Electronic Supplementary Information

Sensitive detection of tumor cells based on aptamer recognition and isothermal exponential amplification

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List of Contents:

1. Table S1 The sequences of DNA oligonucleotides.

DNA	Sequence
Sgc8c	5´- biotin-ATCTAACTGCTGCGCCGCCGGGAAAATACTGTA CGGTTAGA-3´
cDNA	5´- TCTAACCGTACAGTATT-3´
Template Ⅰ	5´- CTGGCGCTTGATGGTATCCAGACTCTAATACTGTA CGGTTAGA-P-3´
Template Ⅱ	5´- CTGGCGCTTGATGGTATCCAGACTCTCTGGCGCTT GATGGTA-P-3´

Notes: The underlined sequences are the recognition site of Nt.BstNBI nicking endonuclease. The letter "P" indicates phosphate at the 3´ end.

2. Optimization of the amount of DNA polymerase, nicking endonuclease, the concentration of Template I and the ratio between Template I and II in the EXPAR assay

(1) Optimization of the amount of DNA polymerase

The amount of Vent (exo-) DNA polymerase was the most critical parameter for the EXPAR. We firstly investigated the influence of the amount of Vent (exo-) DNA polymerase by simultaneously detecting the blank control and 10 pM cDNA. One can see from Fig. S1 that with the increase of the amount of the Vent (exo-) DNA polymerase, the POI values of the blank control and cDNA both reduced indicating that increasing the amount of the Vent (exo-) DNA polymerase could accelerate the process of amplification. The differences of the POI values produced by the blank control and cDNA reached the largest when the DNA polymerase amount was 0.04 U μL^{-1}. After that, using the DNA polymerase at the amount of 0.06 U μL^{-1} and 0.08 U μL^{-1} caused smaller distinction of the POI values between the blank control and cDNA because of the rapid reduction of the POI value of the blank control. Therefore, 0.04 U μL^{-1} is selected as the optimum amount in our EXPAR system.

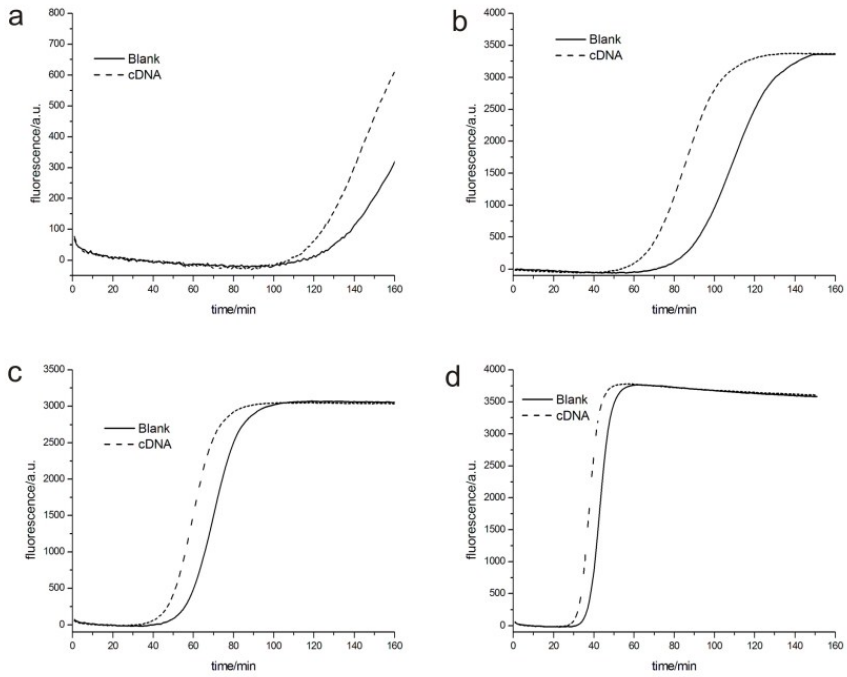

Fig. S1. Optimization of the amount of Vent (exo$^-$) DNA polymerase. DNA polymerase amount: a) 0.02 U μL^{-1}; b) 0.04 U μL^{-1}; c) 0.06 U μL^{-1}; d) 0.08 U μL^{-1}. Other conditions: cDNA, 10 pM; Nicking enzyme, 0.5 U μL^{-1}; Template I and II, both 0.1 μM.

(2) Optimization of the amount of nicking endonuclease

The amount of nicking endonuclease was another pivotal parameter and closely related to the sensitivity and selectivity for detection in EXPAR. It can be seen from Fig. S2 that the POI values of the real-time fluorescence curves produced by the cDNA were gradually shortened with increasing the amount of Nt.BstNBI nicking endonuclease indicating that Nt.BstNBI nicking endonuclease promoted the amplification reaction. The greatest difference of the POI values produced by the blank control and cDNA was obtained at the amount of 0.5 U μL^{-1}. When the amount of nicking endonuclease reached to 0.6 U μL^{-1}, the POI value of the blank control began to decrease obviously. Hence, 0.5 U μL^{-1} is the most appropriate amount of Nt.BstNBI nicking endonuclease for the proposed EXPAR system.

