Supporting Information

Triggered Isothermal PCR by Denaturation Bubble-mediated Strand Exchange Amplification

Chao Shi[a], Fanjin Shang[b], Meiling Zhou[b], Pansong Zhang[c], Yifan Wang[b] and Cuiping Ma[*, b]

[a]College of Life Sciences, Qingdao University, Qingdao, 266071, P. R. China.

[b]Key Laboratory of Sensor Analysis of Tumor Marker, Ministry of Education, College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, P. R. China.

[c]College of Chemical Engineering, Qingdao University of Science and Technology, Qingdao 266042, P. R. China.

***Corresponding author.**

Dr. Cuiping Ma; Tel. (Fax.): +86-84022680. E-mail: **mcp169@163.com**

Materials and methods:

Materials

All primers (**Supplementary Table S1**) were designed by using NUPACK software (http://www.nupack.org/), synthesized by Sangon Biotech (Shanghai, China) and purified by HPLC. The *Bst* 2.0 WarmStart™ DNA polymerase (8 U/μL) were purchased from New England Biolabs. Eva Green was purchased from Bridgen (Beijing, China). RNA pure reagent kit for rapid extraction of ultrapure RNA was ordered from Biomed (Beijing, China). 20-bp DNA ladder and the chemicals used to prepare electrophoresis were purchased from Dalian Takara Company (China). RPMI 1640 cell medium including 10% fetal bovine serum (FBS) was purchased from Sangon Biotech (Shanghai, China). PCloneEZ vector was obtained from CloneSmarter (USA). All chemicals were of analytical grade unless otherwise indicated.

Methods

The reaction system

The optimized SEA reaction was carried out in 10 μL containing 1.0 $\times 10^{-6}$ M P1 and P2, 5.0×10^{-4} M dNTPs, 10% PEG-200, 0.5×Eva Green, 0.8 U *Bst* 2.0 WarmStart™ DNA polymerase, 1×ThermoPol buffer (20 mM Tris-HCl, 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.1% Triton X-100, pH8.8 @ 25℃). The reaction was initiated by adding the different targets without pre-treatment and incubated at 65℃. No template control (NTC) was used as a negative control. The SEA reaction was performed in a CFX96™ Real-Time PCR detection system (Bio-Rad) at 1-min

intervals. 17.5% native PAGE was carried out using tris-acetate-EDTA (TAE) buffer (pH 8.0) for 55 min at 135 V.

RNA Extraction

According to the method described in the literature[29], the total RNA was extracted from *E. coli* by using RNA pure reagent kit for rapid extraction of ultrapure RNA. RNA was verified using agarose gel electrophoresis without denaturing conditions by ethidium bromide staining (data not shown).

Isolation of plasmid DNA

The pBluescript II KS(+) plasmid DNA was extracted according to the method described in the literature[31].

29. Shi, C.; Shen, X.; Niu S.; Ma, C. *J. Am. Chem. Soc.* **2015**, 137(43): 13804-13806.

31. Green, M. R.; Sambrook, J. *Molecular cloning: a laboratory manual*; Cold Spring Harbor Laboratory Press New York, **2012**.

Supplementary Figures

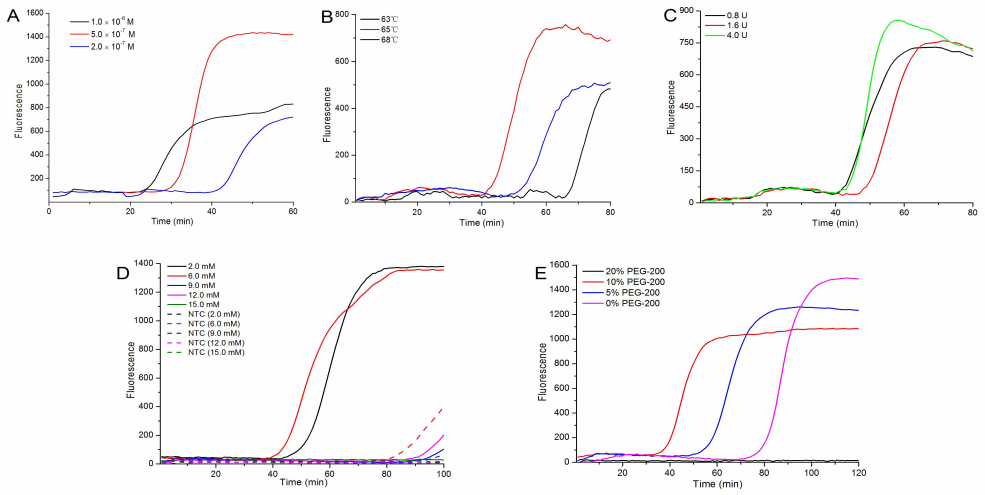

Figure S1. Optimization of SEA reaction.

