Electronic Supporting Information

Multi-site enzymatic repairing amplification (MSERA) enables ultrasensitive detection of terminal deoxynucleotidyl transferase activity

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Experimental section

Materials and reagents. All synthesized nucleotides were HPLC -purified and obtained from Sangon Biotech. Co. Ltd. (Shanghai, China). The sequences of the nucleotides are listed in Table S1. Terminal Deoxynucleotidyl Transferase (TdT), Bst DNA Polymerase, Large Fragment (Bst), Klenow Fragment (3'-5' exo$^-$) (Klenow exo$^-$), Vent DNA Polymerase (3'-5' exo$^-$) (Vent exo$^-$), Sulfolobus DNA polymerase IV, Uracil-DNA Glycosylase (UDG), Endonuclease IV (endo IV), 10×Terminal transferase reaction buffer (50 mM Potassium Acetate, 20 mM Tris-acetate, 10 mM Magnesium Acetate, pH 7.9), cobaltous chloride solution and 10×NEBuffer 2 (50 mM NaCl 10 mM Tris-HCl, 10 mM MgCl2, 1 mM DTT, pH 7.9) were purchased from New England Biolabs (USA) Ltd. (Beijing, China). The dTTP (100 mM), dATP (100 mM), and DNase/RNase-free deionized water were bought from TIANGEN Biotechnology Co., Ltd. (Beijing, China). The dUTP (100 mM) was offered by Takara Bio Inc. The EvaGreen Dye (20×) was bought from Biotium Inc. Sodium pyrophosphate (PP, 99.0%) was bought from Aladdin Co., Ltd. (Shanghai, China). DNase/RNase-free deionized water was used throughout the experimental process.

Multi-site enzymatic repairing amplification reaction. The volume of 20 μL reaction mixture including 2 μL primer P01 (6 μM), 2 μL dTTP (10 mM) , 2 μL dUTP (100 μM) , 8 μL H_2O, 4 μL 10 × TdT buffer, 4 μL $CoCl_2$ (2.5 mM), and an appropriate amount of TdT enzyme was first incubated at 37 ℃ for 90 min. TdT was then inactivated by heating the mixture at 85 ℃ for 20 min, obtaining the solution I. Solution I was than mixed with a 20 μL mixture solution II, containing 2 μL P02 (2 μM), 2 μL dATP (10 mM), 0.5 μL Bst polymerase (8 U/μL), 2 μL UDG (5 U/μL), 1 μL endo IV (10 U/μL),

4 μL 10 × NEBuffer 2 , 4 μL EvaGreen dye and 4.5 μL DNase/RNase-free deionized water. The final MSERA reaction was performed at 37 ℃ in a LightCycler 480 Instrument II (Roche, Germany) with a Roche in situ detection system. The real-time fluorescence intensity was monitored in 60 s intervals using the FAM/SYBR Green channel.

Gel electrophoresis analysis. The products obtained from different steps of MSERA reaction were collected and analyzed through agarose gel electrophoresis. The gel electrophoresis was carried out using 1% agarose gel and 1×TAE buffer at room temperature. Electrophoresis was performed at a constant voltage of 127 V for 40 min. The loading samples (10 μL) were prepared by mixing reaction solution with a 6×loading buffer in each lane. The gel was stained by TORORed® Nucleic Acid Staining Dye (10000×)(Toroivd Co., Ltd., China), and was visualized using Azure C600 (Cycloud, USA).

Real sample application and reproducibility. For the detection of real samples, different amount TdT enzyme (0.2 U, 0.02 U and 0.002 U) was first mixed with human serum to make up a 100-fold diluted human serum detection mixture. The volume of 20 μL reaction mixture including 2 μL primer P01 (6 μM), 2 μL dTTP (10 mM) , 2 μL dUTP (100 μM) , 8 μL H_2O, 2 μL 10 × TdT buffer, 2 μL $CoCl_2$ (2.5 mM), and detention mixture was first incubated at 37 ℃ for 90 min. TdT was then inactivated by heating the mixture at 85 ℃ for 20 min, obtaining the solution I. Further reaction and fluorescence measurement followed the same procedures described above.

