Electronic Supplementary Material (ESI) for Analytical Methods.

Electronic Supplementary Information

Detection of CpG methylation level using methyl-CpG-binding domain fused green fluorescent protein

Marika Fujita[a],‡ Masanori Goto[a],‡ Masayoshi Tanaka[b], and Wataru Yoshida*[a,c]

a. Graduate School of Bionics, Tokyo University of Technology, 1404-1 Katakuramachi, Hachioji, Tokyo, 192-0982, Japan. Email: yoshidawtr@stf.teu.ac.jp

b. Department of Chemical Science and Engineering, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama, Kanagawa, 226-8503, Japan.

c. School of Bioscience and Biotechnology, Tokyo University of Technology, 1404-1 Katakuramachi, Hachioji, Tokyo, 192-0982, Japan

‡ Equal contributions.

Table S1 Oligonucleotides sequences used in this study.

Name	Sequence (5'-3')	Length (mer)
Forward primer for AcGFP1	ACTGAATTCATGGTGAGCAAGGGCG	25
Reverse primer for AcGFP1	ACTGCGGCCGCTCACTTGTA	20
Methylated top strand	Biotin-AAAAAACAGGATXGAGCAGCTACCCT	26
Methylated bottom strand	AGGGTAGCTGCTXGATCCTG	20
Unmethylated top strand	Biotin-AAAAAACAGGATCGAGCAGCTACCCT	26
Unmethylated bottom strand	AGGGTAGCTGCTCGATCCTG	20
Methylated *SEPT9* probe DNA	Biotin-AAAAAATTGACXGXGGGGTCXGACATGATGGC	32
Methylated *SEPT9* target ssDNA	GCCATCATGTXGGACCCXGXGGTCAA	26
Unmethylated *SEPT9* target ssDNA	GCCATCATGTCGGACCCCGCGGTCAA	26
Methylated *BRCA1* probe DNA	Biotin-AAAAAACCCCXGGATGAXGTAAAAGGAAAGAG	32
Methylated *BRCA1* target ssDNA	CTCTTTCCTTTTAXGTCATCXGGGGG	26
Unmethylated *BRCA1* target ssDNA	CTCTTTCCTTTTACGTCATCCGGGGG	26
Methylated LINE-1 probe DNA	Biotin-AAAAAACAATATTXGGGTGGGAGTGA	26
Methylated LINE-1 target ssDNA	TCACTCCCACCXGAATATTG	20
Unmethylated LINE-1 target ssDNA	TCACTCCCACCCGAATATTG	20

X: 5-methylcytosine

ATGTGGAGCCATCCGCAGTTTGAAAAGGCTGAGGACTGGCTGGACTGCCCGGCCCTGGGCCCTGGCTGGAAGCGCCG
CGAAGTCTTTCGCAAGTCAGGGGCCACCTGTGGACGCTCAGACACCTATTACCAGAGCCCCACAGGAGACAGGATCC
GAAGCAAAGTTGAGCTGACTCGATACCTGGGCCCTGCGTGTGATCTCACCCTCTTCGACTTCAAACAAGGCATCTTG
TGCTATCCAGCCCCCAAGGCCCATCCCGTGGCGGTTGCCAGCGAATTCAGACCAATGGTGAGCAAGGGCGCCGAGCT
GTTCACCGGCATCGTGCCCATCCTGATCGAGCTGAATGGCGATGTGAATGGCCACAAGTTCAGCGTGAGCGGCGAGG
GCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCTGTGCCCTGGCCC
ACCCTGGTGACCACCCTGAGCTACGGCGTGCAGTGCTTCTCACGCTACCCCGATCACATGAAGCAGCACGACTTCTT
CAAGAGCGCCATGCCTGAGGGCTACATCCAGGAGCGCACCATCTTCTTCGAGGATGACGGCAACTACAAGTCGCGCG
CCGAGGTGAAGTTCGAGGGCGATACCCTGGTGAATCGCATCGAGCTGACCGGCACCGATTTCAAGGAGGATGGCAAC
ATCCTGGGCAATAAGATGGAGTACAACTACAACGCCCACAATGTGTACATCATGACCGACAAGGCCAAGAATGGCAT
CAAGGTGAACTTCAAGATCCGCCACAACATCGAGGATGGCAGCGTGCAGCTGGCCGACCACTACCAGCAGAATACCC
CCATCGGCGATGGCCCTGTGCTGCTGCCCGATAACCACTACCTGTCCACCCAGAGCGCCCTGTCCAAGGACCCCAAC
GAGAAGCGCGATCACATGATCTACTTCGGCTTCGTGACCGCCGCCGCCATCACCCACGGCATGGATGAGCTGTACAA
GTGA

Fig. S1 DNA sequence of *Strep-tag II-MBD-AcGFP1*.