A. Optimization of the concentration of primer. The reaction mixture contained 1.0×10^{-9} M HCV DNA, 2.0×10^{-7} M, 5.0×10^{-7} M and 1.0×10^{-6} M of each primer, 0.8 U *Bst* 2.0 WarmStart™ DNA polymerase, 5.0×10^{-4} M dNTPs, 0.5×Eva Green and 1×ThermoPol buffer and was incubated at 65℃. The fluorescence signal was rapidly increased when the concentration of each primer was 1.0×10^{-6} M. Therefore, 1.0×10^{-6} M of each primer was adopted for SEA reaction.

B. Optimization of reaction temperature. The SEA was initiated by 1.0×10^{-9} M *E. coli* 16S rRNA , and other assay conditions were the same as above described. The real-time fluorescence curves were measured at 63 ℃ , 65 ℃ and 68 ℃ , respectively. For the same target concentration, the fluorescence intensity was increased higher at 65℃ than that of 63℃ and 68℃. Thus, 65℃ was selected as the optimum reaction temperature for SEA reaction.

C. Effect of the amount of DNA polymerase. With 1.0×10^{-9} M of *E. coli* 16S rRNA target, the fluorescence signals were respectively measured by adding 0.8 U, 1.6 U and 4.0 U *Bst* 2.0 WarmStart™ DNA polymerase. Other experiment conditions were optimum. The fluorescence signals for 0.8 U and 4.0 U *Bst* DNA polymerase were earlier than that of 1.6 U *Bst* DNA polymerase. Considering of effect and cost, 0.8 U *Bst* 2.0 WarmStart™ DNA polymerase was selected for the SEA assay.

D. Effect of the concentration of Mg^{2+}. The above-described optimum assay conditions were used.

The detection target was 1.0×10^{-9} M of *E. coli* 16S rRNA. When both 2.0 and 6.0 mM Mg^{2+} were added, fluorescence signals were greatly increased. Due to the background fluorescence signal of NTC generated by adding 6.0 mM Mg^{2+}, 2 mM Mg^{2+} was chose in SEA reaction.

E. The real-time fluorescence curves for different concentrations of PEG-200. The concentration of the pBlu2KSP target was 1.0×10^{-9} M. When PEG-200 was increased to 10%, Ct value fell roughly 40 cycles for the tested target DNA compared with the negative control, which showed great increase for the amplification efficiency of SEA. Thus, 10% PEG-200 was used for the SEA reaction.

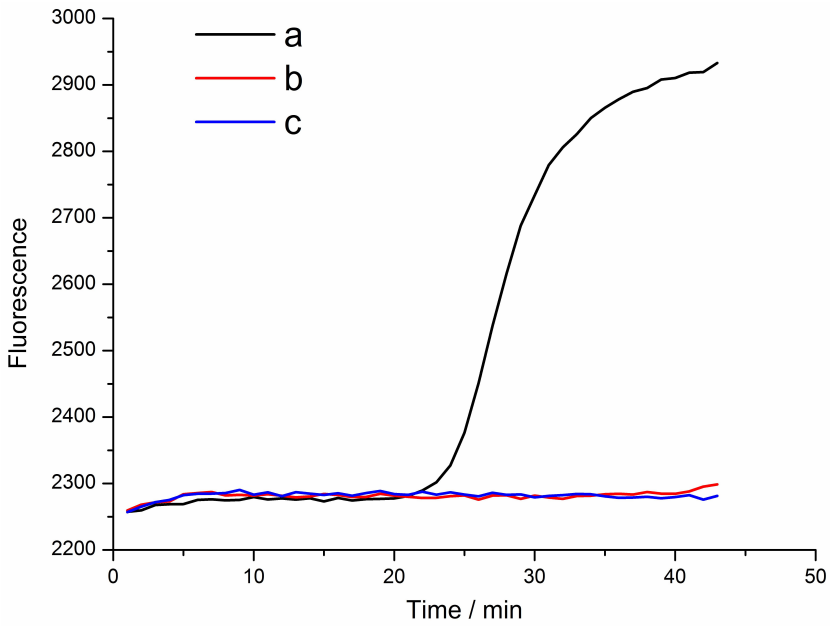

Figure S2. The specificity of our method.

a. real-time fluorescence curve from completely complementary P1; b. real-time fluorescence curve from one-base mismatch P1 (T-A); c. real-time fluorescence curve from one-base mismatch P1 (T-C).

