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# Detection of three-base deletion by exciplex formation with perylene derivatives†

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**Here, we synthesized fluorescent DNA probes labeled with two perylene derivatives for the detection of a three-base deletion mutant. One such probe discriminated the three-base deletion mutant from the wild-type sequence by exciplex emission, and the deletion mutant was identifiable even by the naked eye.**

Recently, insertion/deletion polymorphisms (indels) have been paid much attention as well as single-nucleotide polymorphisms (SNPs) due to their frequent occurrence.[1] Moreover, small (1–4 bp) indels are more frequent than larger indels, and some are related to genetic diseases.[2] Although several methods have been developed to detect indels,[3–7] no fluorescent probes have been developed to detect three-base deletion polymorphisms.

Previously, we engineered perylene-labeled DNA probes that could detect one-base deletion polymorphisms.[8] The general design of perylene-labeled probes that can detect deletion mutants is shown in Scheme 1A. Two perylenes were introduced into the middle of the probe on either side of the target sequence. When the probe hybridized with wild-type DNA, two perylenes (**E** in Scheme 1B) were intercalated between base pairs, and the target base pair(s) interrupted the interaction between the perylenes. However, when the probe hybridized with a deletion mutant, a bulge that includes two perylenes was formed, and the two perylenes, which were in close proximity, interacted. Accordingly, monomer emission from each perylene was quenched, and excimer emission with a longer wavelength appeared. Perylene-labeled probes that were able to detect two-base deletions were constructed by changing the number of bases between perylenes (see Fig. S1A in ESI†). However, three-base deletions could not be detected using perylene-labeled DNA probes containing **E** (see Fig. S1B in ESI†). Only monomer emission was observed from the perylene-labeled DNA probes targeting three-base deletions probably because two **E** perylenes were unable to establish contact across five-base bulges including two dyes. Three-base deletions are called "in-frame" deletions, they do not cause a frameshift, but rather amino acid deletions, and in some cases, an essential residue is lost from a protein. Some in-frame deletions are important as a crucial step in carcinogenesis.[9] Thus, it is necessary to develop novel DNA probes for three-base deletion polymorphisms.

Here, we synthesized two perylene derivatives (**F**, **L** in Scheme 1B) and incorporated them into DNA probes for the detection of three-base deletions. The **F** and **L** moieties are better than the **E** moiety for the detection of three-base deletion for several reasons. (1) Perylene derivatives can emit excimer and exciplex emission.[10–13] Thus, they are utilized as fluorophores for our strategy. (2) These moieties are larger than **E** so that they may form an excimer even in a five-base bulge. (3) **F** and **L** have an extended π system so that the absorption maximum should shift to a longer wavelength than that of **E**. Consequently, the autofluorescence from the sample should be suppressed. In addition, many commercial apparatus are available for the detection of these longer wavelengths. (4) The emission band of **F** substantially overlapped with the absorption bands of **L** (*vide infra*) so that efficient FRET from **F** to **L** led to enlarged apparent Stokes' shift. In this communication, we first investigated spectroscopic behaviors of **F** and **L** in DNA duplexes and then applied these fluorophores to the detection of three-base deletion polymorphisms.

Phosphoramidite monomers with incorporated perylene derivatives were synthesized through a phosphoramidite method as shown in ESI.†[14–16] Sequences synthesized in this study are shown in Scheme 1B. First, we incorporated each dye into the middle of 12 mer DNA oligonucleotides in order to investigate the spectroscopic behaviors of these dyes (**F1a**, **L1a**). In addition, the perylene moiety synthesized previously was also introduced as a control (**E1a**).

Spectroscopic behaviors of these DNAs are shown in Table 1. Absorption ($\lambda_{ab.max}$) and emission maxima ($\lambda_{em.max}$) of the duplexes **F1a**/**N** and **L1a**/**N** were much longer than those of **E1a**/**N** as we designed. In particular, $\lambda_{ab.max}$ of **L1a**/**N** was as long as 484 nm. Interestingly, the **F1a**/**N** emission band overlapped substantially with the **L1a**/**N** absorption band (Fig. 1). Consequently, highly efficient FRET from **F** to **L** was expected. Efficient FRET enables the enlargement of apparent Stokes' shift.[17] Quantum yields ($\Phi$) of these duplexes are also shown in Table 1. **F** and **L** showed comparable quantum yield to **E**; $\Phi$ of the duplexes **F1a**/**N** and **L1a**/**N** were

