Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

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

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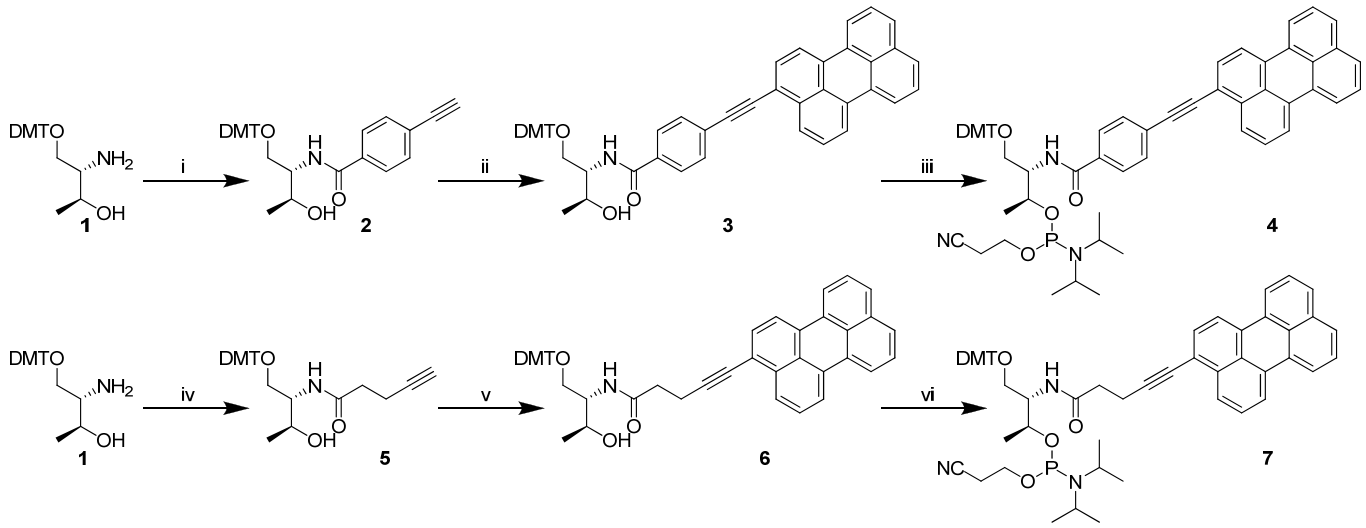


Scheme S1. Synthesis of phosphoramidite monomers bearing dyes. Reagents and conditions; i) 4-ethynylbenzoic acid, PyBOP, Et_3N, CH_2Cl_2, rt, overnight, 75 %; ii) 3-Bromoperylene, CuI, $(Ph_3P)_4Pd$, piperidine, THF, 60°C, 4h, 82 %; iii) $(i\text{Pr})_2NP(Cl)(OCH_2CH_2CN)$, Et_3N, CH_2Cl_2, 0°C , 1h, 93 %; iv) 4-Pentynoic acid, PyBOP, Et_3N, CH_2Cl_2, rt, overnight, 88 %; v) 3-Bromoperylene, CuI, $(Ph_3P)_4Pd$, Et_3N, THF, 60°C, overnight, 77 %; vi) $(i\text{Pr})_2NP(Cl)(OCH_2CH_2CN)$, Et_3N, CH_2Cl_2, 0°C , 1h, 72 %;

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**[1] and 4-ethynylbenzoic acid[2] were synthesized according to the previous reports. 4-Ethynylbenzoic acid (0.68 g, 4.6 mmol) was reacted with PyBOP (2.6 g, 5.0 mmol) in CH_2Cl_2 (40 ml) for 10 min. Then, a solution of Et_3N (20 ml) and compound **1** (1.7 g, 4.2 mmol) in CH_2Cl_2 (10 ml) were added to the above mixture. After vigorous stirring overnight at room temperature, the organic solution was washed with saturated aqueous solution of $NaHCO_3$. The solvent was removed by evaporation, followed by silica gel column chromatography (AcOEt : hexane : Et_3N = 40:60:3, R_f = 0.38) to afford **2** (1.7 g, yield 75 %). ^{1}H-NMR [$CDCl_3$, 500 MHz] δ = 7.76 (d, 1H, J = 8.5 Hz), 7.58 (d, 1H, J = 8 Hz), 7.28-7.20 (m, 7H), 6.81-6.78 (m, 5H), 4.22 (m, 1H), 4.11 (m, 1H), 3.77 and 3.76 (s, 6H), 3.57 (dd, J = 4 Hz, 9.5 Hz, 1H), 3.38 (dd, J = 3 Hz, 9.5 Hz, 1H), 3.12 (br, 1H), 1.20 (d, 3H, J = 6 Hz). ^{13}C-NMR [$CDCl_3$, 126 MHz] δ = 167.1, 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 $C_{34}H_{33}NO_5$ (M^+) 535.2359. Found 535.2363.