Table S1. Sequences (5'-3') of synthetic oligonucleotides used in this study.

Synthetic oligonucleotide	Sequence (5'-3')
P01	ACCTCGAACGACAGCTCC
P02	AAAAA AAAAA AAAAA AAAAA(20A)
P03	Cy5-AAAAA AAAAA AAAAA AAAAA(20A)
P04	AAAAA AAAAA(10A)
P05	AAAAA AAAAA AAAAA AAAAA AAAAA AAAAA(30A)
P06	AAAAA AAAAA AAAAA AAAAAAAAAA AAAAA AAAAA AAAAA(40A)
P07	AAAAA AAAAA AAAAA AAAAAAAAAA AAAAA AAAAA AAAAA AAAAA AAAAA(50A)
P08	AAAAA AAAAA AAAAA AAAAA AAAAA AAAAA AAAAA AAAAA AAAAA AAAAA AAAAA(60A)

Fig. S1. 12% native PAGE electrophoresis results with 0.2 U amount TdT. A) Cy3 and Cy5 channel; B) Cy5 channel; C) Cy3 channel. Line 1: P02(100nM). Line 2: P01(100nM) + TdT(0.2U) + dTTP(500μM) + dUTP(5μM) + P02(250nM) + CoCl2(0.25mM) + TdT reaction buffer(1×). Line 3: Line 2 + Bst large fragment(8U). Line 4: Line 3 + UDG(10U) + Endonuclease IV(10U). Line 5: Line 4 without TdT. Line 6: Line 4 without dUTP. PolyA strands are tagged with Cy5 modification at 5'end (P03) in the electrophoresis. The MSERA reaction time in gel electrophoresis is 30 min.

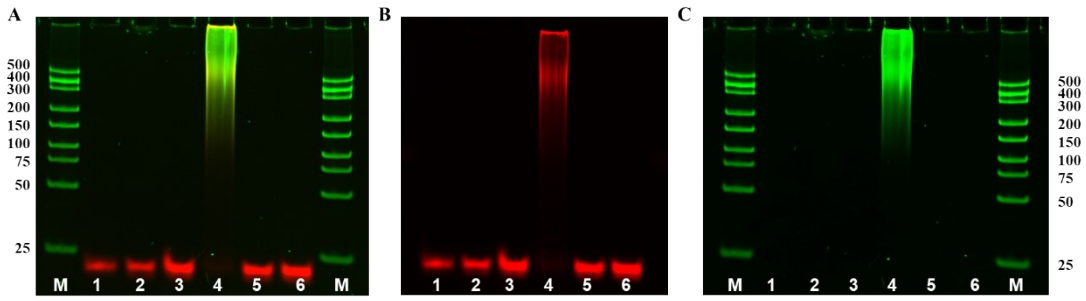
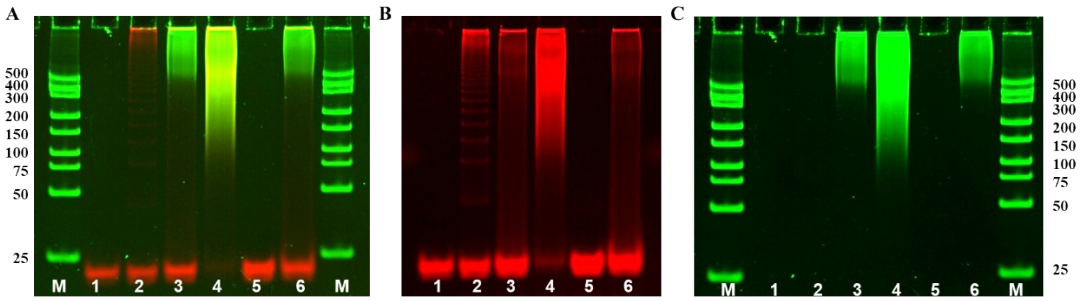


