Electronic Supplementary Information

Development of cascade isothermal amplification approach for sensitive detection of DNA methyltransferase

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Optimization of experimental conditions

To achieve the best performance of the proposed method, we optimized the experimental conditions including the buffer of Dam-catalyzed cleavage reaction, the reaction time of cyclic excision repair-activated cascade isothermal amplification, the concentrations of hairpin template and EXPAR template, the concentration of dNTPs **(**dATP, dGTP, dCTP, dUTP**)**, the amounts of *Bst* DNA polymerase, UDG and Endo IV, and the concentration of signal probe (Fig. S1). To achieve high efficiency of cleavage reaction, we performed the experiment of Dam-catalyzed cleavage of hairpin substrates in CutSmart buffer, Dam buffer and the mixture of above two buffers, respectively. As shown in Fig. S1A, the value of F/F_0 in response to the mixture buffer was much higher than those in response to CutSmart buffer and Dam buffer (F and F_0 are the fluorescence intensity in the presence and in the absence of Dam MTase, respectively). Thus, the mixture buffer of CutSmart and Dam buffers was used in the subsequent experiments.

After Dam MTase-catalyzed cleavage reaction, 4 μL of cleavage products were added into the amplification reaction solution (20 μL) at 37 °C for different reaction time. As shown in Fig. S1B, in the presence of Dam MTase, the fluorescence intensity enhanced with the reaction time. While in the control group without Dam MTase, the fluorescence intensity remained unchanged from 0 to 110 min. However, when the reaction time was more than 110 min, the fluorescence intensity increased in the control group due to the non-specific amplification.[1] Thus, 110 min was selected as the amplification reaction time.

In the DNA lesion repair-directed cascade isothermal amplification, the cleavage products may hybridize with the hairpin templates and function as the primers to initiate the strand displacement amplification (SDA). Thus, the concentration of hairpin templates should be optimized carefully.

As shown in Fig. S1C, the value of F/F_0 enhanced with the increasing concentration of hairpin template from 20 to 50 nM and reached the highest value at 50 nM. Thus, 50 nM hairpin templates were used in the subsequent research.

The concentration of EXPAR template has a crucial effect on the efficiency of the whole amplification.[2] As shown in Fig. S1D, the F/F_0 value increased gradually in the range from 25 to 100 nM, and reached the highest value at 100 nM (F and F_0 are the fluorescence intensity in the presence and absence of 4 U/mL Dam MTase, respectively). Thus, 100 nM EXPAR template was used in the subsequent experiments. In addition, when the concentration of dNTPs (dATP, dGTP, dCTP, dUTP) increased from 25 to 100 μM, the F/F_0 value gradually increased and reached the highest value at 100 μM (Fig. S1E). Thus, we used 100 μM dNTPs in the subsequent experiments.

The cooperation of *Bst* DNA polymerase, UDG and Endo IV is crucial for efficient amplification.[3] We firstly investigated the effect of the amount of *Bst* DNA polymerase on the F/F_0 value at a fixed amounts of UDG (0.7 U) and Endo IV (5 U). As shown in Fig. S1F, the F/F_0 value improved with the increasing amount of *Bst* DNA polymerase from 2.0 to 2.8 U and then reached a plateau beyond the amount of 2.8 U. Thus, 2.8 U of *Bst* DNA polymerase was used in the subsequent experiments. We further investigated the effect of UDG amount on the F/F_0 value with the amount of *Bst* DNA polymerase and Endo IV being fixed at 2.8 and 5 U, respectively. As shown in Fig. S1G, the F/F_0 value enhanced with the increasing amount of UDG from 0.4 to 1 U, and reached the highest value at 1 U. Thus, 1 U of UDG was used in the subsequent experiments. We investigated the effect of Endo IV amount upon on the F/F_0 value with the fixed amounts of *Bst* DNA polymerase (2.8 U) and UDG (1 U). As shown in Fig. S1H, the F/F_0 value improved with the increasing amount of Endo IV from 3 to 5 U, and reached the highest value at 5 U,

followed by the decrease beyond the amount of 5 U due to the high background signal resulting from large amount of Endo IV. Thus, 5 U of Endo IV was used in the subsequent experiments.

