequences of the primers and synthetic templates used in this study. After extension and solid phase purification, the extension products were analyzed with an ABI 377 DNA sequencer, resulting in simultaneous detection of six nucleotide variations by the unique fluorescence signatures of the CFET-labeled extension products (Fig. 5A). The unique fluorescence signatures were spatially resolved in the electropherogram as a result of the different mobilities of the CFET-labeled extension products. In this model experiment, both CFET-1 (FAM) and CFET-2 (T-4-T) distinguished a mimic of the *RBI* mutation V654E, detecting both the wild-type (T) and mutation (A) at the same location in the template. CFET-3 (F-9-T) and CFET-4 (F-13-T) identified a mutation, R661W, with a heterozygous genotype

(C/T). CFET-5 (F-5-A) and CFET-7 (F-10-A) detected another mutation, E675X, with a heterozygous genotype (G/T).

To further validate the SNP detection method using CFET-labeled oligonucleotides and biotin-ddNTPs, we used three CFET-labeled oligonucleotide primers (CFET-1, CFET-3 and CFET-5) to identify three SNPs in a PCR product amplified from exon 20 of the *RBI* gene from patient genomic DNA. The extension reactions were performed in a single tube and the reaction products were analyzed with an ABI 377 DNA sequencer. Three individual homozygous genotypes (T, C and G), verified by DNA sequencing, were unambiguously identified by the three distinct fluorescence signatures from the CFET-labeled SBE products (Fig. 5B): T (FAM, CFET-1), C (F-9-T, CFET-3) and G (F-5-A, CFET-5). Thus, both heterozygous and homozygous genotypes can be identified unambiguously by the unique fluorescence signatures and electrophoretic mobilities of the CFET-labeled SBE products.

In summary, the SBE assay using CFET-labeled oligonucleotides and biotin-ddNTPs in conjunction with solid phase capture described here offers a fast and accurate method for high throughput SNP detection and analysis. This single tube homogeneous assay generated CFET-labeled extension fragments carrying a tag at the 5'-end to give unique fluorescence signatures for each of the multiplex SNPs and a biotin at the 3'-end for solid phase capture, allowing only the extended fragments to be detected. The unique fluorescence signatures, detected by a multicolor fluorescence DNA sequencer, were used to simultaneously identify multiplex SNPs in synthetic DNA templates and a PCR product from the *RBI* gene. Compared to SNP detection methods that require a sizing standard in gel electrophoresis, our method is simple and accurate since each SNP is distinguished by a distinct fluorescence signature with a specific electrophoretic mobility. All the



**Figure 5.** Electropherogram of CFET-labeled SBE products for SNP identification in exon 20 of the *RB1* gene. (A) Detection of six nucleotide variations from synthetic DNA templates. The FAM and T-4-T peaks detected a heterozygous genotype (T/A), while the F-9-T and F-13-T peaks identified a C/T heterozygous genotype. The F-5-A and F-10-A peaks identified another heterozygous genotype (G/T). (B) Detection of three homozygous genotypes (T, C and G) for a PCR product from the *RB1* gene.

primers used in our experiments were 20 bp and this facilitates the hybridization and denaturing processes during analysis. It also simplifies the design of the primers when compared to other melting point-based hybridization assays for SNP analysis (23,24). The oligonucleotide ligation assay (OLA) has been widely used for genetic mutation analysis (25–27). SBE is faster than OLA because the DNA polymerase incorporates ddNTPs specifically in a fraction of a second (28,29). Also, primer design is needed only next to the polymorphic sites in the target DNA template, and the additional oligonucleotides that are required in OLA are unnecessary for SBE (30). The DNA polymerase, such as Thermo Sequenase, employed in SBE is as specific as the *Taq* DNA ligase used in OLA in distinguishing single nucleotide differences at a polymorphic site, and hence accurate discrimination of allelic variants is assured. Other laser-induced multicolor fluorescence electrophoresis systems, such as capillary array DNA sequencers (31) and miniaturized chip-based devices (32,33), can be used to separate the DNA extension fragments and detect the unique fluorescence signatures for high throughput SNP analyses.

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