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Supporting Information

Construction of DNA template sequences to generate fluorescent gold nanoclusters for the sensitive detection of DNA methyltransferase activity bioassay

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Table S1: The oligonucleotides used in this work

DNA probe	Sequences (5'→3')
DNA probe for M.SssI MTase	PO_4-***G***TAGCTTAGTACATTGCTA***CG***TATCTTAGTACATTGATA***C***
DNA probe for Dnmt1	PO_4-***G***TAGCTTAGTACATTGCTA***C***/**m**/***G***TATCTTAGTACATTGATA***C***
T4 PNK	PO_4-TTAGTACATTGCTG***A -PO₄***
EcoR I	PO_4-***TTC***CGGCTGGATGATGTGCCGG*AATTC*CGGCTGGATGATGTGCCGG***AA***

The underline in the dumbbell ring DNA probe sequence marks the recognition and action site of M.SssI MTase in the sequence, the italic marks the recognition site of GlaI. The italic marks the recognition site of T4 PNK in the T4 PNK probe sequence. The italic marks the recognition site of EcoR I in the EcoR I probe sequence.

Optimization of the conditions of the experiment

The efficiency of the assay system is influenced by a number of factors. A series of optimisations have been made to the parameters of the proposed biosensing strategy in order to achieve the best possible results. Firstly, the duration of action of the methyltransferase and endonuclease enzymes has been optimised. Secondly, the amount of endonuclease used was optimised. The amount of endonuclease used directly affects the amount of hairpin DNA released and therefore the fluorescence signal at the output. The optimal methylation and endonuclease action time was 2h

and the optimal amount of endonuclease was 2.5 U, as can be seen from the optimised fluorescence signal plots (Fig. S1. A, B).

It was then considered that the end-addition of tails has a direct effect on the subsequent signal output. Therefore, the time of action of the TdT used in this system was optimised, as the time of action of the TdT affects the length of the DNA stencil strand and plays an important role in the formation of the subsequent gold nanoclusters. The results (Fig. S1. C) show that the optimal TdT action time for the proposed bioassay is 1.5 h. Finally, the amount of TdT used was optimised. The TdT is an efficient enzyme that mediates a rapid tailing reaction in a short time. It is therefore hoped that the optimum amount of TdT can be found through optimisation experiments. The optimal amount of TdT for the proposed bioassay is 20 U as can be seen in Fig. S1. D.