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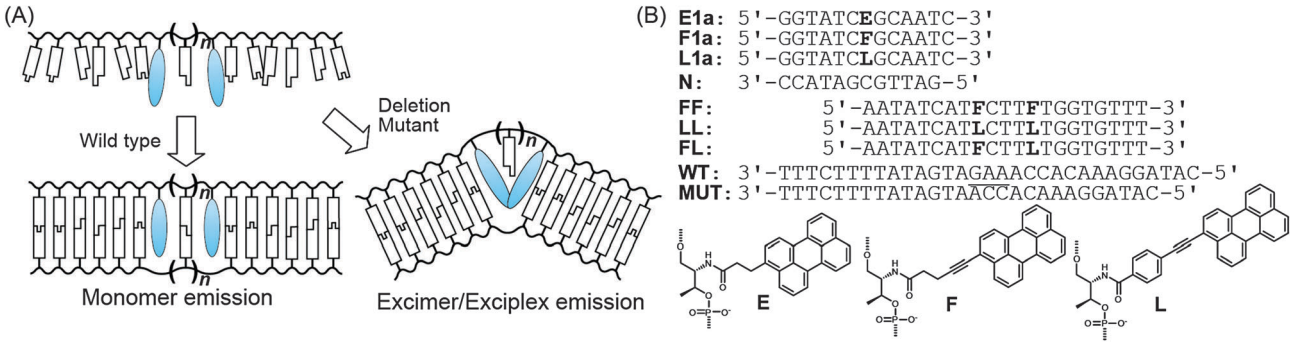
† Electronic supplementary information (ESI) available: Synthetic schemes of phosphoramidite monomer tethering **F** and **L**, fluorescent emission spectra of probes tethering **E**, UV-VIS spectra of duplexes. See DOI: 10.1039/c1cc11041a




**Scheme 1** (A) Schematic illustration of the detection of deletion polymorphisms. *n* represents the number of the bases deleted. (B) Sequences of the modified DNA oligonucleotides synthesized in this study.

**Table 1** Spectroscopic properties of duplexes containing perylene derivatives

| Sequence | $\lambda_{ab.max}$ [a]/nm | $\lambda_{em.max}$ [b]/nm | $\Phi$ [c] | $T_m$ [d]/°C |
|---|---|---|---|---|
| **E1a**/**N** | 452 | 462 | 0.65 | 46.1 |
| **F1a**/**N** | 467 | 479 | 0.56 | 49.7 |
| **L1a**/**N** | 484 | 502 | 0.32 | 37.8 |

[a] Absorption maximum at 20 °C. [b] Emission maximum at 20 °C. [c] Quantum yield determined from the quantum yield of perylene in $N_2$-bubbled cyclohexane (0.78) used as a reference. [d] Solution conditions: [DNA] = 2.0 μM, [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer).

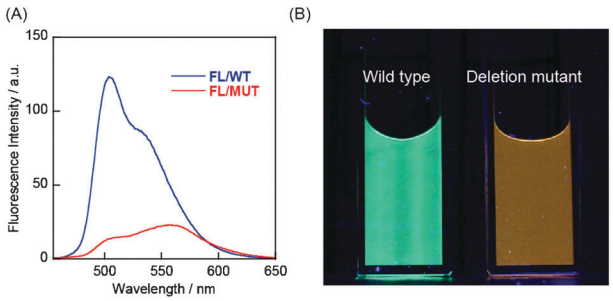



**Fig. 2** (A) Fluorescence emission spectra of **FL**/**WT** and **FL**/**MUT** at 20 °C. Excitation wavelength was 445 nm. Solution conditions were as follows: [Probe] = 1.0 μM, [Target] = 1.2 μM, [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer). (B) A photograph of **FL** with wild type (**FL**/**WT**) or three-base deletion mutant (**FL**/**MUT**) at RT. Excitation wavelength was 400 nm. Solution conditions were as follows: [Probe] = 1.0 μM, [Target] = 1.2 μM, [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer).

0.56 and 0.32, respectively. Accordingly, **F** and **L** moieties are fluorophores with longer wavelength and without losing detection sensitivity than **E**. Because **F** had a larger stacking area than **E**, the melting temperature ($T_m$) of the duplex **F1a**/**N** was higher than that of **E1a**/**N**.[18] Although **L** is larger than **F**, $T_m$ of the duplex **L1a**/**N** was significantly lower than that of the duplex **F1a**/**N**, probably due to steric hindrance.

Next, we attempted to detect a three-base deletion mutant using DNA probes labeled with these fluorophores. The target was the cystic fibrosis transmembrane conductance regulator (CFTR) gene; a specific three-base deletion is responsible for many cases of cystic fibrosis.[4,19] The sequences of probes are shown in Scheme 1B. We incorporated three bases (5′-CTT-3′) with two fluorophores in each probe, and synthesized three probes containing **F** and/or **L** (**FF**, **LL**, **FL**). We hypothesized that when one of these probes hybridized with the wild-type sequence (**WT**), the two fluorophores would intercalate within the DNA duplex and monomer emission would occur, but when a probe hybridized with the mutant sequence (**MUT**), which lacks three bases (5′-AAG-3′), the two fluorophores might interact with each other. Thus, the wild-type sequence and deletion mutant might be distinguished by monomer and excimer/exciplex emissions, respectively, from probe–target duplexes.