Fig. S2. 12% native PAGE electrophoresis results with 2 U amount TdT. A) Cy3 and Cy5 channel; B) Cy5 channel; C) Cy3 channel. Line 1: P02(100nM). Line 2: P01(100nM) + TdT(0.2U) + dTTP(500μM) + dUTP(5μM) + P02(250nM) + CoCl2(0.25mM) + TdT reaction buffer(1×). Line 3: Line 2 + Bst large fragment(8U). Line 4: Line 3 + UDG(10U) + Endonuclease IV(10U). Line 5: Line 4 without TdT. Line 6: Line 4 without dUTP. PolyA strands are tagged with Cy5 modification at 5'end (P03) in the electrophoresis. The MSERA reaction time in gel electrophoresis is 30 min.



Optimization of conditions

The primer concentration range of 50nM to 500nM was first optimized. The results showed no significant difference between different concentrations. However, when the concentration is 300nM, the result will still be better than other concentrations. The final primer concentration was chosen to be 300nM.

To improve the efficiency of the detection method, TdT extension time was optimized. The extension times were set at 30, 60 ,90, and 120 min. As shown in figure, when the extension time is not less than 90 min, our method achieved the best results.

In this experiment, the ratio of dTTP to dUTP is significant. We fixed the dTTP concentration of 500μM and optimized different dUTP concentration. Results showed that when concentration of dUTP was too low, there almost no signal, indicate low efficiency of the reaction. In contrast, when the ratio of dTTP to dUTP was 10 to 1, both signal and background were very high. In this case, TU template generated by Bst polymerase induced de novo polymerization contains more dU, resulting in much more amplification site in non-specific reaction, which caused the appearance of higher non-specific background. When the concentration of dTTP was equal to dUTP, the generated polyT was too short, thus the efficiency was low and signal was weak. Thus, we chose 5μM dUTP as reaction conditions.

The concentration of polyA was also optimized. As figure showed, when concentration of polyA was 0.1μM, the performance of our method was the best. So the concentration of polyA was determined to be 0.1μM.

The optimization of the length of primer polyA was also performed. As shown in

the figure, the performance of polyA containing 20 to 60 numbers A is similar, while 10 nt polyA has poor signal. Thus, we use 20 nt polyA as the final condition.

For the optimization of UDG amount, we found that when 5 U amount of UDG was added, the performance of MSERA was the best.

Fig.S3. Experiments for Optimization of oligo primer P01 concentration. a) Different colors represent different concentration. Solid lines represent the group that add TdT (0.2U), while dash lines represent the background signal. b) Column figure shows the time gap between signal and background, represented by ΔPOI.

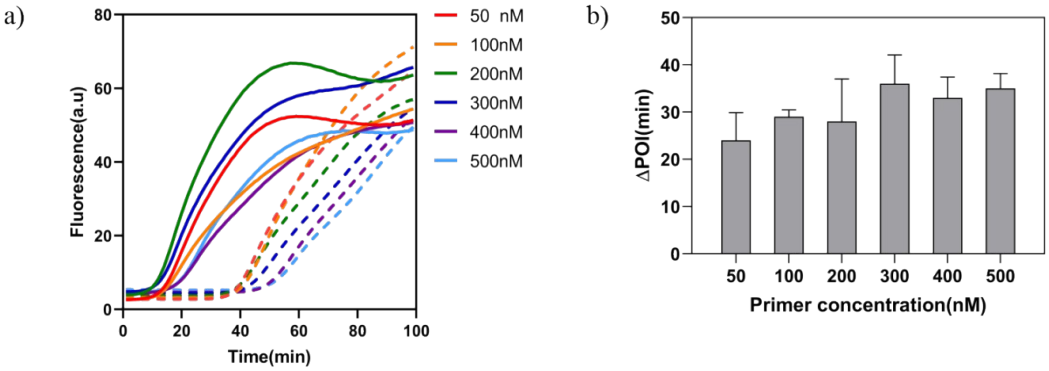


Fig.S4. Experiments for Optimization of TdT extension time. a) Different colors represent different concentration. Solid lines represent the group that add TdT(0.2U), while dash lines represent the background signal. b) Column figure shows the time gap between signal and background, represented by ΔPOI. * indicates P value less than 0.05.