Compound 3.

3-Bromoperylene was synthesized according to the literature.[3] 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 **2** (0.70 g, 1.3 mmol) in dry THF 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 ($CHCl_3$: hexane : AcOEt :Et_3N = 33: 33:33:3, R_f = 0.37) gave **3** (0.84 g, yield 82 %).

^{1}H-NMR [$CDCl_3$, 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 = 8 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). ^{13}C-NMR [$CDCl_3$, 126 MHz] δ = 167.3, 158.9, 144.6, 135.8, 135.6, 134.7, 134.7, 133.9, 132.4, 131.9, 131.7, 131.5, 131.0, 130.7, 130.2, 130.2, 128.7, 128.6, 128.5, 128.2, 127.5, 127.4, 127.3, 127.2, 126.8, 126.7, 126.1, 121.2, 120.9, 120.8, 119.7, 113.6, 94.7, 91.0, 87.1, 68.9, 65.5, 55.5, 54.4, 20.4. HRMS(FAB) Calcd for $C_{54}H_{43}NO_5$ (M^+) 785.3141. Found 785.3112.

Compound 4.

Et_3N (0.16 ml, 1.2 mmol) and 2-cyanoethyldiisopropylchlorophosphoramidite (0.10 ml, 0.46 mmol) were added to a solution of compound **3** (0.18 g, 0.23 mmol) in CH_2Cl_2 (5.0 ml) at 0 °C. Then the mixture was stirred for 60 min at 0 °C. Then, $CHCl_3$ was added to the reaction mixture and was washed with saturated aqueous solution of $NaHCO_3$ and that of NaCl. After drying over $MgSO_4$, the solvent was removed by evaporation, followed by silica gel column chromatography ($CHCl_3$: Et_3N = 100:3) to afford **4** (0.21 g, yield 93.0 %). ^{1}H-NMR [$CDCl_3$, 500 MHz] δ = 8.25 (d, 1H, J = 8.5 Hz), 8.22 (d, 1H, J = 7.5 Hz), 8.17 (m, 1H), 8.11 (d, 1H, J = 7.5 Hz), 7.83 (d, 2H, J = 8.5 Hz), 7.79 (d, 1H, J = 8.5 Hz), 7.73-7.67 (m, 5H), 7.58 (t, 1H, J = 7.5 Hz), 7.49-7.44 (m, 4H), 7.33-7.26 (m, 7H), 6.83-6.80 (m, 4H), 6.59 and 6.39 (d, 1H, J = 8.5 Hz), 4.49 (m, 1H), 4.40 (m, 1H), 3.85-3.54 (m, 12H), 2.65-2.58 (m, 2H), 1.30-1.11 (m, 15H). ^{31}P-NMR [$CDCl_3$, 500 MHz] δ = 147.8. HRMS(FAB) Calcd for $C_{63}H_{61}N_3O_6P$ ($M+H^+$) 986.4298. Found 986.4280.

Compound 5.

4-Pentynoic acid (0.12 g, 1.2 mmol) was reacted with PyBOP (0.62 g, 1.2 mmol) in CH_2Cl_2 (10 ml) for 10 min. Then, a solution of Et_3N (5 ml) and compound **1** (0.41 g, 1.0 mmol) in CH_2Cl_2 (10 ml) were added to the above mixture. After vigorous stirring overnight,

the organic solution was washed with saturated aqueous solution of $NaHCO_3$. The solvent was removed by evaporation, followed by silica gel column chromatography (AcOEt : hexane : Et_3N = 50:50:3, R_f = 0.13) to afford **5** (0.43 g, yield 88 %). ^{1}H-NMR [$CDCl_3$, 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). ^{13}C-NMR [$CDCl_3$, 126 MHz] δ = 171.5, 158.9, 144.6, 135.7, 135.5, 130.2, 130.2, 128.3, 128.2, 127.3, 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 $C_{30}H_{33}NO_5$ (M^+) 487.2359. Found 487.2367.