The DNA sequence of *Strep-tag II*, *MBD*, and *AcGFP1* are shown as orange, blue, and red, respectively.

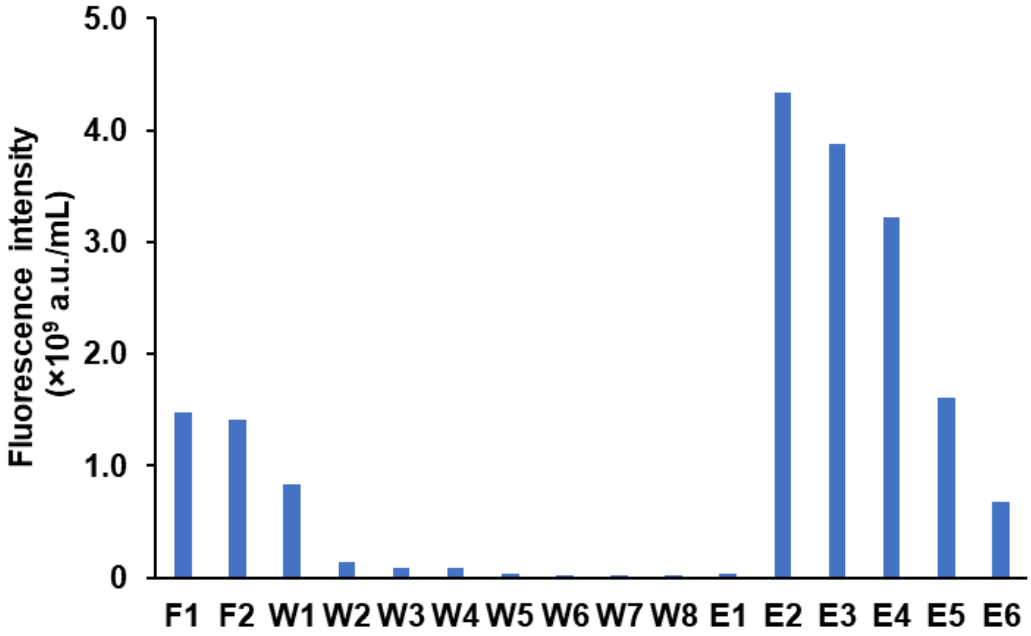

Fig. S2 Fluorescence intensity of each fraction in the purification of MBD-AcGFP1.

The fluorescence intensity of flow-through fractions (F1-F2), wash fractions (W1-W8), and elution fractions (E1-E6) were measured using 5 µL of samples with 45 µL of 1× PBS.