Supplementary Table

Supplementary Table S1. Sequences of nucleic acids used in this work.

Templates/primers		Sequences（5′-3′）
***E.coil* 16S rDNA（[a]J01859.1 [b]935-1107)**		CAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCG AAGAACCTTACCTGGTCTTGACATCCACGGAAGTTTTC AGAGATGAGAATGTGCCTTCGGGAACCGTGAGACAGG TGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTT GGGTTAAGTCCCGCAACGAGCG **(173 nt)**
	Taq DNA polymerase	CGCTCGTTGCGGGACTTAACCC
Prrimer P1	Vent DNA polymerase Vent (exo$^-$) DNA polymerase *Bst* 2.0 WarmStart DNA polymerase	CGCTCGTTGCGGGACTTAACC
	Taq DNA polymerase	GGCTGTCGTCAGCTCGTGTTGTG
Primer P2	Vent DNA polymerase Vent (exo$^-$) DNA polymerase *Bst* 2.0 WarmStart DNA polymerase	GCTGTCGTCAGCTCGTGTTG
***E.coil* 16S rDNA（[a]J01859.1 [b]250-795)**		GTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTA GCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGA GACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGG AATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGC CGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTT TCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCT CATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCC GTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGT TAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGT TTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGG GAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGA GGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTA GAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCC TGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGG AGCAAACAGGATTAGATACC **(546 nt)**
Primer P1-45		GTGTGGCTGGTCATCCTCTCAGAC
Primer P2-45		CTAGGCGACGATCCCTAGCTG
Primer P1-66		GTGTGGCTGGTCATCCTCTCAGAC
Primer P2-66		GTAGGTGGGGTAACGGCTCAC
Primer P1-87		ACTGCTGCCTCCCGTA

Primer P2-87	CTAGGCGACGATCCCTAGCT
pBluescript II KS(+) ([a]X52327.1 [b]559-612)	ATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGAC GTTGTAAAACGACGGC **(54 nt)**
Primer P1	GCCGTCGTTTTACAACGTCGTGA
Primer P2	ATTAAGTTGGGTAACGCCAGGGT
***E.coil* 16S rRNA（[a]J01859.1 [b]1057-1107)**	GCUGUCGUCAGCUCGUGUUGUGAAAUGUUGGGUUA AGUCCCGCAACGAGCG **(51 nt)**
Primer P1	CGCTCGTTGCGGGACTTAACC
Primer P2	GCTGTCGTCAGCTCGTGTTG
Hepatitis C Virus (HCV) （[a]D10749.1 [b]283-332）	GTGGTACTGCCTGATAGGGTGCTTGCGAGTGCCCCGGG AGGTCTCGTAGA (50nt)
Primer P1	ACCTCCCGGGGCACT
Primer P2	GCCTGATAGGGTGCTTGCG
Primer P1 (T-A)	ACCTCCCGGGGCAC*A*
Primer P1 (T-C)	ACCTCCCGGGGCAC*C*

[a] GenBank accession number [b] The position of specific sequence in genomic DNA

The dotted line in sequence of target represented the complementary sequence of Primer P1. The underlined portion was the complementary sequence of Primer P2.

Supplementary Table S2. The sequencing data of 87-bp amplification products.

Nucleic acids		Sequences (5′-3′)
87-bp tandem repeats with variable numbers	458 bp	ACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCT CTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCAACTTCCCCACT GCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTC AGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCAACTTCCCCACTGCT GCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGA CCAGCTAGGGATCGTCGCCT*T*GGTGAGCCGTTACCCCACCAGCTGCATCAGGCTTGCG CCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTT CCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCT*T*G
	677 bp	ACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCT CTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCAACTTCCCTACTG CTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCA GACCAGCTAGGGATCGTCGCCT*T*GGTGAGCCGTTACCCCACCAACTTCCCCACTGCTG CCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGAC CAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCAACTTCCCCACTGCTGCCTC CCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAG CTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCAACTTCCCTACTGCTGCCTCCCG TAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTA GGGATCGTCGCCTAGGTGAGCCGTTACCCCACCAACTTCCCCACTGCTGCCTCCCGTAG GAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGG ATCGTCGCCT*T*GAAGGGCGACACGCGAATTCG

The gray shaded region represented the complementary sequence of 87-bp amplification product. The dotted line represented the insertion of unexpected bases. The mutated bases of amplification products were shown in red and italic.