Compound 6.

To a solution of copper(I) iodide (0.18 g, 0.94 mmol) and tetrakis(triphenylphosphine)palladium(0) (79 mg, 0.068 mmol) in dry THF (10 ml) and piperidine (20 ml) was added 3-bromoperylene (0.48 g, 1.4 mmol). After the vigorous stirring at room temperature under nitrogen for 30 minutes, a solution of compound **5** (0.59 g, 1.2 mmol) in dry THF was added to the above mixture at 60 °C. The reaction mixture was refluxed for 3.0 hours and then the solvent was removed by evaporation. Column chromatography on silica gel ($CHCl_3$: hexane : AcOEt : Et_3N = 33:33:33:3, R_f = 0.14) gave **6** (0.68 g, yield 77 %). ^{1}H-NMR [$CDCl_3$, 500 MHz] δ = 8.21 (m, 2H), 8.16 (m, 2H), 8.06 (d, 1H, *J* = 8 Hz), 7.70 (dd, 2H, *J* =2.5 Hz, 8 Hz), 7.57 (d, 1H, *J* = 7.5 Hz), 7.50 (m, 3H), 7.36 (m, 2H), 7.30-7.05 (m, 7H), 6.77 (m, 4H), 6.27 (d, 1H, *J* = 8 Hz), 4.12 (m, 1H), 4.01 (m, 1H), 3.73 (s, 6H), 3.40 (dd, *J* = 4.5 Hz, 10 Hz, 1H), 3.33 (dd, *J* = 3.5 Hz, 9.5 Hz, 1H), 2.95 (m, 2H), 2.64 (m, 2H), 1.14 (d, 3H, *J* = 6.5 Hz). ^{13}C-NMR [$CDCl_3$, 126 MHz] δ = 171.7, 158.8, 144.6, 135.7, 135.6, 135.0, 134.8, 131.6, 131.5, 131.3, 131.1, 131.0, 130.2, 130.1, 128.7, 128.7, 128.4, 128.2, 128.1, 127.3, 127.2, 126.9, 126.8, 126.4, 120.9, 120.9, 120.7, 120.7, 119.8, 113.5, 94.6, 87.0, 80.3, 68.5, 65.2, 55.4, 55.4, 54.8, 54.6, 53.8, 46.0, 36.2, 20.2, 16.7. HRMS(FAB) Calcd for $C_{50}H_{43}NO_5$ (M^+) 737.3141. Found 737.3141.

Compound 7.

Et_3N (0.16 ml, 1.1 mmol) and 2-cyanoethyldiisopropylchlorophosphoramidite (0.10 ml, 0.44 mmol) were added to a solution of compound **6** (0.16 g, 0.22 mmol) in CH_2Cl_2 (1.0 ml) and the solution was stirred for 60 min at 0 °C. Then, $CHCl_3$ was added to the reaction mixture and was washed with saturated aqueous solution of $NaHCO_3$ and that of NaCl. After drying over $MgSO_4$, the solvent was removed by evaporation, followed by silica gel column chromatography ($CHCl_3$: hexane : Et_3N = 50:50:3, R_f = 0.58) to afford **7** (0.15 g, yield 72 %). ^{1}H-NMR [$CDCl_3$, 500 MHz] δ = 8.21 (d, 2H, *J* = 7.5 Hz), 8.16 (m, 2H), 8.05 (t, 1H, *J* = 7.5 Hz), 7.70 (dd, 2H, *J* =2 Hz, 8 Hz), 7.52-7.46 (m, 4H), 7.41 (m, 2H), 7.28-7.19 (m, 7H), 6.80-6.76 (m, 4H), 5.98 and 5.83 (d, 1H, *J* = 9.5 Hz), 4.39 (m, 1H), 4.26 (m, 1H), 3.73 (s, 6H), 3.63-3.35 (m, 4H), 3.25 (m, 1H), 3.12 (m, 1H), 2.93 (m, 2H), 2.60 (m, 2H), 2.37-2.24(m, 2H), 1.35-1.07 (m, 15H). ^{31}P-NMR [$CDCl_3$, 500 MHz] δ = 149.0, 148.6. HRMS(FAB) Calcd for $C_{59}H_{60}N_3O_6P$ (M^+) 937.4220. Found 937.4216.

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 reversed-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. 6886 (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 N_2-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 (T_m) was determined from the maximum in the first derivative of the melting curve. Both the heating and cooling curves were measured, and the T_m obtained from them coincided with each other to within 2.0 °C.

