Electronic Supplementary Material (ESI) for Analyst.

Highly Sensitive Fluorescence Assay for Methyltransferase Activity by Exonuclease-aided Signal Amplification

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Table S1. Comparison of the different methods for assay of DAM activity.

Methods	Analytical Time (min)	Detection Limit (U/mL)	Biological Samples analyzed	References	Years
Fluorescent DNA hairpin probe	30	0.8	NO	1	2007
Gold nanoparticle-based colorimetric assay	120	-	NO	2	2009
Colorimetric assay by methylation-responsive DNAzyme-based signal amplification	155	0.25	NO	3	2010
Electrochemical assay by gold nanoparticle amplification	240	0.12	NO	4	2011
Fluorescent assay by hairpin-shaped DNAzyme signal amplification	180	0.8	NO	5	2012
Bioluminescence assay by methylation-resistant cleavage	340	0.08	NO	6	2012
Rolling circle amplification-induced chemiluminescence	120	0.000129	NO	7	2013
Colorimetric assay by methylation-blocked cascade amplification	77	0.4	NO	8	2014
Fluorescence approach by rolling circle amplification	225	0.18	NO	9	2014
Fluorescent assay by methylation-sensitive cleavage coupled with exonuclease-aided signal amplification	30	0.0025	*E. coli cells*	This work	2015

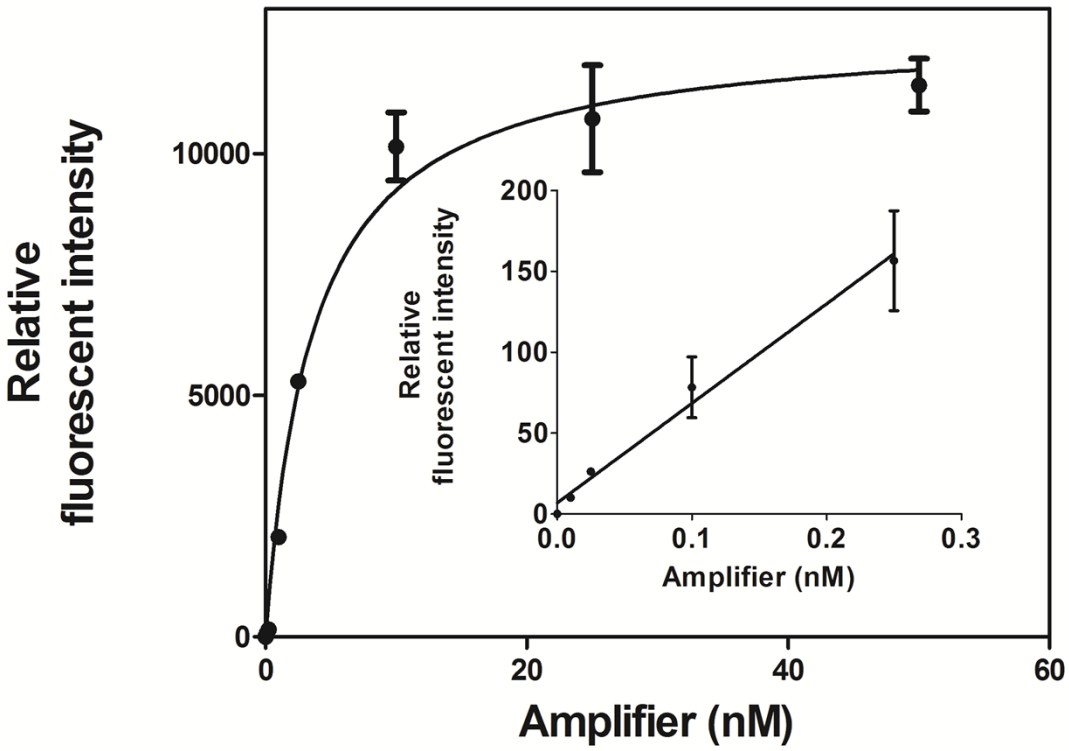

Figure S1. Relative fluorescent intensity with different amounts of Amplifier.

