Supporting Information (SI)

A universal two-step strategy for multiple DNA MTase activity: Enhancing sensitivity through CRISPR/Cas12a-assisted hyperbranched rolling circle amplification (CA-HRCA)

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Experimental reagents

HiScribe T7 RNA synthesis kit, Dam methyltransferase, CpG methyltransferase (M.SssI), DpnI restriction endonuclease, HhaI restriction endonuclease, Bst DNA Polymerase (Large Fragment) and T4 DNA ligase were available from New England Biolabs (Beijing, China). LbCas12a was obtained from Guangzhou Meige Biological Technology Co., Ltd. 5-fluorouracil, Acryl/Bis 30% Solution (29:1), ammonium persulfate, BSA, TBE buffer, TE buffer, DEPC-treated H_2O, ddH_2O, DNA marker, loading buffer, 4S Red Plus Nucleic Acid Stain and dNTP (25 Mm) were purchased from Sangon Biotech (Shangehai) Co., Ltd. Normal human serum was obtained from Solarbio (Beijing, China). N, N, N ′, N ′-Tetramethylethylenediamine (TEMED) was purchased from Shanghai Macklin Biochemical Co., Ltd.

Gel electrophoresis analysis

The feasibility of this strategy and the preparation of crRNA were verified by 10% polyacrylamide gel electrophoresis. The electrophoresis was performed for 100 min at

110 V in 1×TBE buffer and then stained in 1×TS-GelRed. Finally, the gel is visualized by a gel Imaging system. A distinct band corresponding to the prepared crRNA was observed in lane 1 (Fig. S1).

Preparation of crRNA

First, the mixture of crRNA template (10 μM) and T7 promoter (10 μM) was denatured at 95 ° C for 5 min, and then slowly cooled to room temperature at 0.1 ° C /s. The mixture was added with 2 μL T7 RNA polymerase and 10 μL NTP mix (20 mM) and incubated at 37 ℃ for 17 h. Then 2 μL DNase I and 3 μL DNase I buffer were added to the transcripts and incubated at 37 ℃ for 1 h to digest the crRNA template. Finally, the transcribed crRNA was purified with miRNA purification kit and quantified with Nano Drop 2000C (Thermo Fisher).

Table S 1 The nucleic acid sequences used in this work

名称	序列 (5'-3')
Dumbbell probe	CAG TTC CTC GAT CTT GAT AGG GAG ACA AGA TCG AGG AAC TGG TGT GTG *CGC G*AA GCA AGA ACA GAG ACT T*CG CG*C ACA CAC -NH_2
Padlock probe	P – CAC ACA CAC ATA CGC GAA GTC TCT GTT CTT GCT TCG CTT TGA ATA AGA GAT ATC AAC ATT ATC TTG TCT CCC TAT CAA GAC ACA CAC CC
Ligation probe	TGT GTG TGT GGG GTG TGT G-NH_2
Primer	TTT GAA TAA GAG ATA TCA ACA TTA
crRNA template	TAA TGT TGA TAT CTC TTA TTA TCT ACA CTT AGT AGA AAT TAC CCT ATA GTG AGT CGT ATT AAT TTC
crRNA	UAA UUU CUA CUA AGU GUA GAU **AAU AAG AGA UAU CAA CAU UA**
T7 promoter	GAA ATT AAT ACG ACT CAC TAT AGG G
F-Q reporter	HEX-TATTATT-BHQ1