1. Y. Hara, T. Fujii, H. Kashida, K. Sekiguchi, X. Liang, K. Niwa, T. Takase, Y. Yoshida and H. Asanuma, *Angew. Chem. Int. Ed.*, 2010, **49**, 5502-5506.
2. E. Yashima, T. Matsushima and Y. Okamoto, *J. Am. Chem. Soc.*, 1997, **119**, 6345-6359.
3. H. Maeda, Y. Nanai, K. Mizuno, J. Chiba, S. Takeshima and M. Inouye, *J. Org. Chem.*, 2007, **72**, 8990-8993.
4. H. Kashida, T. Takatsu and H. Asanuma, *Tetrahedron Lett.*, 2007, **48**, 6759-6762.

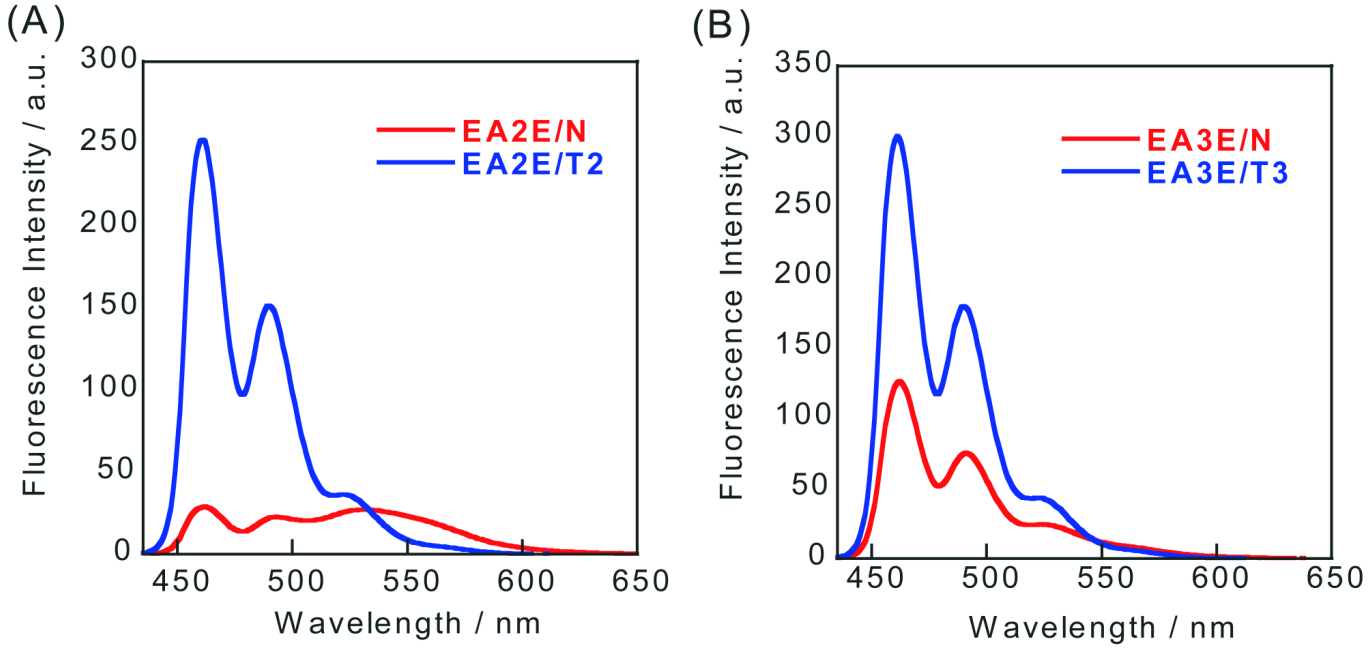
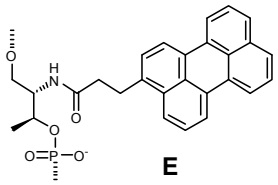


Fig. S1. Detection of (A) two-base and (B) three-base deletion polymorphisms by use of **E**. Solution conditions were as follows: [DNA] = 5.0 μM, [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer), 0 °C. Excitation wavelength: 425 nm.