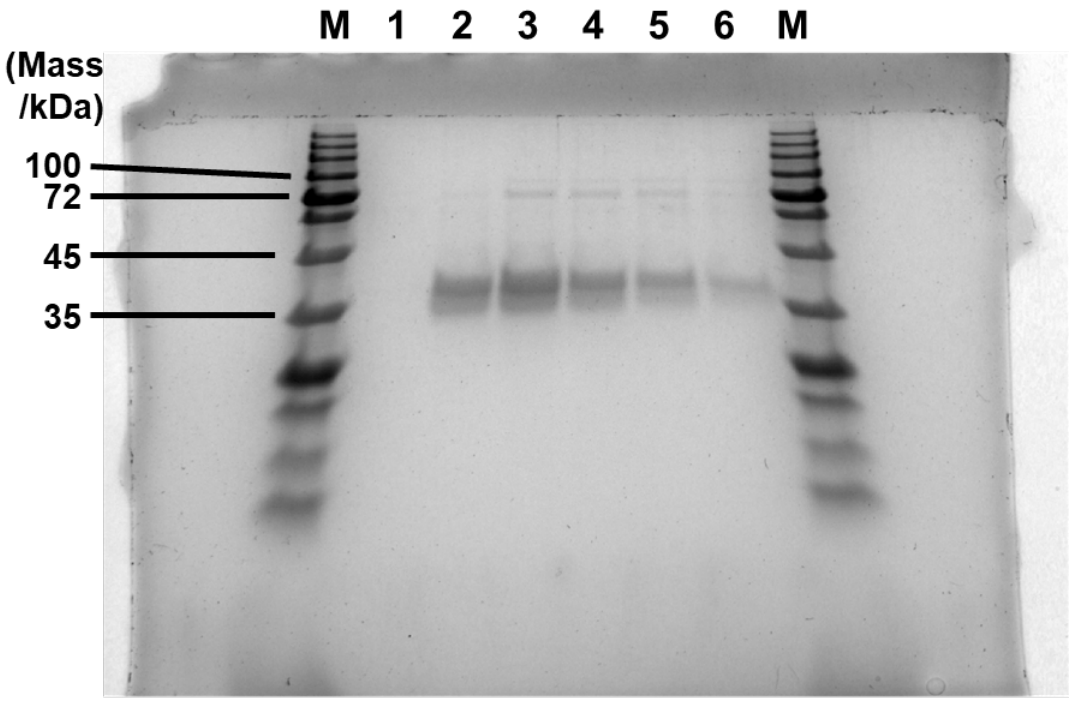

Fig. S3 SDS-PAGE analysis of the elution fractions.

The elution fractions (1-6) were analyzed using 12% SDS-PAGE. Molecular weight of MBD-AcGFP1 is 3.8×10^4.

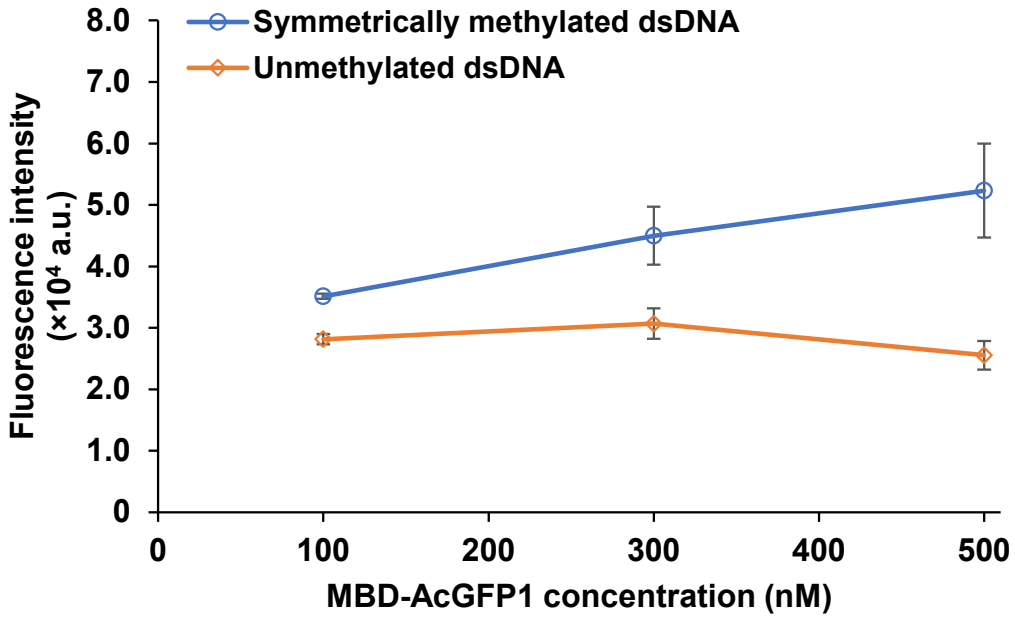

Fig. S4 Optimization of the concentration of the MBD-AcGFP1.