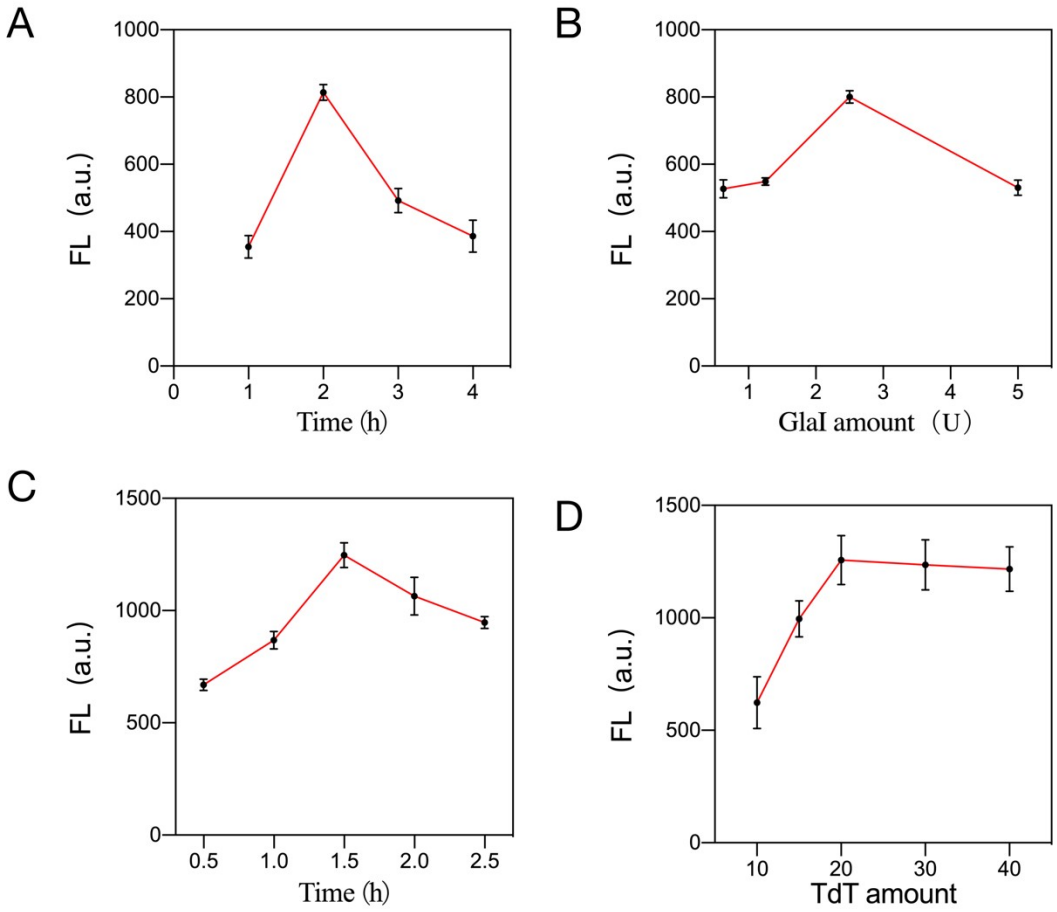

Fig. S1. Optimisation of assay system characteristics. (A) Optimization of methylation/enzyme action time. (B) Optimization of the amount of endonuclease GlaI. (C) Optimization of end-transferase tailing time. (D) Optimization of the amount of end-transferase

Comparison of methods for the detection of methyltransferase activity

Table S2 Comparison of methods for the detection of methyltransferase activity

Method	range(U/mL)	LOD(U/mL)	Ref
ECL	0.5 – 125	0.2	(1)
ECL	5 – 80	0.45	(2)
ECL	0-40	0.083	(3)
ECL	5-25	0.04	(4)
ECL	1-80	0.316	(5)
PEC	1–100	0.3	(6)
Fluorescence	1–20	0.18	(7)
ECL	1-40	0.3	(8)
Colorimetry	0.2-60	0.067	(9)
Colorimetry	0.08-50	0.069	(10)
Colorimetry	0.5-10	0.05	(11)
Fluorescence	0.1-100	0.077	This work

Recovery experimental results in human serum samples

Table S3 Recovery experimental results in human serum samples

Add (U/mL)	Found (U/mL)	Recovery (%)	RSD (%)
5	4.98	99.53	2.09
10	10.52	105.20	4.38
20	20.68	103.40	1.14
50	48.99	97.98	3.68