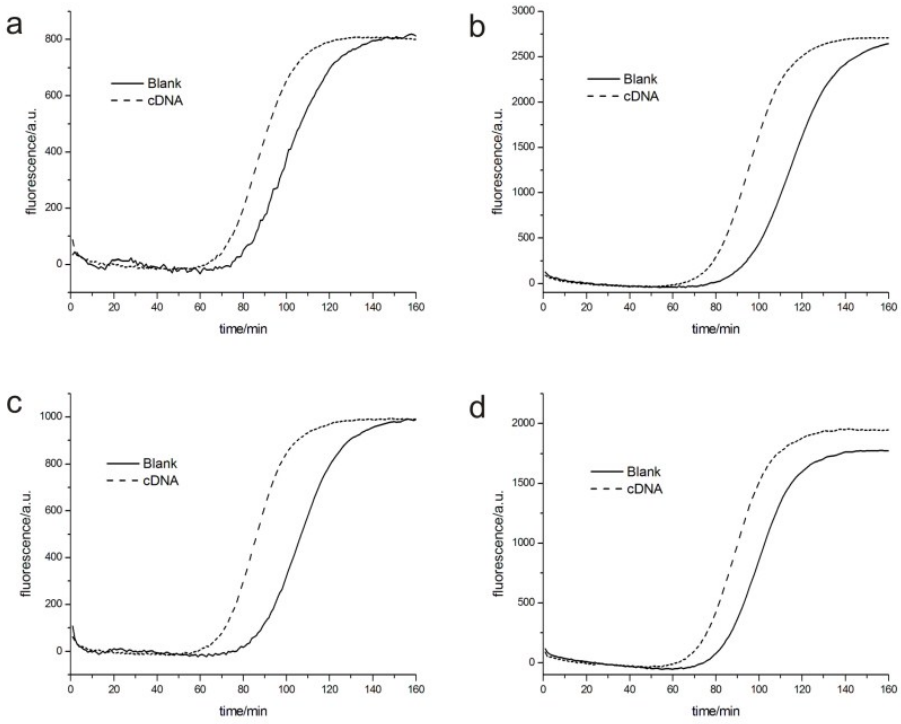

Fig. S2. Optimization of the amount of Nt.BstNBI nicking endonuclease. Nicking endonuclease amount: a) 0.3 U μL^{-1}; b) 0.4 U μL^{-1}; c) 0.5 U μL^{-1}; d) 0.6 U μL^{-1}. Other conditions: cDNA, 10 pM; Vent (exo^-) DNA polymerase, 0.04 U μL^{-1}; Template I and II, both 0.1 μM.

(3) Optimization of the concentration of Template I

The concentration of Template I was another important parameter in this EXPAR system. As shown in Fig. S3, with the increase of the concentration of Template I, the fluorescence intensities in the real-time fluorescence curves produced by the blank control and cDNA both increased gradually. However, the POI values were not obviously influenced. This phenomenon demonstrated that the concentration of Template I mainly affected fluorescence signal intensity rather than amplification speed in this EXPAR assay. When the concentration of Template I was more than 0.1 μM, increase of fluorescence intensity became very slowly. Taking into account that the fluorescence signal intensity produced by 0.1 μM Template I was high enough for detection, we chose this concentration as the optimum in this work.

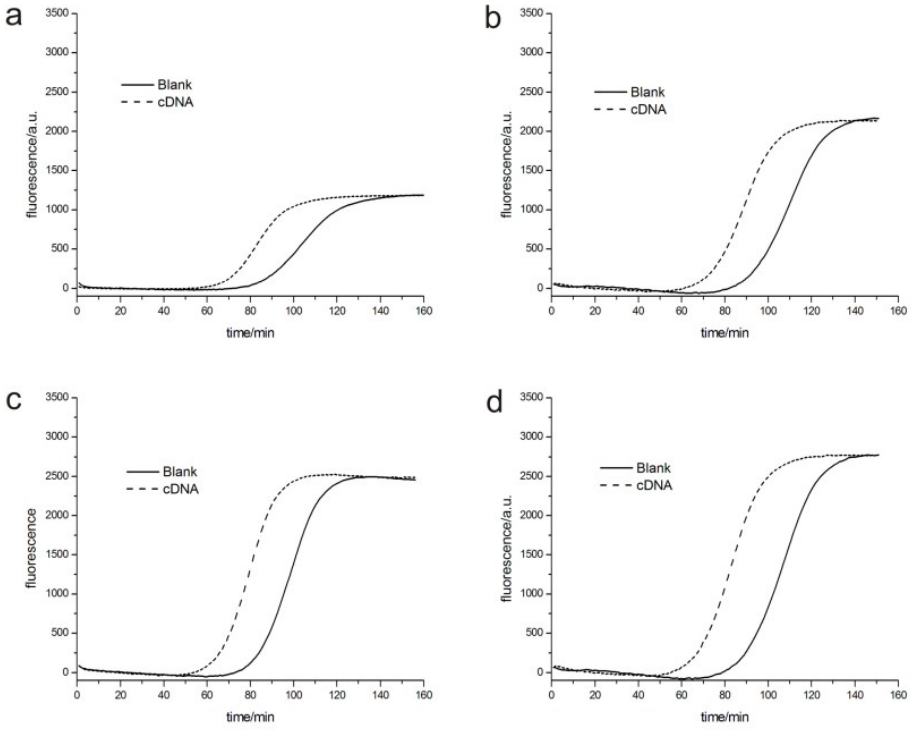

Fig. S3. Optimization of the concentration of Template I. Template I concentration: a) 0.02 μM; b) 0.04 μM; c) 0.08 μM; d) 0.1 μM. Other conditions: cDNA, 10 pM; DNA polymerase, 0.04 U μL^{-1}; Nicking enzyme, 0.5 U μL^{-1}; Template II, 0.1 μM.