First, we evaluated the probes containing only **F** or **L**. The results of hybridization of the **FF** probe with **WT** or **MUT** sequences are shown in Fig. S2A. The duplex **FF**/**WT** showed strong monomer emission of **F** at 476 and 509 nm, indicating that the two **F**s did not interact because of the intervening base pairs. However, **FF** did not show excimer emission with **MUT**, but decreased monomer emission due to quenching,

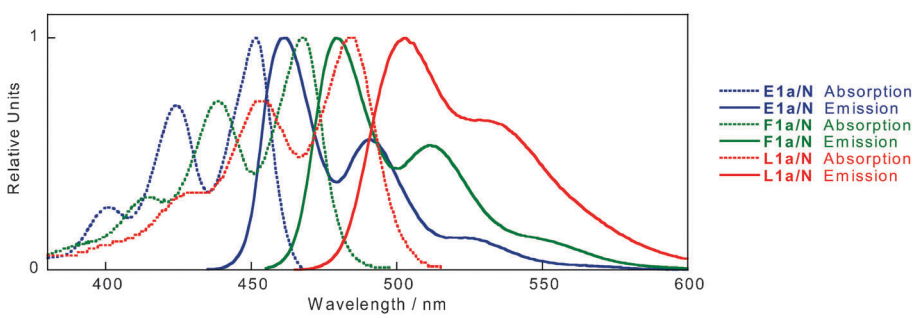



**Fig. 1** Emission and absorption spectra of **E1a**/**N**, **F1a**/**N** and **L1a**/**N** at 20 °C. Each spectrum was normalized at the absorption or emission maximum.

**Table 2** Detection ability and duplex stability of probe DNA

| | $I_{ex}/I_{mon}$[a] | | $T_m$[b]/°C | |
|---|---|---|---|---|
| Probe | **WT** | **MUT** | **WT** | **MUT** |
| **FF** | 0.11[c,e] | 0.14[c,e] | 57.5 | 40.6 |
| **LL** | 0.25[d,f] | 0.33[d,f] | 41.0 | 34.1 |
| **FL** | 0.25[d,e] | 1.46[d,e] | 49.2 | 42.6 |

[a] Ratio of the intensity of excimer emission to monomer emission at 20 °C. [Probe] = 1.0 μM, [Target] = 1.2 μM, [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer). [b] [Probe] = [Target] = 2.0 μM, [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer). [c] $I_{550}/I_{476}$. [d] $I_{570}/I_{507}$. [e] Excited at 445 nm. [f] Excited at 455 nm.

demonstrating that **F** moieties could not interact with each other in the five-base bulge. Similarly, the duplex **LL**/**MUT** showed no excimer emission, although strong monomer emission at 506 nm was observed with the duplex **LL**/**MUT** (Fig. S2B). Thus, probes with homo **FF** or **LL** pairs did not detect the three-base deletion.

In contrast, the probe with one **F** and one **L** moiety (**FL**) exhibited exciplex emission from a five-base bulge (Fig. 2). When **FL** was hybridized with **WT**, only monomer emission of **L** was observed at 507 nm. These results indicated that **F** and **L** were intercalated between base pairs and direct interaction between **F** and **L** was suppressed. Furthermore, complete disappearance of the emission band of **F** at 476 nm demonstrates that highly efficient FRET occurred from **F** to **L**. In contrast, when **FL** hybridized with the three-base deletion mutant (**MUT**), monomer emission of **L** decreased, and a new band appeared at 556 nm concurrently.[20] This new band corresponded to the exciplex between **F** and **L** that formed inside the five-base bulge.[21] The ratio of intensity at 570 nm to that at 507 nm was 1.46 with the duplex **FL**/**MUT** whereas that with **FL**/**WT** was 0.25 (Table 2). Three-base deletion was successfully detected with **FL**.[22] Importantly, the three-base deletion was identifiable even by the naked eye (Fig. 2B); the colors of **FL**/**WT** and **FL**/**MUT** were green and orange, respectively.

Hetero-dimerization was also substantiated from the UV-VIS spectrum; although spectra were rather complicated due to overlaps of the absorption band of **F** and **L** (see Fig. S3 in ESI†), absorption spectrum of **FL**/**MUT** was completely different from that of **FL**/**WT**. In particular, both hypochromism and hyperchromism were observed at 450–500 nm and 400–450 nm, respectively, with **FL**/**MUT** samples, indicating that **F** and **L** were interacting excitonically to form a heterodimer with **FL**/**MUT**. Spectral changes of **FF**/**MUT** and **LL**/**MUT** were much smaller than that of **FL**/**MUT**. Only slight hypochromism was observed. In addition, $T_m$ of **FL**/**MUT** was higher than those of **FF**/**MUT** and **LL**/**MUT** whereas $T_m$ of **FL**/**WT** was lower than that of **FF**/**WT** (Table 2). These results indicate that **F** and **L** moieties in **FL** could form a dimer in the bulge, but those in **FF** and **LL** could not. Consequently, strong exciplex emission was observed only in **FL**/**MUT**.

In conclusion, we engineered a fluorescent probe containing size-expanded perylene derivatives, which have larger molecular sizes than **E**. As a result, a three-base deletion polymorphism was successfully detected by monitoring exciplex emissions from a perylene-labeled DNA probe. In addition, the spectral difference between wild-type and mutant samples was easily visible to the naked eye.

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