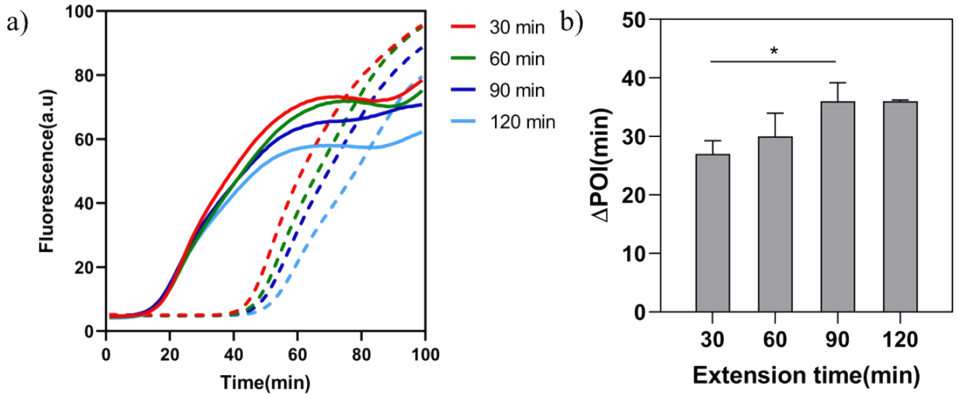


Fig.S5. Experiments for Optimization of dUTP concentration. Different colors represent different concentration. Solid lines represent the group that add TdT(0.2U), while dash lines represent the background signal.

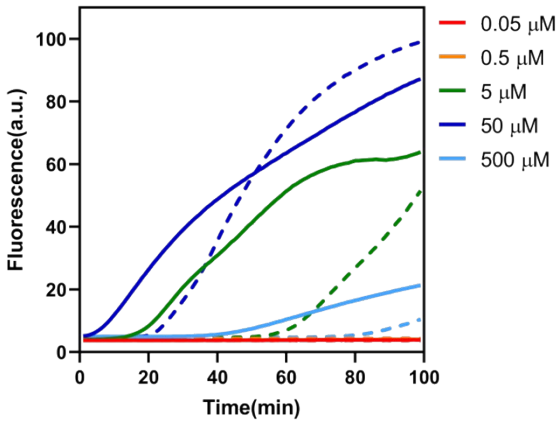


Fig.S6. Experiments for Optimization of polyA concentration. Different colors represent different concentration. Solid lines represent the group that add TdT(0.2U), while dash lines represent the background signal.

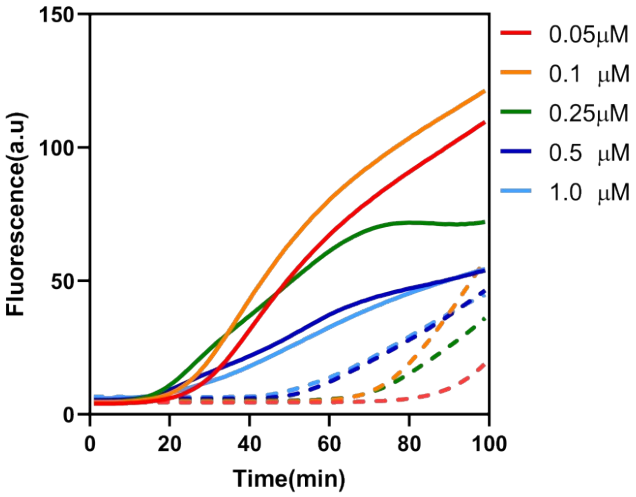


Fig.S7. Experiments for Optimization of polyA length. Different colors represent different length. Solid lines represent the group that add TdT(0.2U), while dash lines represent the background signal.