EA2E : 5'-GGTATC**E**AA**E**GCAATC-3'
EA3E : 5'-GGTATC**E**AAA**E**GCAATC-3'
T2 : 3'-CCATAGTTCGTTAG-5'
T3 : 3'-CCATAGTTTCGTTAG-5'
N : 3'-CCATAGCGTTAG-5'



E

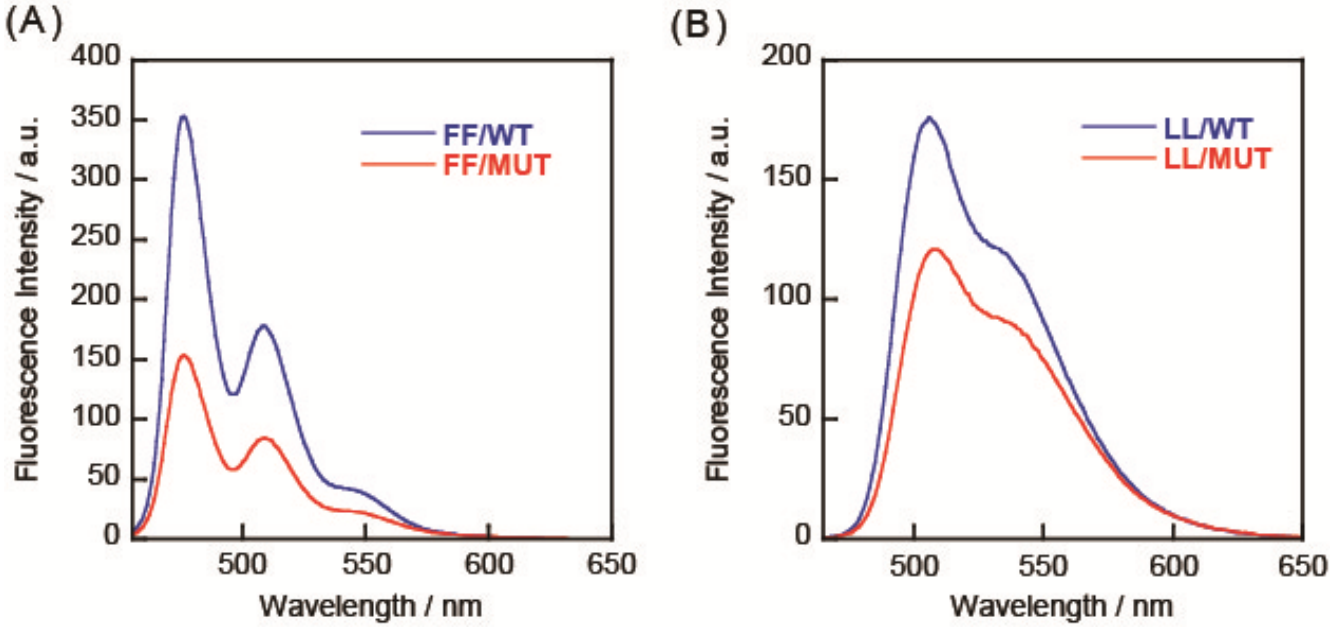


Fig. S2 Fluorescent emission spectra of (A) **FF** and (B) **LL** with **WT** (blue line) or **MUT** (red line) at 20 °C. Excitation wavelength was (A) 445 nm or (B) 455 nm. Solution conditions were as follows: [Probe] = 1.0 μM, [Target] = 1.2 μM [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer).

