Supporting Information

for

Cytosine-bulge-dependent fluorescence quenching for real-time hairpin primer PCR

F. Takei, X. Chen, G. Yu, T. Shibata, C. Dohno and K. Nakatani*

The Institute of Scientific and Industrial Research, Osaka University, 8-1 Mihogaoka, Ibaraki, Osaka 567-0047, Japan

Contents

Table S1. Sequence of oligomers used in these studies

M1:	5’-TCCA***T***_GCAAC-3’
M2:	5’-TCCAG_***T***CAAC-3’
U1:	5’-TCCAT_GCAAC-3’
U2:	5’-TCCAG_TCAAC-3’
3:	5’-GTTGCCATGGA-3’
4:	5’-GTTGCATGGA-3’
5:	5’-GTTGACCTGGA-3’
6:	5’-GTTGACTGGA-3’
7:	5’-GTTGCTATGGA-3’
8:	5’-GTTGCGATGGA-3’
9:	5’-GTTGCAATGGA-3’

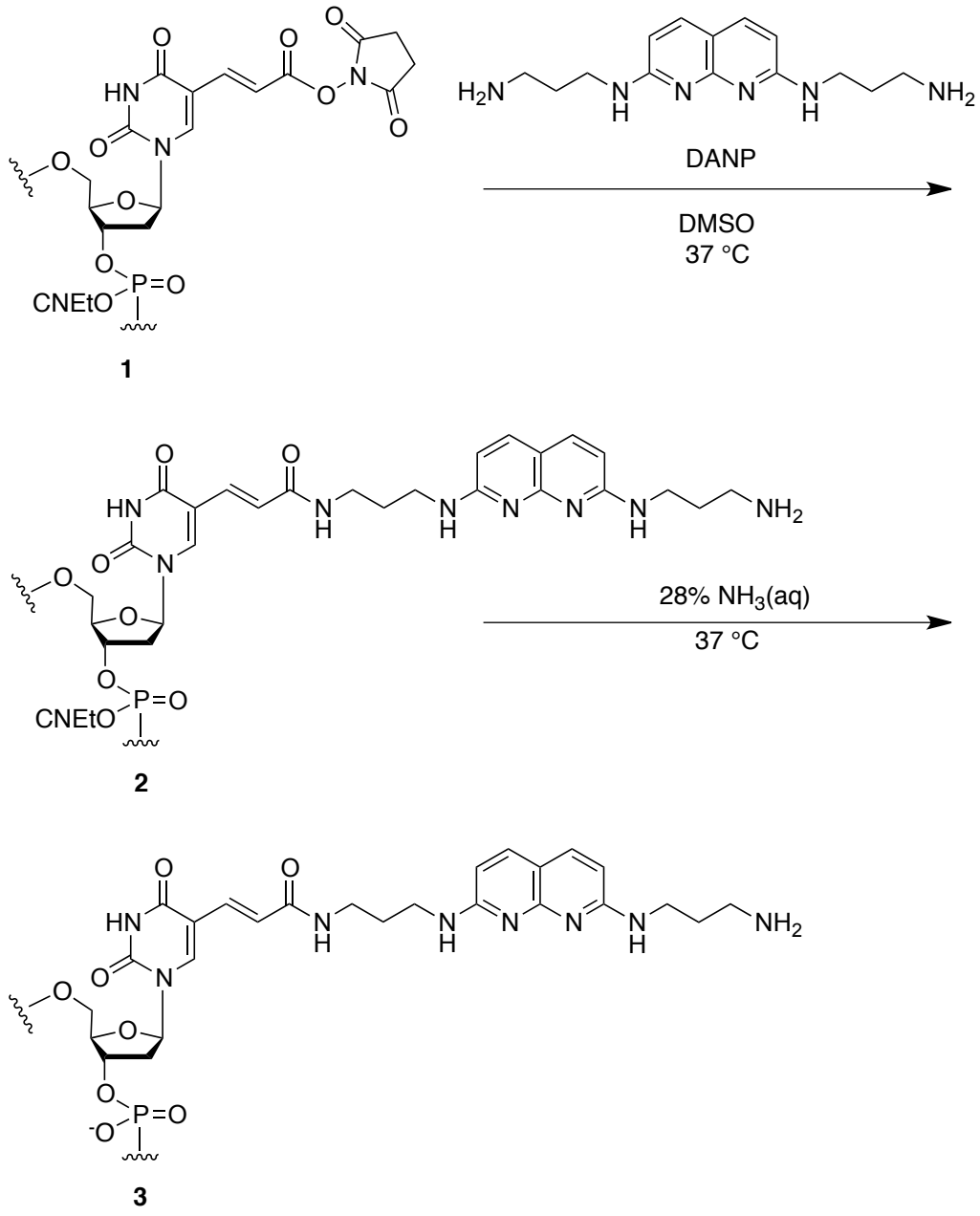

