Supporting Information

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

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**Materials.**
All the conventional phosphoramidite monomers, CPG columns, reagents for DNA synthesis and Poly-Pak II cartridges were purchased from Glen Research. Other reagents for the synthesis of phosphoramidite monomer were purchased from Tokyo Kasei Co., Ltd, and Aldrich.

**Compound 2.**
Compound 1 and 4-ethylbenzoic acid were synthesized according to the previous reports. 4-Ethylbenzoic acid (0.68 g, 4.6 mmol) was reacted with PyBOP (2.6 g, 5.0 mmol) in CH₂Cl₂ (40 ml) for 10 min. Then, a solution of Et₃N (20 ml) and compound 1 (1.7 g, 4.2 mmol) in CH₂Cl₂ (10 ml) was added to the above mixture. After vigorous stirring overnight at room temperature, the organic solution was washed with saturated aqueous solution of NaHCO₃. The solvent was removed by evaporation, followed by silica gel column chromatography (AcOEt : hexane : Et₃N = 40:60:3, Rf = 0.84) to afford 2 (1.7 g, yield 82 %).

**Compound 3.**
3-Bromoperylene was synthesized according to the literature. To a solution of copper(I) iodide (0.23 g, 1.2 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.10 g, 0.090 mmol) in dry THF (20 ml) and piperidine (40 ml) was added 3-bromoperylene (0.52 g, 1.6 mmol). After the vigorous stirring at 60 °C under nitrogen for 30 minutes, a solution of compound 1 in dry THF (5.0 ml) was added to the above mixture. After vigorous stirring overnight, the reaction mixture was washed with saturated aqueous solution of NaHCO₃ and that of NaCl. After drying over MgSO₄, the organic solution was washed with saturated aqueous solution of NaHCO₃. The solvent was removed by evaporation, followed by silica gel column chromatography (CHCl₃ : hexane : AcOEt :Et₃N = 33:33:33:3, Rf = 0.38) to afford 3 (0.70 g, yield 75 %). 1H-NMR [CDCl₃, 500 MHz] δ = 8.31 (d, 1H, J = 8.5 Hz), 8.29 (d, 1H, J = 7.5 Hz), 8.24 (t, 1H, J = 7 Hz), 8.19 (d, 1H, J = 8 Hz), 7.84 (d, 2H, J = 8.5 Hz), 7.79 (d, 1H, J = 7 Hz), 7.75-7.71 (m, 4H), 7.64 (t, 1H, J = 7.5 Hz), 7.52 (t, 2H, J = 8 Hz), 7.39 (m, 2H), 7.31-7.22 (m, 9H), 6.85 (d, 1H, J = 9 Hz), 6.83-6.79 (m, 4H), 4.24 (m, 1H), 4.14 (m, 1H), 3.78 and 3.78 (s, 6H), 3.60 (dd, J = 4 Hz, 9.5 Hz, 1H), 3.40 (dd, J = 3.5 Hz, 10 Hz, 1H), 3.15 (br, 1H), 1.23 (d, 3H, J = 6.5 Hz). 13C-NMR [CDCl₃, 126 MHz] δ = 167.3, 158.9, 144.5, 135.7, 135.5, 134.6, 132.6, 130.1, 130.1, 128.3, 128.1, 127.3, 127.2, 125.7, 113.6, 87.2, 83.0, 79.8, 69.0, 65.7, 55.4, 54.2, 20.3. HRMS(FAB) Calcd for C₃₄H₃₃NO₅ (M+H+) 535.2359. Found 535.2363.

**Compound 4.**
Et₃N (0.16 ml, 1.2 mmol) and 2-cyanoethylisopropylchlorophosphoramidite (0.10 ml, 0.46 mmol) were added to a solution of compound 3 (0.18 g, 0.23 mmol) in CH₂Cl₂ (5.0 ml) at 0 °C. Then the mixture was stirred for 60 min at 0 °C. Then, CHCl₃ was added to the above mixture at 60 °C. The reaction mixture was refluxed for 3 hours and then the solvent was removed by evaporation. Column chromatography on silica gel (CHCl₃ : hexane : AcOEt :Et₃N = 33:33:33:3, Rf = 0.37) gave 4 (0.84 g, yield 82 %).