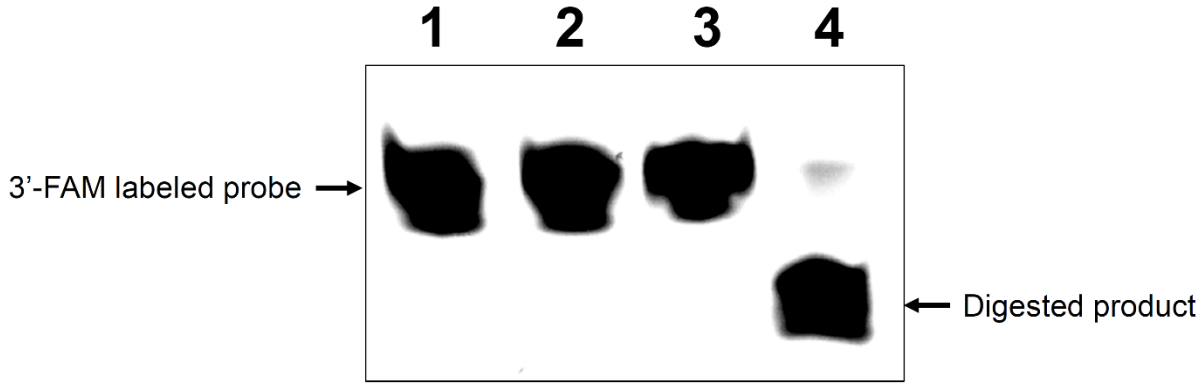

Figure S2. Gel electrophoresis analysis of the digestion of substrate upon DAM methylation and Dpn I digestion. 3'-FAM labeled probe (5'-TATACGCACCGTCCTACGATCCGTTTTTCGGATCGTAGGACGGTGCGTATAAATG-FAM-3') was used for the evaluation. The reaction mixture (20 μL) consisted of 50 nM 3'-FAM labeled probe, 160 μM SAM, and 1 U of DAM. The reaction buffer contained 20 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 50 mM KCl, and 1 mM DTT. The experiment was performed at 37 °C for 20 min. The products were separated by 10% denaturing polyacrylamide gel electrophoresis (PAGE) in the presence of 7 M urea and quantitated on a Molecular Imager PharosFX system (Bio-Rad Hercules, CA). The direction of running gel is from top to bottom. Lane 1, 3'-FAM probe alone; lane 2, 3'-FAM labeled probe + Dpn I; lane 3, 3'-FAM labeled probe + DAM; lane 4, 3'-FAM labeled probe + DAM + Dpn I.

