Electronic Supplementary Material (ESI) for Analyst.
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Electronic Supporting Information
for

A Signal-on Fluorescent Assay for DNA Methyltransferase Activity Using a Methylation-resistant Endonuclease

A. Experimental section

Materials and Instrumentation

The *Dam* MTase (*Escherichia coli*), *Dpn*II endonuclease, SAM, and corresponding buffer solutions were purchased from New England Biolabs, Inc. (UK). SYBR Green I (SG) was purchased from Lonza Rockland, Inc. (USA). 5-Fluorouracil, gentamicin, benzylpenicillin, ampicillin, and mitomycin were purchased from Sigma-Aldrich (USA) and used without further purification. Ultrapure water with an electrical resistance larger than 18.2 MΩ was obtained using a Milli-Q system (Millipore, USA) and used throughout the study. The oligonucleotides used in this study were synthesized and purified by Bioneer Corporation (Daejeon, South Korea). The sequences were as follows:

Oligo A- 5'-ATGTTG**GATC**GATG**GATC**GATG**GATC**GAGA-3'

Oligo B- 5'-TCTC**GATC**CATC**GATC**CATC**GATC**CAACAT-3'

All the fluorescence measurements were performed using a quartz cuvette (with a path length of 10 mm and an inner width of 1 mm) and an LS55 fluorescence spectrometer (Perkin Elmer, UK). The emission spectra were collected from 510 to 650 nm, with an

excitation wavelength of 497 nm at room temperature. The excitation and emission slit widths were set at 5 nm, with a scanning speed at 600 nm/minute. The fluorescence intensity at 525 nm was chosen as the optimal experimental condition to evaluate the performance of the proposed assay.

The gel image was captured using the LAS-3000 imaging system (Fujifilm, Japan).

Fluorescence assay for *Dam* MTase activity

The double-stranded DNA (dsDNA) was prepared by mixing equal concentrations of Oligo A and Oligo B in the hybridization buffer (10 mM Tris-HCl, 50 mM NaCl, and 1 mM EDTA, pH 7.8). The mixture was heated to 90 ^{o}C for 5 minutes, cooled slowly to room temperature, and stored at 4 ^{o}C for further use.

The methylation reaction was performed in a 20-μl reaction volume in a centrifuge tube. The typical mixture of the methylation reaction consisted of 2 μl of 10X *Dam* buffer (500 mM Tris-HCl, 100 mM EDTA, and 50 mM 2-mercaptoethanol, pH 7.5), 1 μl of dsDNA (10 μM), 2 μl of S-adenosylmethionine (SAM, 800 μM), various concentrations of *Dam* MTase, and corresponding volumes of H_2O to 20 μl. The mixture was first incubated at 37 ^{o}C for 60 minutes, and the cleavage reaction of *Dpn*II was then performed in the same centrifuge tube. To minimize the influence of the methylation reaction buffer on the cleavage reaction, the volume of the cleavage reaction was increased to 50 μl. The added 30 μl of typical mixture was composed of 5 μl of 10X *Dpn*II reaction buffer (500 mM Bis-Tris-HCl, 1000 mM NaCl, 100 mM $MgCl_2$, and 10 mM DTT, pH 6.0), 4 μl of *Dpn*II

(10U/μl), and 21 μl of H_2O. The cleavage reaction was performed at 37 ^oC for 30 minutes. Lastly, 5 μl of a 500X SG solution and 45 μl of H_2O were added, followed by fluorescence measurements after 5 minutes.

Influence of certain drugs on *Dam* MTase activity

As *Dpn*II was also used in this proposed assay, it was therefore necessary to investigate the influence of *Dam* MTase inhibitors on *Dpn*II activity. The experiment for the effect of drugs on *Dpn*II activity was performed in a 20-μl reaction volume by mixing 1 μl dsDNA (10 μM), 2 μl 10X *Dpn*II reaction buffer (500 mM Bis-Tris-HCl, 1000 mM NaCl, 100 mM $MgCl_2$, and 10mM DTT, pH 6.0), 2 μl drug (100 μM), 4 μl *Dpn*II (10 U/μl) and 11 μl H_2O in a centrifuge tube. The mixture was incubated at 37 ^oC for 60 minutes, and then 5 μl of 500X SG solution and 75 μl H_2O were added, followed by fluorescence measurements after 5 minutes.