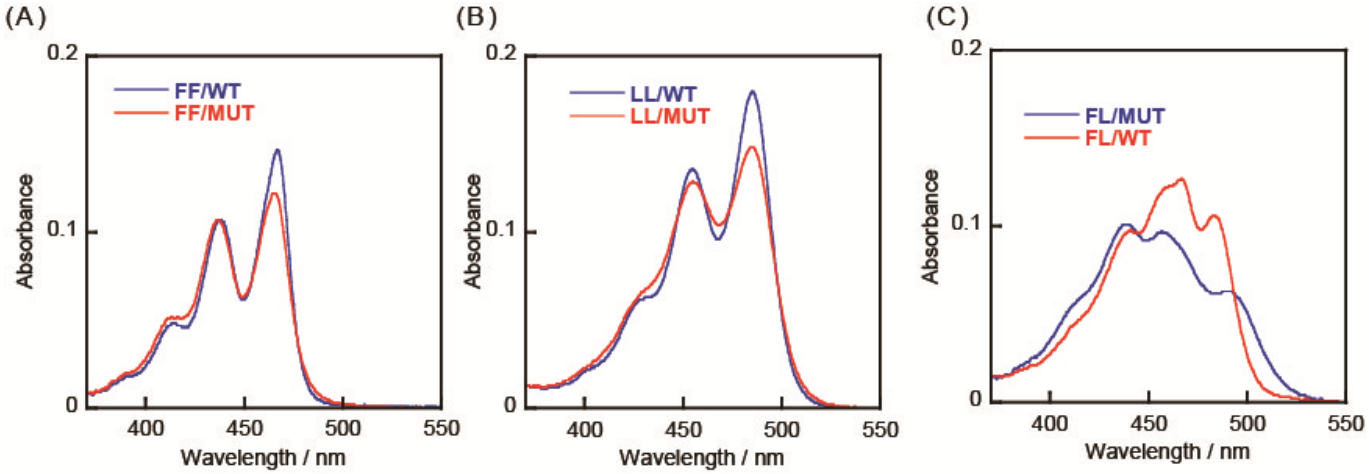
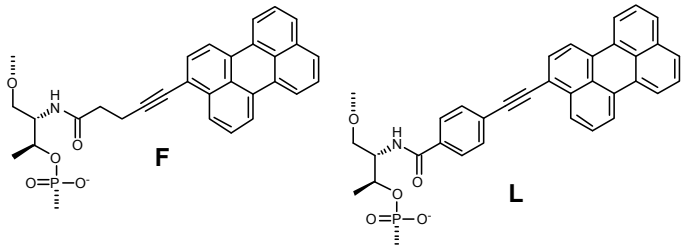


Fig. S3. UV-VIS spectra of (A) **FF**, (B) **LL** and (C) **FL** with **WT** (blue line) or **MUT** (red line). Solution conditions were as follows: [DNA] = 2.0 μM, [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer).

FF: 5'-AATATCAT**F**CTT**F**TGGTGTTT-3'
LL: 5'-AATATCAT**L**CTT**L**TGGTGTTT-3'
FL: 5'-AATATCAT**F**CTT**L**TGGTGTTT-3'
WT: 3'-TTTCTTTTATAGTAGAAACCACAAAGGATAC-5'
MUT: 3'-TTTCTTTTATAGTAACCACAAAGGATAC-5'



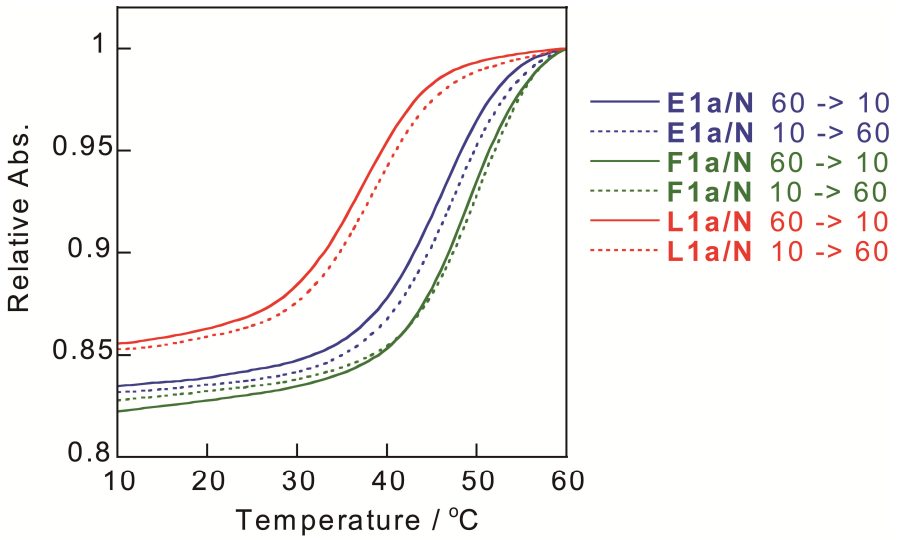


Fig. S4. Melting Curves of **E1a/N**, **F1a/N** and **L1a/N**. Both heating and cooling curves are shown. Solution conditions were as follows: [DNA] = 2.0 μM, [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer).