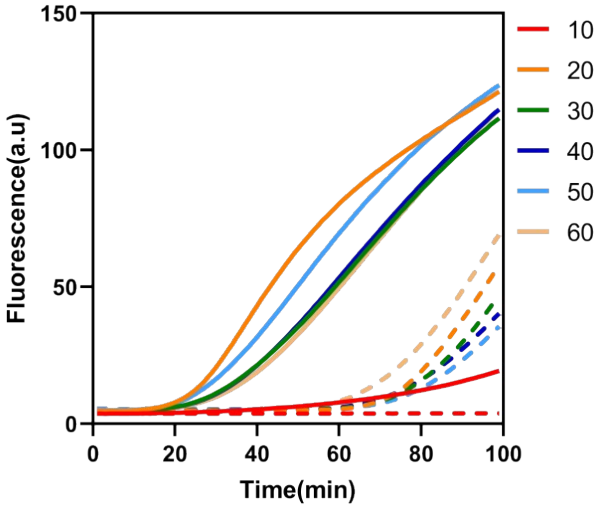


Fig.S8. Experiments for Optimization of UDG amount. a) Different colors represent different amount. Solid lines represent the group that add TdT(0.2U), while dash lines represent the background signal.

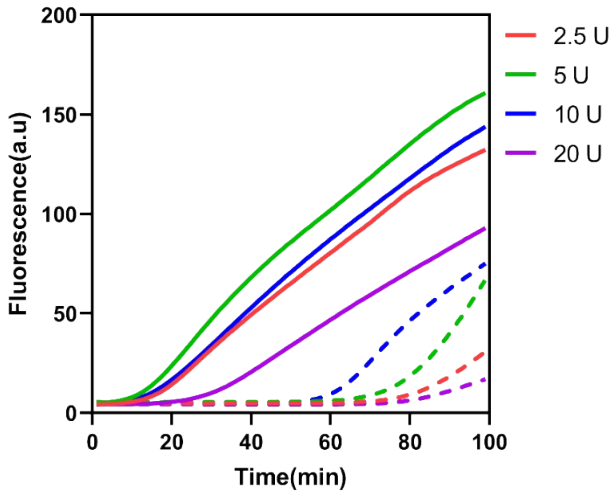


Table S2. Comparison of this work with other TdT activity detection methods.

Methods description	**Detection limit (U)**	**Linear range (U)**	**Reference**
G-quadruplexes	0.05	0.3 ~ 4	[1]
AgNC-based biosensors	0.0318	0 ~ 3	[2]
iridium(III)-based i-motif probe	0.0125	0 ~ 0.4	[3]
protein-inorganic hybrid nanoflowers	4.6×10^{-4}	2×10^{-3} ~ 2	[4]
symbiotic multi-DNA machines	2×10^{-4}	2×10^{-4}~ 0.2	[5]
DNA-AgNCs/GO/GCE	4×10^{-4}	0.002 ~ 0.45	[6]
Multichannel Exponential Amplifications	1.1×10^{-4}	1.1×10^{-4} ~ 55	[7]
Single quantum-dot based nanosensor	4×10^{-5}	0 ~ 0.4	[8]
TdT-yielded DNA-CuNCs	5×10^{-4}	2.5×10^{-3} ~ 0.8	[9]
Long polyT-templated copper nanoparticles	0.0375	0 ~ 5	[10]
Randomly arrayed G-quadruplexes	2.64	30.8 ~ 616	[11]
MSERA	**1.67×10^{-5}**	**2×10^{-5}~0.2**	**This work**

Table S3. The recovery determination of MSERA in artificial serum sample (n=3) using 1% health human serum.

Samples	Added U	Found U	Recovery (%)	RSD (%)
Serum 1	**0.002**	**0.0020**	**101.71**	**1.28**
Serum 2	**0.02**	**0.0192**	**96.16**	**2.03**
Serum 3	**0.2**	**0.2112**	**105.59**	**5.15**

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