The influence of drugs on *Dam* MTase activity was then evaluated. All the inhibition experiments were performed under conditions similar to those used for the *Dam* MTase activity assay, except for the addition of 5 μM of different drugs to each sample. Briefly, the inhibition reactions were performed in a 20-μl reaction volume in a centrifuge tube. Before adding 5 μl of *Dam* MTase (8 U/μl), 1 μl of different drugs (100 μM) was introduced to the typical mixture (containing 2 μl 10X Dam buffer [500 mM Tris-HCl, 100 mM EDTA, and 50 mM 2-mercaptoethanol, pH 7.5], 1 μl 10 μM dsDNA, 2 μl 800μM

SAM, 9 µl H_2O). The reaction was then incubated at 37 ^oC for 60 minutes with all the ensuing steps being similar to the *Dam* MTase assay.

The relationship between the 5-fluorouracil concentration and *Dam* MTase activity was also investigated. The procedure of these experiments was similar as those above, except for the addition of various concentrations of 5-fluorouracil to each sample.

Assay of *Dam* MTase activity by gel electrophoresis

A 15-µl solution containing 1X *Dam* methyltransferase buffer, 5 µM dsDNA, 80 units *Dam* MTase, and 80 µM SAM was incubated at 37 ^oC for 3 hours. The sample was then increased in volume to 30 µl by adding another 15 µl of solution containing 80 units of *Dpn*II and 2X *Dpn*II reaction buffer. After incubating for an additional 3 hours, the sample was applied to a 4% agarose gel to separate the cleaved products from the substrate. The electrophoresis was performed in 1X TBE (pH 8.0) at 100 V for 40 minutes.

B. Supporting data

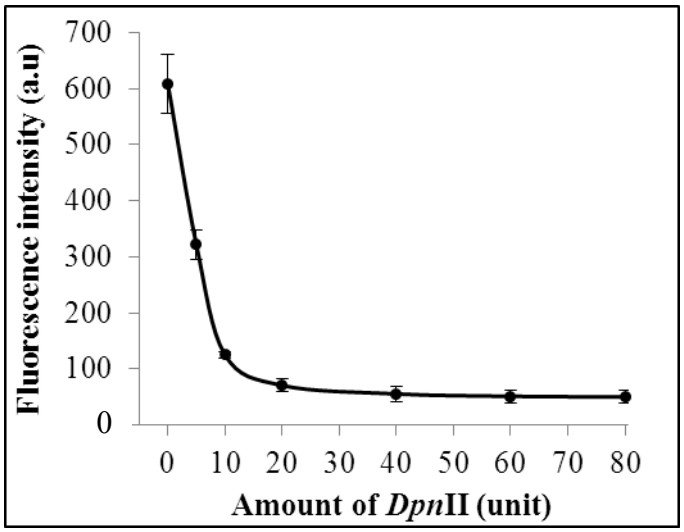

Figure S1. Relationship between the fluorescence intensity and amount of *Dpn*II used. A 20-µl solution containing 0.5 µM dsDNA, 1X *Dpn*II reaction buffer, and various amounts of *Dpn*II was incubated for 1 hour. The volume of the solution then was increased to 100 µl by the addition of 75 µl of H_2O and 5 µl of 500X SG solution. Fluorescence measurements were then performed after 5 minutes. The error bars represent the standard deviations of three repeated experiments.

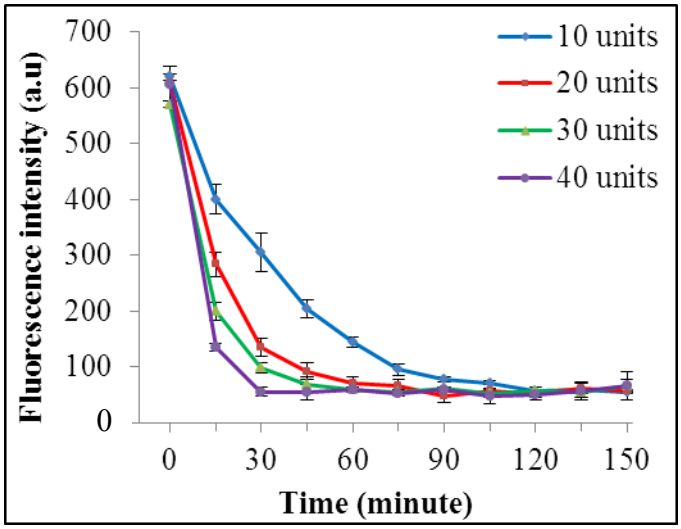

Figure S2. Relationship between fluorescence intensity and *Dpn*II reaction time at 10, 20, 30, and 40 units. These experiments were performed in a manner similar that above, except that the fluorescence intensity was measured every 15 minutes. The error bars represent the standard deviations of three repeated experiments.