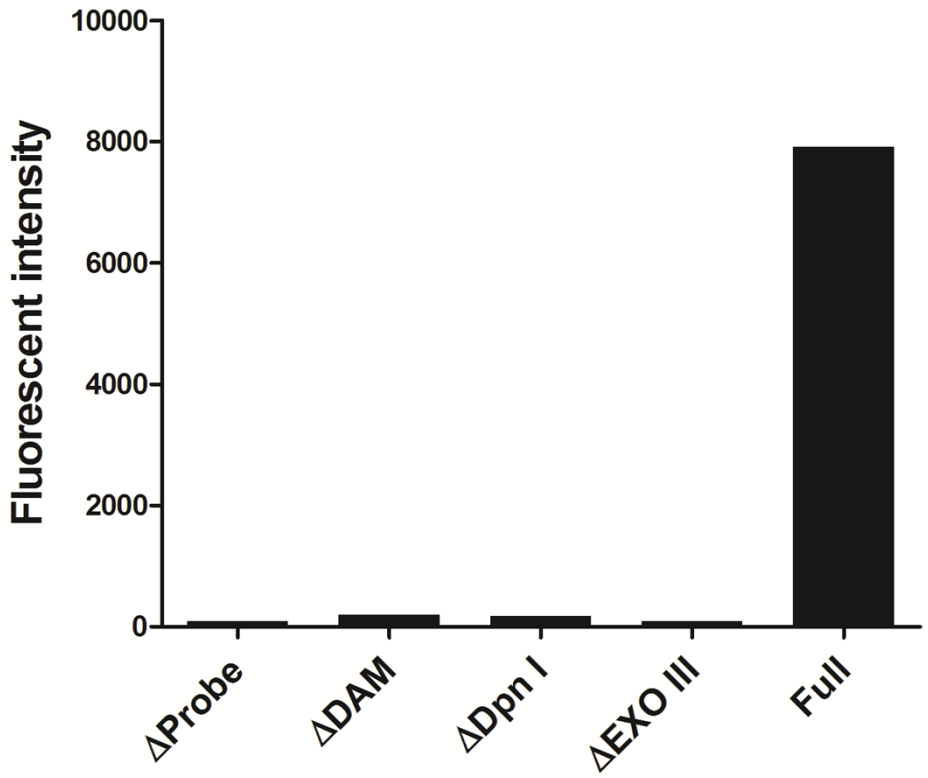

Figure S3. Relative fluorescence intensity in response to different controls. Column 1, the reaction mixture with omitting hairpin probe; column 2, the reaction mixture with omitting DAM; column 3, the reaction mixture with omitting Dpn I; column 4, the reaction mixture with omitting EOX III; column 5, the reaction mixture containing all the above components.

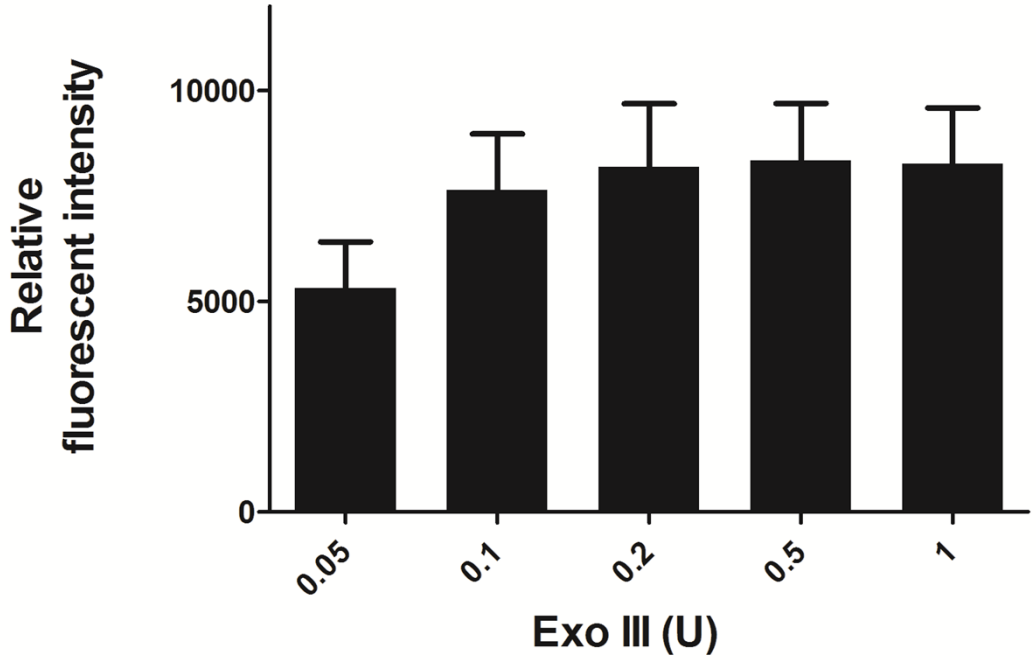

Figure S4. Relative fluorescence intensity in response to different concentrations of Exo III.

References

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