The concentration of signal probe has a crucial effect upon the detection sensitivity. As shown in Fig. S1I, the F/F_0 value increased gradually in the range from 200 to 350 nM, and reached the highest value at 350 nM. Thus, 350 nM signal probe was used in the subsequent experiments.

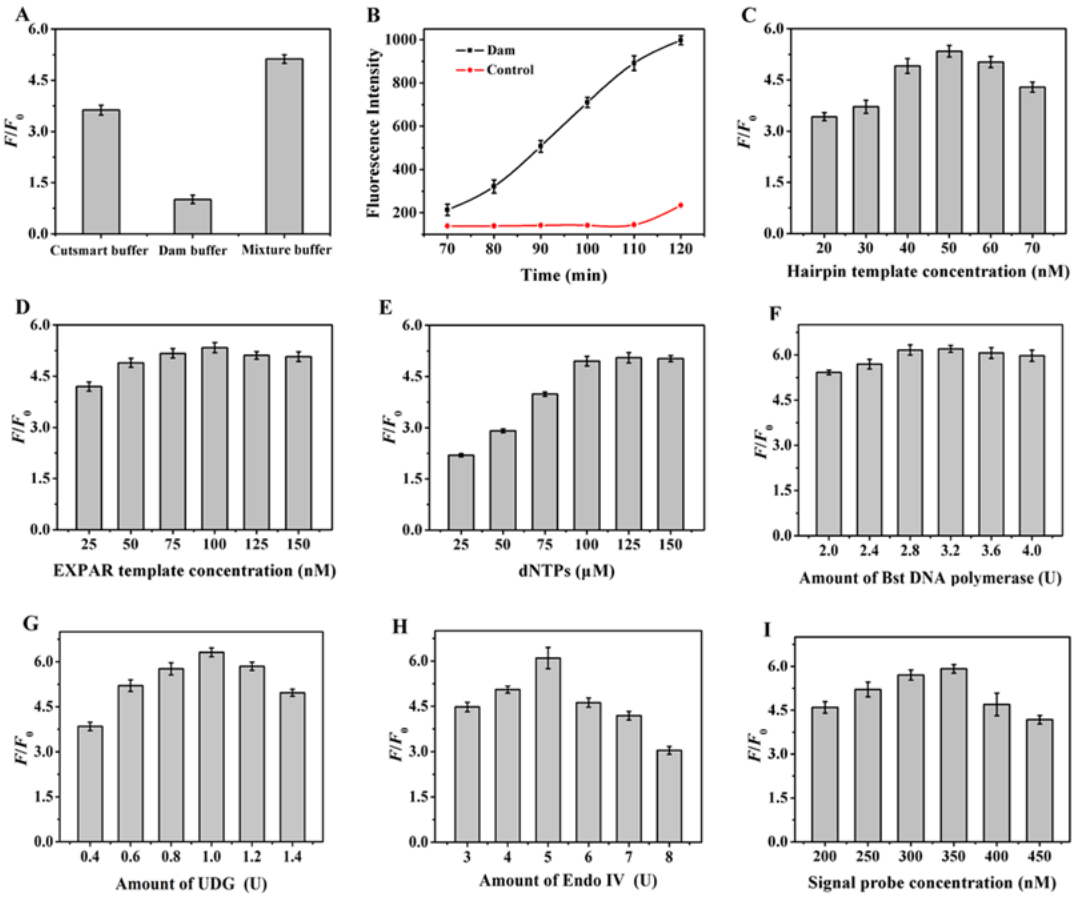

Fig. S1 (A) Variance of F/F_0 value with the cleavage reaction buffer. (B) Variance of fluorescence intensity with the reaction time. (C) Variance of F/F_0 value with different-concentration hairpin template. (D) Variance of F/F_0 value with different-concentration EXPAR template. (E) Variance of F/F_0 value with different-concentration dNTPs. (F) Variance of F/F_0 value with different amount of *Bst* DNA polymerase. (G) Variance of F/F_0 value with different amount of UDG. (H)

Variance of F/F_0 value with different amount of Endo IV. (I) Variance of F/F_0 value with different-concentration signal probe. F and F_0 are the fluorescence intensity in the presence and absence of 4 U/mL Dam MTase, respectively. Error bars show the standard deviation of three experiments.

References

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