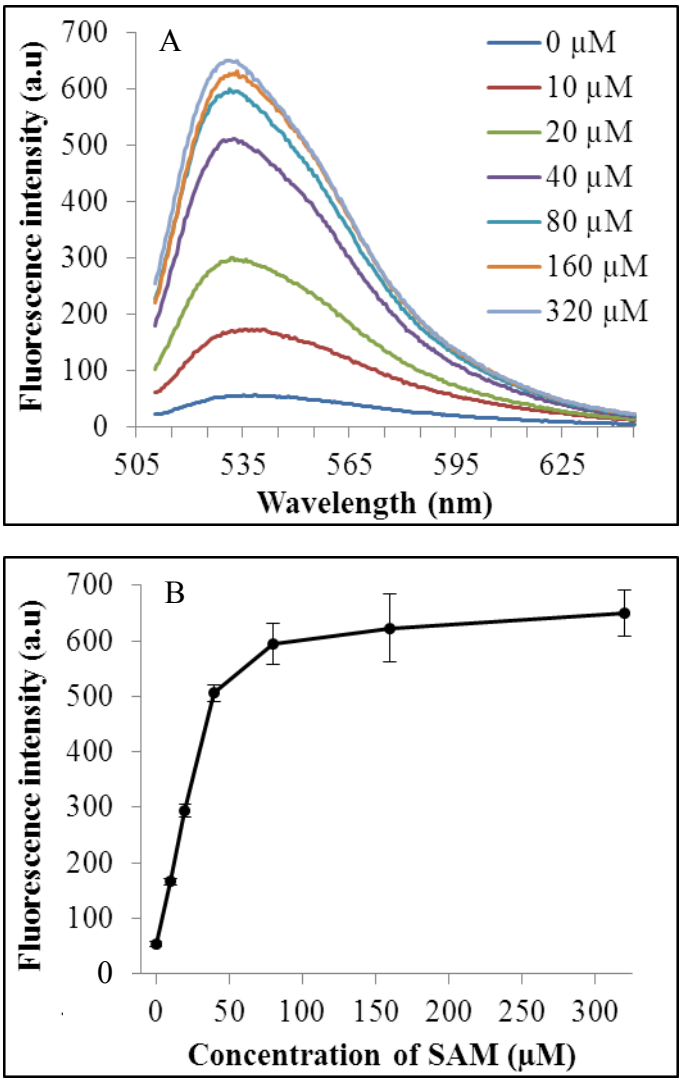

Figure S3. Fluorescence emission spectra (A) and calibration curve (B) of the solutions upon exposure to different concentrations of SAM. These experiments were performed in a manner similar to the fluorescence assay for *Dam* MTase activity, except using different SAM concentrations. The error bars represent the standard deviations of three repeated experiments.

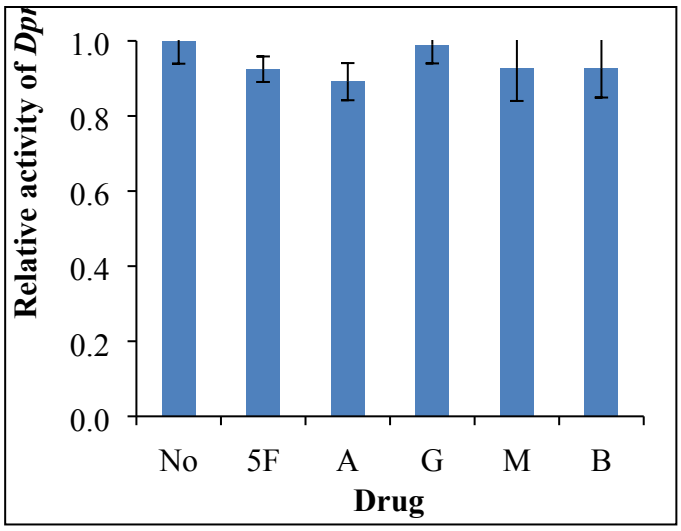

Figure S4. Effect of different drugs on *Dpn*II activity. In these experiments, we defined the relative activity of 40 units of *Dpn*II without the addition of drugs as 1. The experiments were performed in a manner similar to that in Figure S1, with the addition of certain concentration of drug into each sample. The different drugs, 5F (5-fluorouracil), A (ampicillin), G (gentamicin), M (mitomycin), and B (benzylpenicillin) were used at the same concentration (10 μM). The error bars represent the standard deviations of three repeated experiments.