Fig. S1 Synthetic scheme of DANP-anchored DNA.

Sequence of C-bulge DNAs

5'-TCCA***T***_GCAAC-3'
3'-AGGTAX̄CGTTG-5'
(**X** = C, T, G, A)

	Sequence
M1/3:	5'-***T***_G-3' 3'-AC̄C-5'
M1/7:	5'-***T***_G-3' 3'-AT̄C-5'
M1/8:	5'-***T***_G-3' 3'-AḠC-5'
M1/9:	5'-***T***_G-3' 3'-AĀC-5'

T : DANP-anchored T

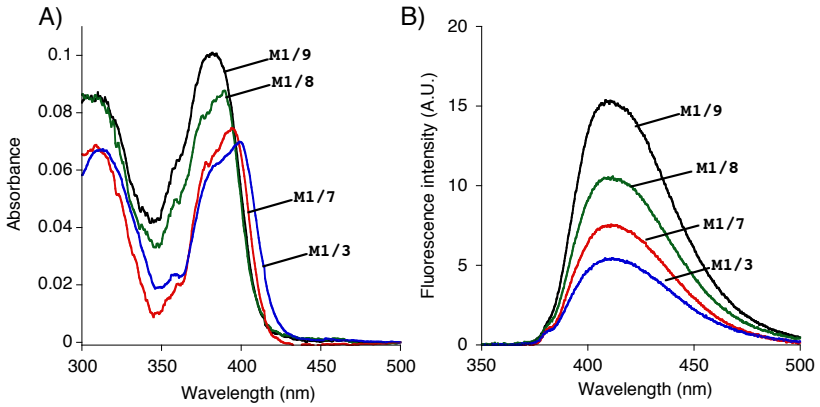

Fig. S2 A) UV and B) Fluorescence spectra of **M1/3** (4.5 μM) (blue), **M1/7** (4.5 μM) (red), **M1/8** (4.5 μM) (green), and **M1/9** (4.5 μM) (black) in sodium phosphate buffer (pH 7.0, 10 mM) and sodium chloride (100 mM). Excitation wavelength was at 365 nm.

