Supporting Information

Highly Sensitive Detection of Uracil-DNA 
Glycosylase Activity Based on Self-Initiating 
Multiple Rolling Circle Amplification

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1. Gel Electrophoresis Analysis of the Preparation of the cPLP.

In this method, cyclized padlock probe (cPLP) is pre-prepared. To verify the successful circularization of the cPLP, polyacrylamide urea gel electrophoresis was performed. As shown in Figure S1, there is obvious difference in the migration rates before and after the preparation of the cPLP, resulting from the conformational change of the DNA. Moreover, the single band after the preparation of the cPLP, verifying that the linear DNA could be fully digested following the incubation with exonucleases I and III. The results of the gel electrophoresis confirmed the successful circularization of the cPLP.

Figure S1. Agarose gel electrophoresis results of before and after the preparation of the cPLP. Lane 1: before the preparation of the cPLP; Lane 2: after the preparation of the cPLP; Lane 3: 20 bp DNA marker.

2. Optimization of the Experimental Conditions.

In the detection of UDG by the self-initiating multiple rolling circle amplification (SM-RCA) reaction, various factors, including concentration of template probe (TP) and cPLP, amount of phi29 DNA polymerase and endonuclease IV (Endo IV), reaction time of RCA, and the ratio of dTTP to dUTP, affected the performance of the assay and were studied.

The concentration of TP has the important effect on the SM-RCA. As shown in Figure S2a, the fluorescence ratio obviously decreased with the increasing of the
Figure S2. Effect of the concentration of TP (a) and cPLP (b), the amount of Endo IV (c) and phi29 DNA polymerase (d), the dTTP/dUTP (e), and the RCA reaction time (f) on the detection of UDG. Sample/Blank was the fluorescence intensity ratio of SM-RCA product with and without UDG. The concentration of UDG is $5 \times 10^{-4}$ U/mL. Sequential detection was carried out as described in the experimental section.

The concentration of TP from 0.01 to 1 nM, and almost kept stable in the ranges 1–10 nM. This may be attributed to the fact that as TP increased, the fluorescence intensity of blank increased, resulting in a low ratio of sample to blank. Therefore, 0.01 nM TP was selected for the subsequent study.
Figure S2b showed that the concentration of cPLP affected the performance of SM-RCA. With the increasing the concentration of cPLP from 1 to 10 nM, the fluorescence ratio was significantly improved, suggesting that the efficiency of SM-RCA was enhanced with the increasing of cPLP. In the concentration ranges 10–100 nM, the fluorescence ratio gradually reduced. This may be attributed to the fact that as cPLPs increased, the fluorescence intensity of blank increased quickly, resulting in a low ratio of sample to blank. Thus, we chose the optimized concentration of cPLP as 10 nM.

The amount of Endo IV plays an important role in SM-RCA. Due to its unique digestion mechanism of apurinic/apyrimidinic (AP) endonuclease, Endo IV can induce multiple primers by cleaving the AP sites in DNA. As shown in Figure S2c, as the amount of Endo IV increased, the fluorescence ratio of sample to blank gradually increased and reached a maximum at 5 U. As the amount of Endo IV increased from 5 to 15 U, the fluorescence ratio decreased gradually. Therefore, the optimized amount of Endo IV was chosen as 5 U in the SM-RCA reaction.

Figure S2d showed the effect of phi29 DNA polymerase on the SM-RCA system. The fluorescence ratio increased accordingly with the increasing of phi29 DNA polymerase from 1 to 5 U, and decreased in the ranges 5–15 U. Thus, we selected 5 U phi29 DNA polymerase for the experiment study.

In SM-RCA reaction, the amount of dUTP is directly related to the amount of U bases embedded in the DNA products, which affects the efficiency of SM-RCA. Therefore, we optimized the ratio of dTTP and dUTP in SM-RCA reaction. As shown in Figure S2e, the fluorescence intensity ratio (Sample/Blank) increased step by step from 9:1 to 1:9, suggesting that more and more U bases bound to the DNA product from RCA reaction to further initiate the SM-RCA with the increase of dUTP. Moreover, the Sample/Blank increased less from 1:9 to 1:19. This may be attributed to the fact that the dTTP/dUTP ratio of 1:9 was sufficient for the U base to be embedded in the DNA product to induce the SM-RCA reaction. Therefore, dTTP/dUTP ratio of 1:9 was selected for the subsequent study.
We also optimized the reaction time of SM-RCA to obtain the better performance of the SM-RCA. As shown in Figure S2f, increasing of the reaction time gave rise to a significantly enhancement of Sample/Blank from 45 to 60 min, and decreased obviously from 60 to 90 min. These results showed that the SM-RCA was gradually accomplished with increasing the RCA time to 60 min. After 60 min, the fluorescence intensity of blank increased quickly, resulting in a low ratio of sample to blank. Therefore, 60 min of RCA time was chosen for the subsequent study.

3. Real Sample Detection

| Sample | added/10^{-5} U | found/10^{-5} U | Recovery/% | RSD/% |
|--------|-----------------|-----------------|------------|-------|
| 1 cell | 1.00            | 1.05 ± 0.05     | 105.0 ± 5.0 | 4.6   |
|        | 2.00            | 1.88 ± 0.05     | 94.0 ± 2.5  | 2.8   |
|        | 5.00            | 4.86 ± 0.08     | 97.2 ± 1.6  | 1.6   |
| 2 cells| 1.00            | 0.86 ± 0.04     | 86.0 ± 4.0  | 5.1   |
|        | 2.00            | 2.04 ± 0.08     | 102.0 ± 4.0 | 3.8   |