(4) Optimization of the ratio between Template I and II

This proposed EXPAR system contained two templates. Hence the ratio between Template I and II affected the amplification efficiency. We detected the influence of different ratio of Template I and II by comparing the POI values of the real-time fluorescence curves produced by the blank control and cDNA in the EXPAR system. We fixed the concentration of Template I as 0.1 μM, and then changed the concentration of Template II. As shown in Fig. S4, with increase of the concentration of Template II, the POI value of the cDNA was slightly shorted, and the difference of POI values between the blank control and the cDNA reached its maximum at the ratio of 1:1. When the ratio between Template I and II became to 1:1.5 and 1:2, the differences of the POI values were reduced gradually. Therefore, 1:1 is selected to be the optimum ratio between Template I and II in the EXPAR system.

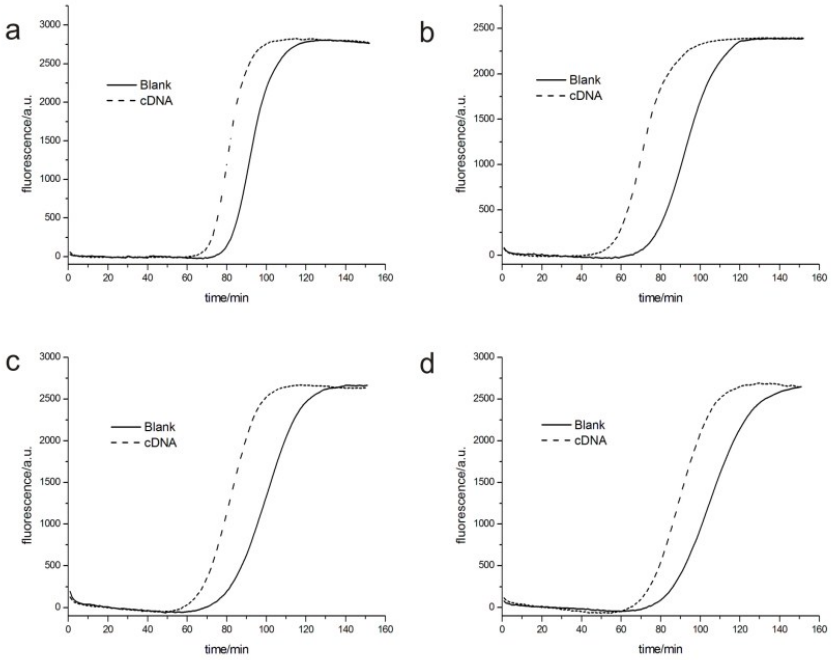

Fig. S4. Optimization of the ratio between Template I and Template II by fixing the concentration of Template I at 0.1 μM. The ratio between Template I and II: a) 1:0.5; b) 1:1; c) 1:1.5; d) 1:2. Other conditions: cDNA, 10 pM; DNA polymerase, 0.04 U μL^{-1}; Nicking enzyme, 0.5 U μL^{-1}.

Table S2. Comparison between the proposed EXPAR-based assay and other reported techniques for detection of tumor cells.

Ref.*	Aptamer based techniques for detection of tumor cells	Detection Limit (cells)	No. of steps	Cell line
9	Flow Cytometry and silver nanocluster fluorescence	150	2	CCRF-CEM
10	Nanoparticle-based collection and fluorescence	~	3	CCRF-CEM
11	Nanorod-based fluorescence	~	3	CCRF-CEM
12	Nanostructure-based Surface-enhanced Raman scattering	~10	3	A549
13	Rolling cycle amplification and chemiluminescence	163	5	Ramos
14	Magnetic relaxation	10	3	CCRF-CEM
15	Nanoparticles and fluorescence	6.6 (calculated)	3	CCRF-CEM
16	Enzyme-catalyzed silver deposition and electrochemistry	10	5	Ramos
17	Electrochemistry	2 (calculated)	5	HepG2
18	Dual-aptamer modification and electrochemistry	1	3	MEAR
19	DNA cycle-amplifying and electrochemiluminescence	162 (calculated)	5	Ramos
21	PCR and FRET	0.13 (calculated)	3	HL-60
23	Cyclic enzymatic signal amplification and Colorimetric	40	3	CCRF-CEM
The proposed methods	EXPAR and real-time fluorescence	10	3	CCRF-CEM

Note:* The references are cited in the text of the paper.