E1a : 5'-GGTATC**E**GCAATC-3'
F1a : 5'-GGTATC**F**GCAATC-3'
L1a : 5'-GGTATC**L**GCAATC-3'
N : 3'-CCATAGCGTTAG-5'

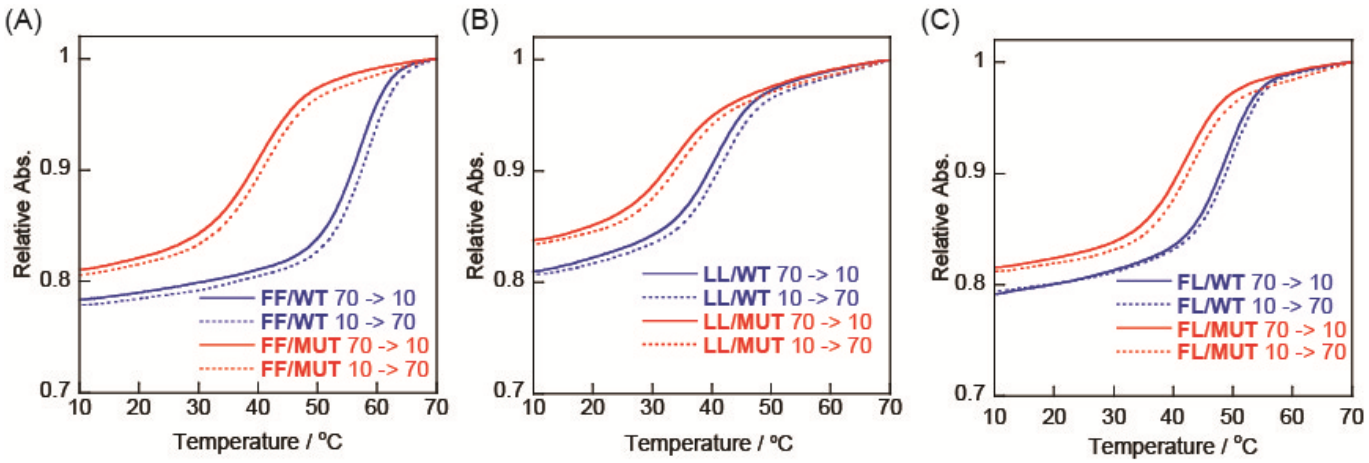


Fig. S5. Melting Curves of (A) **FF**, (B) **LL** and (C) **FL** with **WT** (blue line) or **MUT** (red line). Both heating and cooling curves are shown. Solution conditions were as follows: [DNA] = 2.0 μM, [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer).

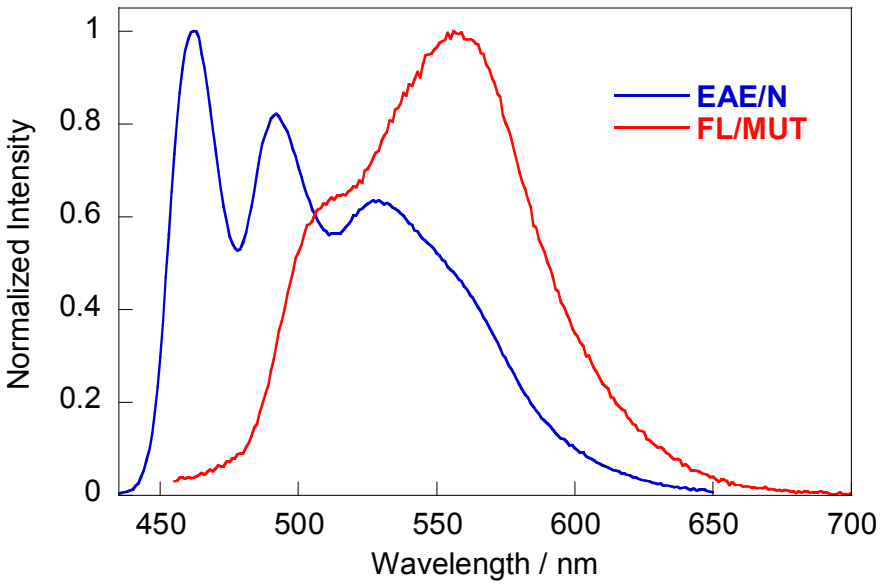


Fig. S6. Comparison of the excimer emission of **E** and the exciplex emission of **F** and **L**. Solution conditions were as follows: [**FL**] = 1.0 μM, [**MUT**] = 1.2 μM, [**EAE**] = [**N**] = 5.0 μM, [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer).