**Compound 5.**
4-Pentynoic acid (0.12 g, 1.2 mmol) was reacted with PyBOP (0.62 g, 1.2 mmol) in CH₂Cl₂ (10 ml) for 10 min. Then, a solution of Et₃N (5 ml) and compound 1 (0.41 g, 1.0 mmol) in CH₂Cl₂ (10 ml) was added to the above mixture. After vigorous stirring overnight,
the organic solution was washed with saturated aqueous solution of NaHCO₃. The solvent was removed by evaporation, followed by silica gel column chromatography (AcOEt : hexane : Et₂N = 50:50:3, Rf = 0.13) to afford 5 (0.43 g, yield 88 %). ¹H-NMR [CDCl₃, 500 MHz] δ = 7.39-7.21 (m, 9H), 6.84 (m, 4H), 6.23 (d, 1H, J = 8.5 Hz), 4.10 (m, 1H), 3.95 (m, 1H), 3.79 (s, 6H), 3.44 (dd, J = 4 Hz, 9.5 Hz, 1H), 3.31 (dd, J = 3.5 Hz, 9.5 Hz, 1H), 2.99 (br, 1H), 2.54 (m, 2H), 2.45 (m, 2H), 1.98 (t, 1H, J = 2.5 Hz), 1.14 (d, 3H, J = 6 Hz). ¹³C-NMR [CDCl₃], 126 MHz δ = 171.5, 158.9, 144.6, 135.7, 135.5, 130.2, 130.2, 128.3, 128.2, 128.7, 113.5, 87.1, 83.2, 69.8, 68.8, 65.5, 55.5, 53.7, 35.7, 20.1, 15.2. HRMS(FAB) Calcd for C₃₀H₃₃NO₅ (M⁺) 487.2359. Found 487.2367.

Compound 6. To a solution of copper(I) iodide (0.15 g, yield 72 %). ¹H-NMR [CDCl₃, 500 MHz] δ = 6.27 (d, 1H, J = 7.5 Hz, 1H), 2.95 (br, 1H), 2.54 (m, 2H), 2.45 (m, 2H), 1.98 (t, 1H, J = 2.5 Hz), 1.14 (d, 3H, J = 6 Hz). ¹³C-NMR [CDCl₃], 126 MHz δ = 171.5, 158.9, 144.6, 135.7, 135.5, 130.2, 130.2, 128.3, 128.2, 128.7, 113.5, 87.1, 83.2, 69.8, 68.8, 65.5, 55.5, 53.7, 35.7, 20.1, 15.2. HRMS(FAB) Calcd for C₃₀H₃₃NO₅ (M⁺) 487.2359. Found 487.2367.

Synthesis of the modified DNA involving perylene.

Synthesis of phosphoramidite monomer bearing E was reported previously. 4 The modified DNAs were synthesized on an automated DNA synthesizer (ABI-3400 DNA synthesizer, Applied Biosystems) by using phosphoramidite monomers bearing dye molecules and other conventional ones. Coupling efficiency of the monomers corresponding to modified residues was as high as the conventional ones as judged from the coloration of released trityl cation. After the recommended work-up, they were purified by reverse-phase HPLC and characterized by MALDI-TOFMS (Autoflex II, BRUKER DALTONICS).

MALDI-TOFMS for:

**F1a:** Obsd. 4141 (Calcd. for [F1a+H⁺]: 4142). **L1a:** Obsd. 4188 (Calcd. for [L1a+H⁺]: 4190). **E1a:** Obsd. 4118 (Calcd. for [E1a+H⁺]: 4118). **FF:** Obsd. 6791 (Calcd. for [FF+H⁺]: 6791 ). **LL:** Obsd. 6868 (Calcd. for [LL+H⁺]: 6887). **FL:** Obsd. 6840 (Calcd. for [FL+H⁺]: 6839).