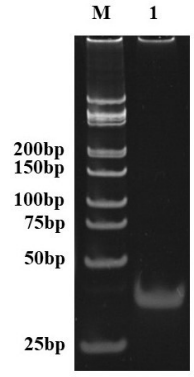

Fig. S1 crRNA electrophoresis

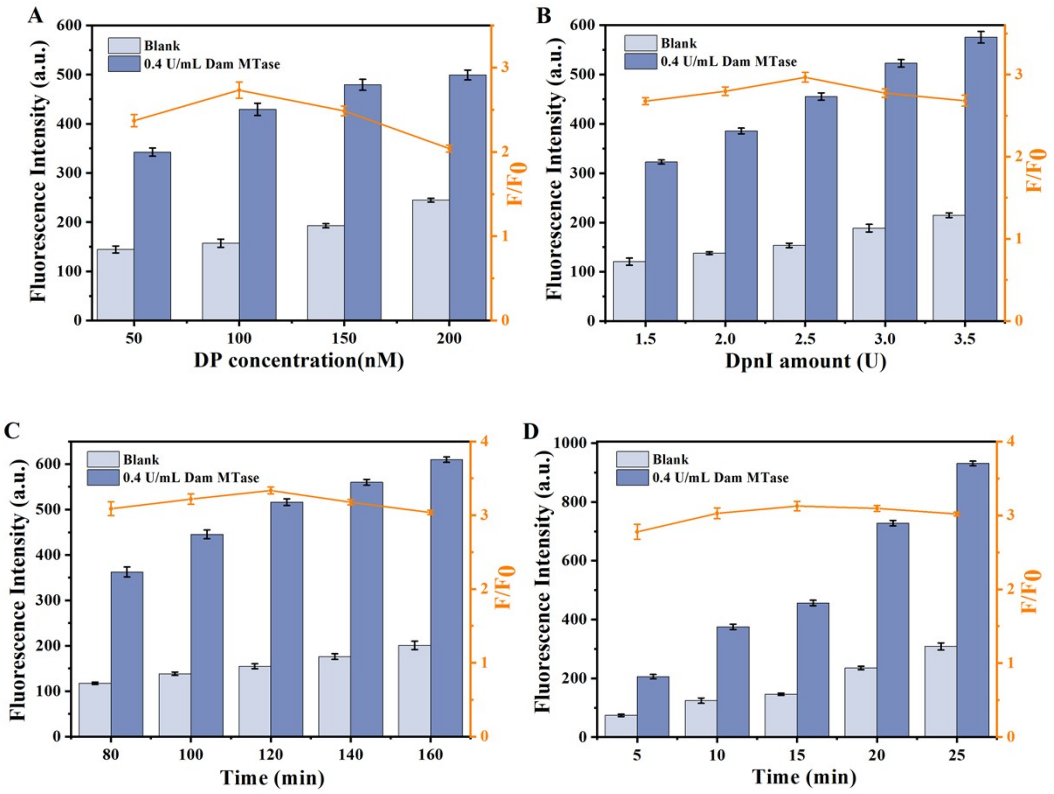

Fig. S2 Effects of DP concentration (A), DpnI amount (B), amplification time (C) and CRISPR/Cas12a incubation time (D) on Dam MTase detection in CA-HRCA system (N = 3).

Table S2 Comparison with other methods for DNA MTase activity detection

Method	Linear range (U/mL)	LOD (U/mL)	Time （h）	Ref.
Functionalized Metal Organic Framework (Electrochemical Biosensor)	0.002 - 1	0.001	7.1	[28]
silver core-gold satellite nanocomposites-modified silicon wafer (SERS Biosensor)	0.05 - 50	2.8×10^{-3}	16.4	[29]
Au Nanocube (SERS Biosensor)	1×10^{-4} - 0.5	8.65×10^{-5}	6.3	[30]
DNA nanodevice-functionalized hemin/G-quadruplex DNAzyme (colorimetric Biosensor)	0.1 - 20	0.3	3	[31]
Hyperbranched Rolling Circle Amplification (Fluorescence Biosensor)	2.5 - 70	1.8	3.6	[32]
Cascade Signal Amplification (Fluorescence Biosensor)	0.1 - 10	0.002	2.5	[33]

Autonomous Concatenated DNA Circuit (Fluorescence Biosensor)	0 - 0.004	1.2×10^{-4}	4.8	[34]
Cascade Assembly Amplification (Fluorescence Biosensor)	0.005 - 500	1.0×10^{-3}	5.2	[35]
SDA–RCA reaction (Fluorescence Biosensor)	0.02 - 4	0.0067	8.3	[36]
Dendritic rolling circle amplification and Mg^{2+}-dependent DNAzyme (Fluorescence Biosensor)	0.1 - 10	0.36	5.15	[37]
CRISPR/Cas12a-assisted hyperbranched rolling circle amplification (CA-HRCA)	0.001 - 0.1, 0.1 - 4	7.6×10^{-4}	2.6	This work

The procedure for the determination of LOD :

"$S/N = (average_{sample} - average_{blank})/SD_{blank}$ [1]

The signal-to-noise ratio (S/N) is 3. SDblank presents the associated standard deviation of blank samples. LOD = (averagesample − averageblank), which is calculated by the linear fitting equation."

[1] L. Guo, Y. Xu, A. R. Ferhan, G. Chen, D.-H. Kim (2013) Oriented Gold Nanoparticle Aggregation for Colorimetric Sensors with Surprisingly High Analytical Figures of Merit, J. Am. Chem. Soc. 2013, 135, 33, 12338–12345