The methylated top strand and unmethylated top strand were mixed with the methylated bottom strand and unmethylated bottom strand at 1.25 μM in a binding buffer (1× PBS with 2 mM $MgCl_2$) to prepare symmetrically methylated and unmethylated dsDNA, respectively. The dsDNA (125 pmol) was immobilized on the streptavidin-coated wells, and MBD-AcGFP1 (100, 300, or 500 nM) was added. The fluorescence intensity of the bound MBD-AcGFP1 was measured (n = 3, mean ± SD).

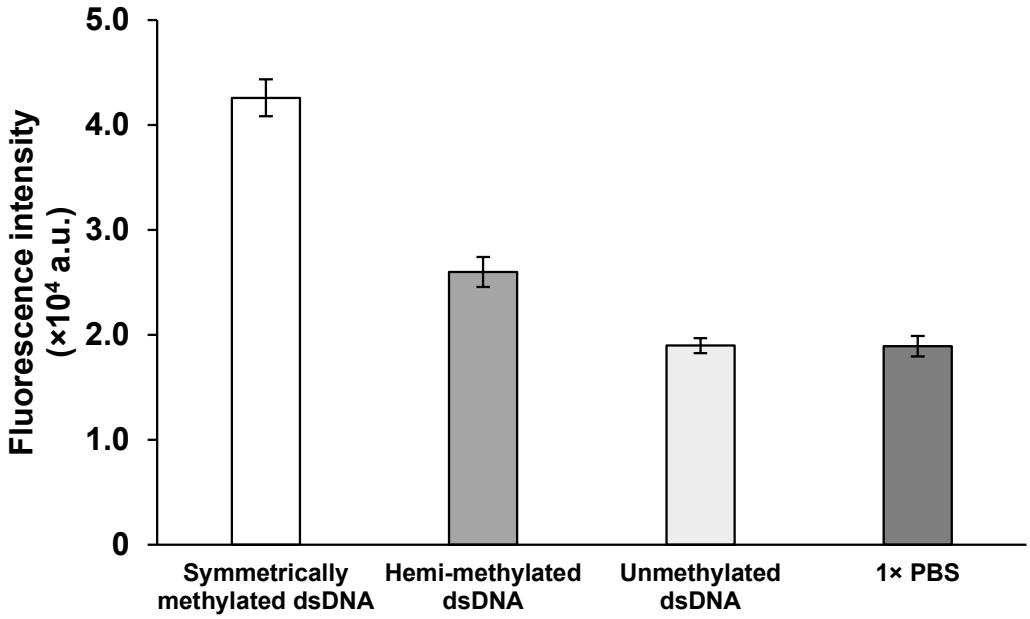

Fig. S5 Raw fluorescence intensity of the binding assay of MBD-AcGFP1 against symmetrically, hemi-, and unmethylated dsDNA.

Fluorescence intensity of 1× PBS in the streptavidin-coated well, which contains neither any DNA nor MBD-AcGFP1 was measured as the background intensity.