**Spectroscopic measurements**
The UV-visible spectra were measured on a Shimadzu model UV-1800 with a 10 mm quartz cell. It was equipped with a programmed temperature-controller. Conditions of the sample solutions were as follows (unless otherwise noted): [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer), [DNA] = 2.0 μM. Fluorescence spectra were measured on a JASCO model FP-6500 with a microcell. The sample solutions were as follows: [NaCl]=100 mM, pH 7.0 (10 mM phosphate buffer), [probe]=1.0 μM, [Target] = 1.2 μM. Quantum yield were determined from the quantum yield of perylene in N₂-bubbled cyclohexane (0.78) as a reference.

**Measurement of melting temperature**
The melting curve of duplex DNA was obtained with the above apparatus by measuring the change of absorbance at 260 nm versus temperature. The melting temperature (Tm) was determined from the maximum in the first derivative of the melting curve. Both the heating and cooling curves were measured, and the Tm obtained from them coincided with each other to within 2.0 °C.


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Fig. S1. Detection of (A) two-base and (B) three-base deletion polymorphisms by use of E. Solution conditions were as follows: [DNA] = 5.0 μM, [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer), 0 °C. Excitation wavelength: 425 nm.

**EA2E**: 5'-GGTATCEAAECAATC-3'

**EA3E**: 5'-GGTATCEAAAEGCAATC-3'

**T2**: 3'-CCATAGCGTTAG-5'

**T3**: 3'-CCATAGTTTCGTTAG-5'

**N**: 3'-CCATAGCGTTAG-5'

N
e

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Fig. S2 Fluorescent emission spectra of (A) FF and (B) LL with WT (blue line) or MUT (red line) at 20 °C. Excitation wavelength was (A) 445 nm or (B) 455 nm. Solution conditions were as follows: [Probe] = 1.0 μM, [Target] = 1.2 μM [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer).
**Fig. S3.** UV-VIS spectra of (A) **FF**, (B) **LL** and (C) **FL** with **WT** (blue line) or **MUT** (red line). Solution conditions were as follows: [DNA] = 2.0 μM, [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer).

**FF:**
5′-AATATCATFCTTFTGGTGT-3′

**LL:**
5′-AATATCATLCTTLTGGTGT-3′

**FL:**
5′-AATATCATFCTTLTGGTGT-3′

**WT:**
3′-TTTCTTTTTATAGTAGAAACCACAAAGGATAC-5′

**MUT:**
3′-TTTCTTTTTATAGTAACCACAAAGGATAC-5′

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Fig. S4. Melting Curves of E1a/N, F1a/N and L1a/N. Both heating and cooling curves are shown. Solution conditions were as follows: [DNA] = 2.0 μM, [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer).

E1a : 5′-GGTATC E GCAATC-3′
F1a : 5′-GGTATC F GCAATC-3′
L1a : 5′-GGTATC L GCAATC-3′
N : 3′-CCATAGCGTTAG-5′
Fig. S5. Melting Curves of (A) FF, (B) LL and (C) FL with WT (blue line) or MUT (red line). Both heating and cooling curves are shown. Solution conditions were as follows: [DNA] = 2.0 μM, [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer).
**Fig. S6.** Comparison of the excimer emission of E and the exciplex emission of F and L. Solution conditions were as follows: [FL] = 1.0 μM, [MUT] = 1.2 μM, [EAE] = [N] = 5.0 μM, [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer).

EAE : 5′-GGTATCEAECAATC-3′
N : 3′-CCATACGTTAG-5′
Fig. S7. Ratio of the intensity of exciplex emission to monomer emission at lower probe concentrations. Concentration of the target (WT or MUT) was 1.2 times higher than that of FL for each sample. Solution conditions were as follows: [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer), 20 °C.
Fig. S8. Ratio of the intensity of exciplex emission to monomer emission at various target/probe ratios. Plots at low concentration are magnified in the inset. The concentration of FL was fixed to 1.4 μM. Solution conditions were as follows: [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer), 20 °C.