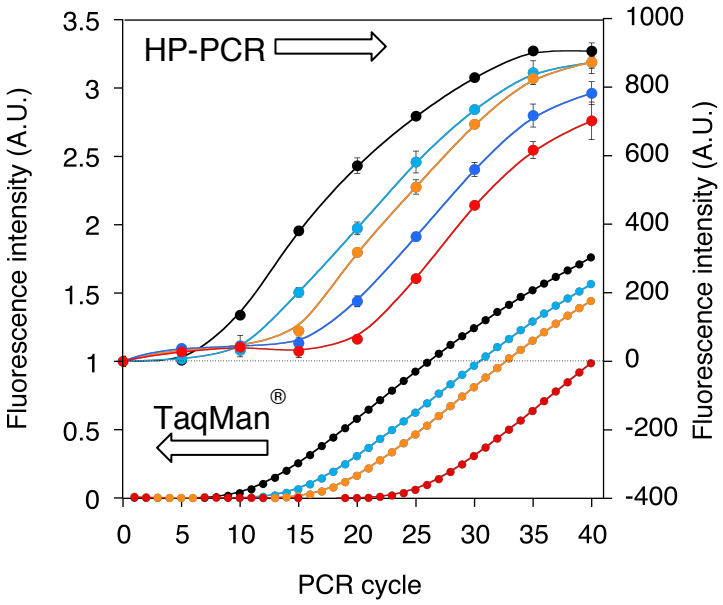

Fig. S3 Fluorescence intensity of PCR solution using HP-PCR and TaqMan® PCR with different concentrations of template pUC18. Key: 500 pM (black), 50 pM (light blue), 5 pM (orange), 0.5 pM (blue), 0.05 pM(red). TaqMan® PCR on the pUC18 plasmid was performed with a forward primer (5'-d(CAG GAA ACA GCT ATG ACC ATG ATT)-3', a reverse primer (5'-d(GTT GTA AAA CGA CGG CCA GTG)-3', and a TaqMan® probe (5'-d(FAM-TTG CAT GCC TGC AGG T-quencher-MGB), which were designed and synthesized by Life Technologies JAPAN. The amplification reaction was carried out with 0.5 μM of each primer, TaqMan® Fast Advanced Master Mix (Life Technologies, JAPAN), and pUC18 in a final volume of 25 μL. The amplification protocol was 50 °C for 2 min, 95 °C for 10 min, and 40 cycles of 95 °C for 15 s, and 60 °C for 60 s on a StepOne™ Real-Time PCR Systems (Applied Biosystems®).

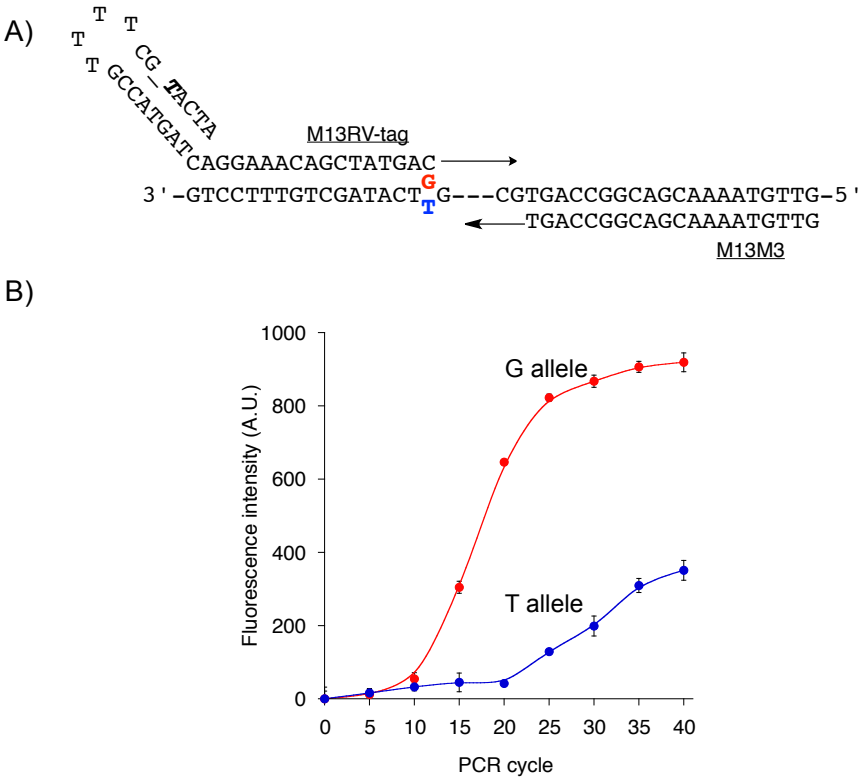

Fig. S4 A) Alignment of primers on wild- and mutant-alleles. B) Fluorescence intensity of PCR solutions using alleles G (red) and T (blue) templates.