Pre-experiment: detection of M.SssI MTase activity in cell lysate

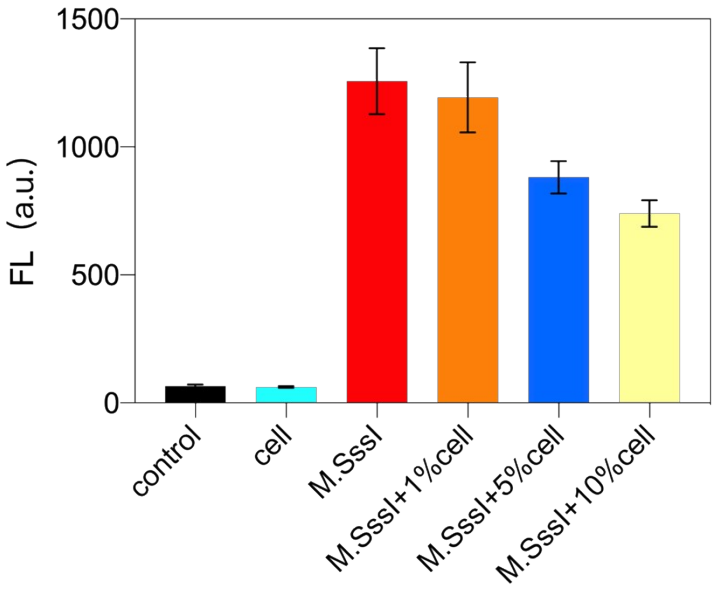

Fig. S2. Pre-experiment: detection of M.SssI MTase activity in cell lysate

Recovery experimental results in cell lysate

Table S4 Recovery experimental results in cell lysate

Add (U/mL)	Found (U/mL)	Recovery (%)	RSD (%)
5	5.73	114.6	8.18
10	10.72	107.23	17.62
20	19.90	99.48	11.12

Detection of methyltransferase activity in different human-derived cells.

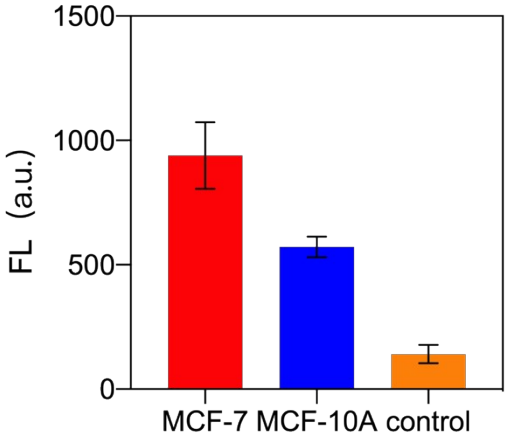

Fig. S3. Detection of methyltransferase activity in different human-derived cells

Inhibitor screening pre-experiment

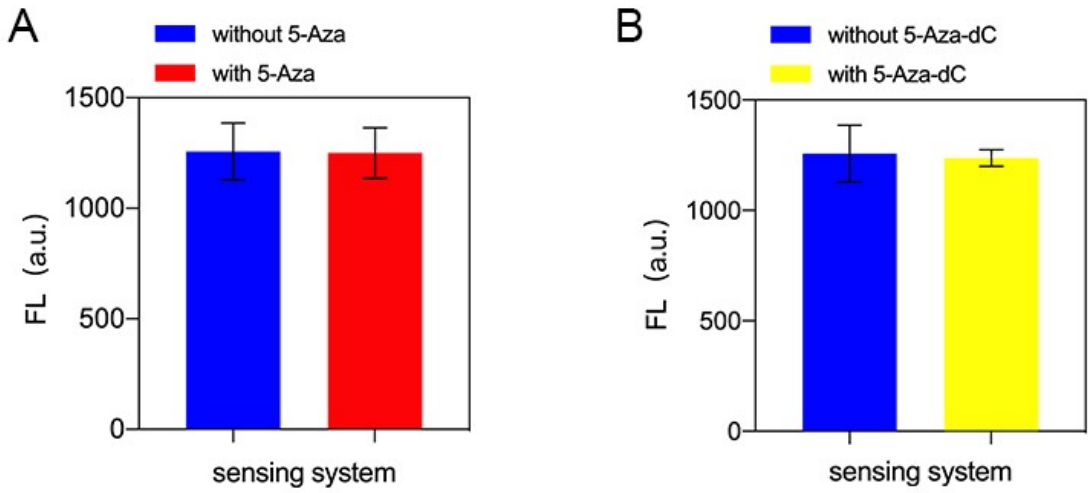

Fig. S4. (A) Inhibitor screening pre-experiment: inhibitor (5-Aza) added after methylation and endocytosis. (B) Inhibitor screening pre-experiment: inhibitor (5-Aza-dC) was added after methylation and endocytosis

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