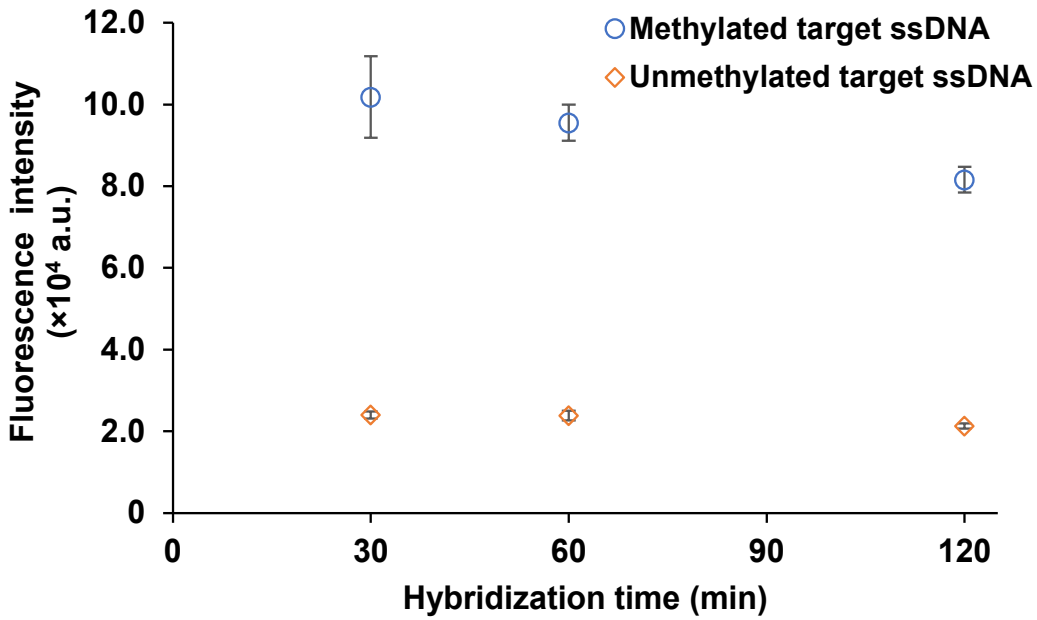

Fig. S6 Optimization of the hybridization time of the methylated *SEPT9* target ssDNA to the probe DNA.
Methylated *SEPT9* probe DNA (125 pmol) was immobilized onto streptavidin-coated wells. The *SEPT9* methylated or unmethylated target ssDNA (125 pmol) was added to the well and incubated at 25°C for 30, 60, or 120 min. After washing the wells, 500 nM MBD-AcGFP1 was added and incubated at 25°C for 30 min. After washing the wells, fluorescence intensity of the bound MBD-AcGFP1 was measured (n = 4, mean ± SD).

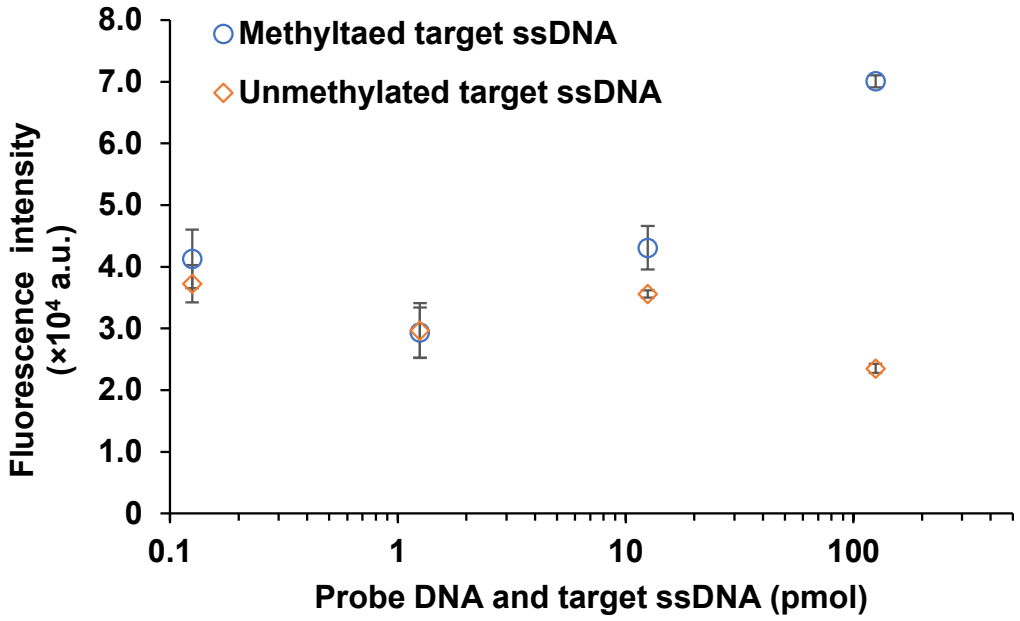

Fig. S7 Optimization of the amount of the methylated *SEPT9* probe DNA and target ssDNA.

Methylated *SEPT9* probe DNA (0.125, 1.25, 12.5, or 125 pmol) was immobilized onto streptavidin-coated wells. The *SEPT9* methylated target ssDNA (0.125, 1.25, 12.5, or 125 pmol) was added to the 0.125–125 pmol of corresponding methylated *SEPT9* probe DNA-immobilized wells and incubated at 25°C for 30 min. After washing the wells, 500 nM MBD-AcGFP1 was added and incubated at 25°C for 30 min. After washing the wells, fluorescence intensity of the bound MBD-AcGFP1 was measured (n = 4, mean ± SD).