Experimental Section

Synthesis of modified DNA (M1, M2) and primer M13RV-tag

DNAs including *N*-hydroxysuccinimide ester on uridine were synthesized and purchased from Japan Bio Service (Saitama, Japan). The modified DNAs were treated with 0.1 mM DANP in dimethyl sulfoxide solution and incubated at 37 °C for approximately 3 hours. After removing the dimethyl sulfoxide, the DNA was cleaved from the CPG and deprotected by using a 28% ammonia solution at room temperature. The resulting mixture was evaporated and purified by reverse-phase high-performance liquid chromatography on a COSMOSIL 5C_{18}-MS-II column (10 × 150 mm), and eluted with 5–17% (20 min) acetonitrile in 0.1 M triethylammonium acetate, pH 7.0, at a flow rate 3.0 mL/min. Mass spectra of DANP-anchored DNAs were recorded on a JEOL AccuTOF JMS-T 100N mass spectrometer. DNA M1: $[M+H]^+$ calcd. 3285.4, observed 3285.4; DNA M2: $[M+H]^+$ calcd. 3285.4, observed 3283.4; M13RV-tag: $[M+H]^+$ calcd. 11356.5, observed 11354.3

Measurement of melting temperature (T_m)

All T_ms of the DANP-bound DNA (4.5 μM, final duplex concentration) were measured in 10 mM sodium cacodylate buffer (pH 7.0) containing 100 mM NaCl. Absorbance profiles were measured at 260 nm using a Shimadzu UV-2550 spectrometer equipped with a temperature controller using a 1-cm path length cell. The absorbance of the samples was monitored at 260 nm from 2 to 90 °C with a heating rate of 1 °C/min.

Measurement of fluorescence spectra

Fluorescence spectra were measured with SHIMADZU RF-5300PC spectrofluorophotometer. All DNA (4.5 μM, final duplex concentration) measurements were taken in 10 mM sodium phosphate buffer (pH 7.0) containing 100 mM NaCl. The excitation wavelength was 365 nm, 380 nm, or 400 nm.

Measurement of UV-vis spectra

UV-vis spectra were measured with a BECKMAN COULTER DU 800 spectrophotometer. All DNA (4.5 μM, final duplex concentration) measurements were taken in 10 mM sodium phosphate buffer (pH 7.0) containing 100 mM NaCl.

Allele-specific PCR on pUC18 plasmid

Allele-specific PCR on the pUC18 plasmid was performed with a forward primer, M13M3 (5'-d(GTT GTA AAA CGA CGG CCA GT)-3'), and a reverse primer, M13RV-tag (5'-d(ATC A***T***G CTT TTG CCA TGA TCA GGA AAC AGC TAT GAC)-3'). The amplification reaction was carried out with 0.5 μM of each primer, 5 μM of DANP, Taq PCR Master Mix Kit (Qiagen), and pUC18 or mutant pUC18 (100 pg/μL) in a final volume of 40 μL. The amplification protocol was 95 °C for 1 min, and

40 cycles of 95 °C for 10 s, 55 °C for 30 s, and 72 °C for 30 s on a TP600 PCR Thermal Cycler (TAKARA). All PCR products were analyzed by native PAGE (8% polyacrylamide gel stained with SYBR gold). Fluorescence intensities of PCR solutions after certain cycles were measured on a BERTHOLD Mithras LB 940 microplate reader with 400-nm excitation and 450-nm emission filters.

Sequencing of PCR products

The DNA sequencing of the PCR product of M13M3 and M13RV-tag was determined from Fasmac (Japan) using pMD19 T-cloning vector (TaKaRa) with M13M4 and M13RV primers. The result of DNA sequencing showed

5′-TGTAA AACGA CGGCC AGT GCCAA GCTTG CATGC CTGCA GGTCG
ACTCT AGAGG ATCCC CGGGT ACCGA GCTCG AATTC GTAAT CATGG
TCATA GCTGT TTCCT G ATCAT GGCAA AAGC**A** TGATA-3′.

Bold underline of T and A descended from T-cloning vector and Taq polymerase, respectively. Sequences shown with single underlines are M13M3 (GTAA AACGA CGGCC AGT) and antisense of M13RV (G TCATA GCTGT TTCCT G), and the sequence shown with a underline is antisense-DANP-anchored tag sequence (ATCAT GGCAA AAGC**A** TGAT). The nucleotide opposite the DANP-anchored T was A, which showed with bold and *Italic*.