Electronic Supplementary Information

Single-ribonucleotide repairing-mediated ligation-dependent cycling signal amplification for sensitive and specific detection of DNA methyltransferase

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1. Optimization of *Taq* DNA ligase amount

Taq DNA ligase is responsible for the ligation reaction and plays a critical role in single-ribonucleotide repairing-mediated ligation-dependent cycling signal amplification. Thus, the amount of *Taq* DNA ligase should be optimized. As shown in Fig. S1, the fluorescence intensity enhances with the increasing amount of *Taq* DNA ligase and reaches the highest value at the amount of 15 U, followed by the decrease beyond the amount of 15 U. Therefore, the optimal amount of *Taq* DNA ligase is determined to be 15 U.

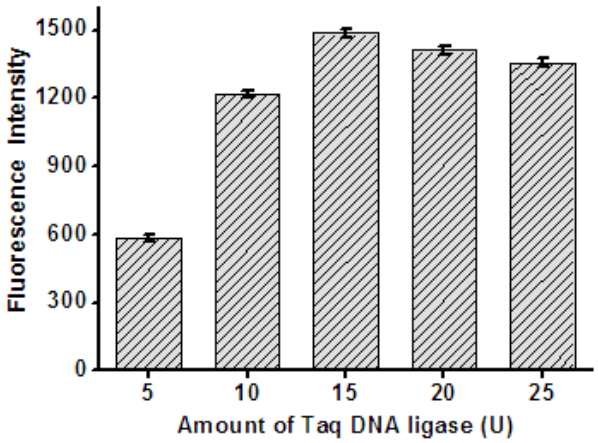

Fig. S1 Variance of fluorescence intensity with different amount of *Taq* DNA ligase. Error bars show the standard deviation of three independent experiments.

2. Optimization of RNase HII amount

RNase HII-catalyzed single-ribonucleotide repair is the key step in the whole amplification reaction and directly determines whether single-ribonucleotide repairing-mediated ligation-dependent cycling signal amplification can be successfully carried out or not. Thus, the amount of RNase HII should be optimized. As shown in Fig. S2, the fluorescence intensity enhances with the increasing amount of RNase HII from 1 to 2 U, followed by the decrease beyond the amount of 2 U. Therefore, 2 U of RNase HII is used in the subsequent research.

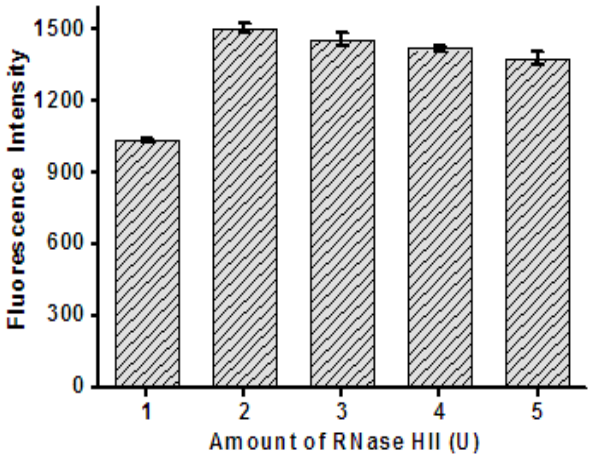

Fig. S2 Variance of fluorescence intensity with different amount of RNase HII. Error bars show the standard deviation of three independent experiments.

3. Optimization of the reaction time of single-ribonucleotide repairing-mediated ligation-dependent cycling signal amplification

The reaction time has a significant impact on the performance of single-ribonucleotide repairing-mediated ligation-dependent cycling signal amplification. We monitored the variance of fluorescence intensity with reaction time. As shown in Fig. S3, the fluorescence intensity enhances with reaction time from 10 to 60 min and reaches the maximum value at 60 min, followed by the decrease after 60 min. Therefore, 60 min is determined to be the optimal reaction time.

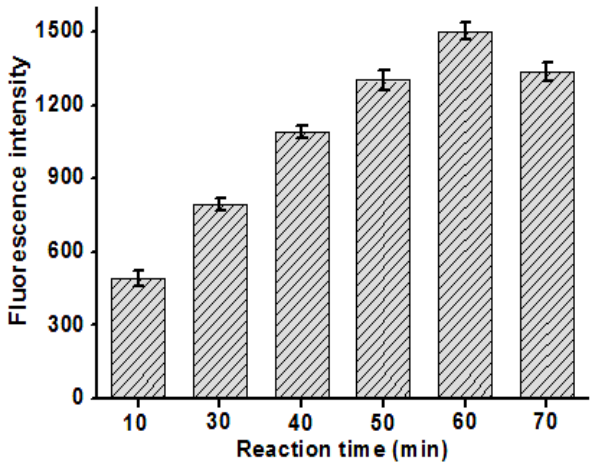

Fig. S3 Variance of fluorescence intensity with reaction time. Error bars show the standard deviations of three independent experiments.

4. Optimization of the signal probe concentration

The signal probe can hybridize with the reaction products of enzymatic repairing-mediated ligation-dependent multiple SDA and subsequently be cleaved by RNase HII to generate a distinct fluorescence signal. Thus, the concentration of signal probe should be optimized. As shown in Fig. S4, the fluorescence intensity enhances with the increasing concentration of signal probe from 500 to 900 nM and reaches the plateau above the concentration of 900 nM. Therefore, 900 nM is selected as the optimal concentration of signal probe.

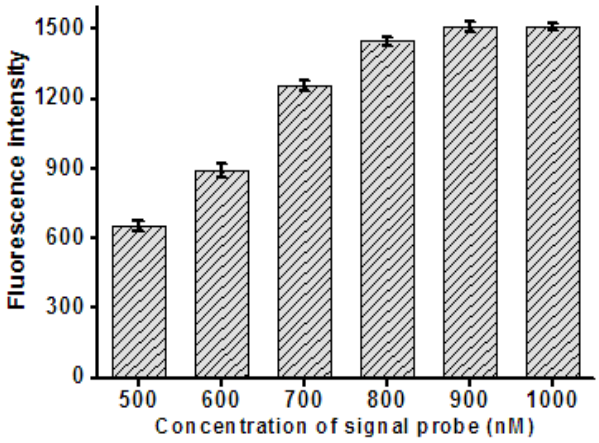

Fig. S4 Variance of fluorescence intensity with different-concentration signal probe. Error bars show the standard deviations of three independent experiments.

5. Detection of Dam MTase activity in the spiked cancer cell extracts

To further investigate the feasibility of the proposed method for complex real sample analysis, we measured the recovery of Dam MTase by spiking different-concentration Dam MTase (1-40 U/mL) to the A549 cancer cell extracts. As shown in Table S1, the recovery ratio is calculated to 96.3% - 110.7% with a relative standard deviation (RSD) of 0.19% - 1.68%, consistent with the values (recovery ratio of 97.5% - 112.8% and RSD of 0.16% - 2.16%) obtained by using the spiked human serum samples (Table 1). These results demonstrate that the proposed method can be applied for the accurate quantification of Dam MTase activity in complex real samples.

Table S1. Recovery studies in the spiked A549 cancer cell extract

sample	added (U/mL)	measured (U/mL)	recovery (%)	RSD (%)
1	1	1.31	102.6	0.19
2	5	4.83	99.4	0.37
3	10	11.42	110.7	0.49
4	20	18.64	96.3	1.68
5	40	42.33	107.4	0.87