EAE : 5'-GGTATC**E**A**E**GCAATC-3'
N : 3'-CCATAGCGTTAG-5'

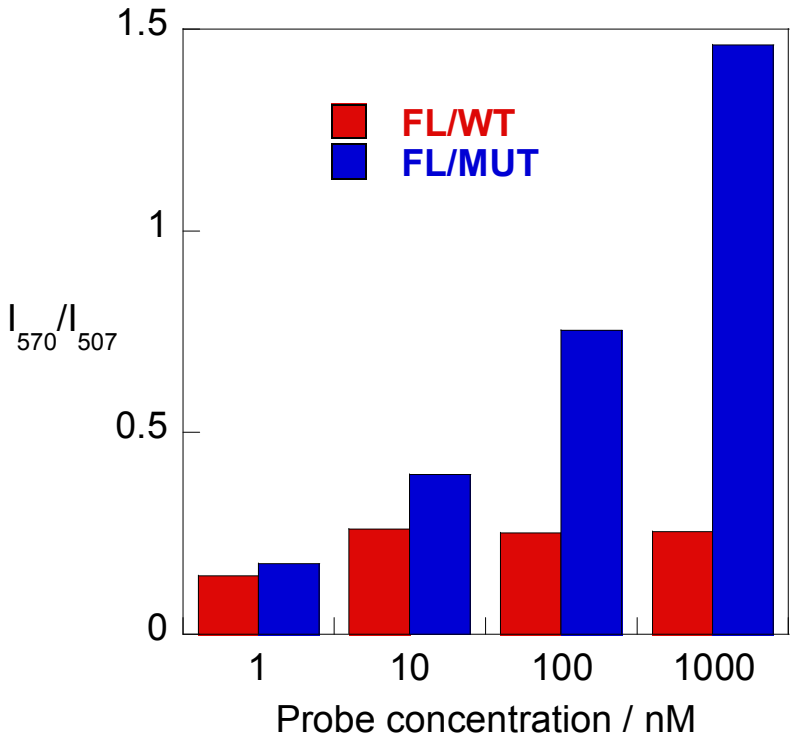


Fig. S7. Ratio of the intensity of exciplex emission to monomer emission at lower probe concentrations. Concentration of the target (**WT** or **MUT**) was 1.2 times higher than that of **FL** for each sample. Solution conditions were as follows: [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer), 20 °C.

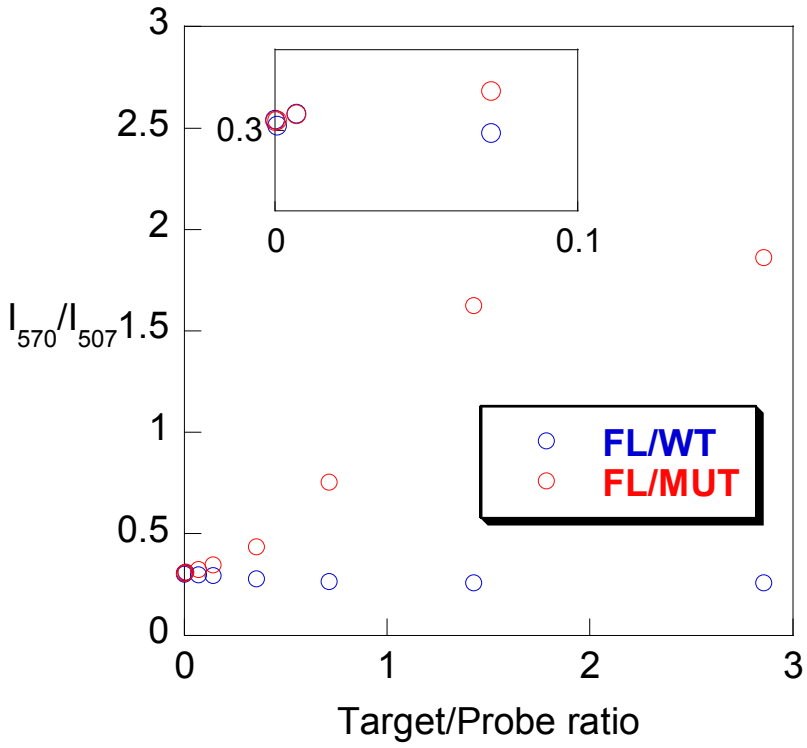


Fig. S8. Ratio of the intensity of exciplex emission to monomer emission at various target/probe ratios. Plots at low concentration are magnified in the inset. The concentration of **FL** was fixed to 1.4 μM. Solution conditions were as follows: [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer), 20 °C.