Supporting information

Rapid DNA detection and one-step RNA detection catalyzed by *Bst* DNA polymerase and the narrow-thermal-cycling

Mengzhe Li,[#,†] Mengmeng Liu,[#,†] Cuiping Ma,[*,‡] and Chao Shi[*,†]

†Qingdao Nucleic Acid Rapid Testing International Science and Technology Cooperation Base, College of Life Sciences, Department of Pathogenic Biology, School of Basic Medicine, Qingdao University, Qingdao, 266071, PR China

‡Key Laboratory of Optic-electric Sensing and Analytical Chemistry for Life Science, MOE, Shandong Provincial Key Laboratory of Biochemical Engineering, College of Marine Science and Biological Engineering, Qingdao University of Science and Technology, Qingdao, 266042, PR China

Corresponding Author

*sc169@163.com (C. Shi)

*mcp169@163.com (C. Ma)

[#]M. Li and M. Liu contributed equally.

Experimental Section

1. Materials

Listeria monocytogenes (*L. monocytogenes*) stored in our laboratory was used as the detection target in this work. All primers and other oligonucleotides in Table S1 were designed with the NUPACK web tool (http://www.nupack.org/), DNAMelt Web Server (http://unafold,rna.albany.edu/?q=DINAMelt), and MFE primer 3.0 (https://mfeprimer3.igenetech.com), and synthesized in Sangon Biotech (Shanghai, China). *Bst* 2.0 Warmstart DNA Polymerase used in this method was obtained from New England Biolabs (MA, US). Chemicals for electrophoresis gels and 20-bp DNA ladders were bought from Takara Biomedical Technology Co., Ltd (Beijing, China).

Table S1 Synthetic oligonucleotides in this work

Name	Sequence (5'→3')
P1[a]	GTCATTGGAAACTGGAAGACTG
P2[a]	CCACTCTCCTCTTCTGCAC
M1P2[b]	CCACTCTCCTCTTCTGCGA
M2P2[b]	CCACTCTCCTCTTCTGGAA
M3P2[b]	CCACTCTCCTCTTCTGCAA
50-nt synthesized DNA[c]	GGGTCATTGGAAACTGGAAGACTGGAGTGCAG AAGAGGAGAGTGGAATTC
50-nt synthesized RNA[d]	GGGUCAUUGGAAACUGGAAGACUGGAGUGCA GAAGAGGAGAGUGGAAUUC

[a]Primers for ASEA reactions. The forward and reverse primers were P1 and P2, respectively.

[b]Mismatched P2 were used in the specificity assay of ASEA. The red nucleotide bases represented mutations corresponding to P2.

[c,d]50-nt synthetic DNA was located at the position of 639-688 in *Listeria monocytogenes* 16S rDNA (GenBank No.: M58822.1), and 50-nt synthetic RNA was the correspondence with the synthetic DNA sequence. The double underlined

sequence of synthetic DNA or RNA was same to P1, and the wavy lined sequence of synthetic DNA or RNA was complementary to the sequence of P2.

2. The optimization of ASEA

The 50-nt synthesized DNA of *L. monocytogenes* was used. The inherent optimal reaction temperature for primers in the previous SEA assay was used as the renaturation temperature (T_r, 62℃) in ASEA. Then, a denaturation temperature (T_d) was introduced to develop thermal cycles within a narrow temperature range. Each thermal cycle consisted of incubating the reaction mixture at T_d for 1 s and immediately reducing the temperature to T_r for another 1 s. As *Bst* DNA polymerase could be inactivated at 80℃ for some time, the T_d was optimized below 80℃ (from 70 to 76℃) using the CFX Connect™ RealTime PCR System (Bio-Rad, CA) to determine the reaction efficiency. Next, the concentrations of primers and *Bst* DNA polymerase in the ASEA assay were optimized in a total 20 μL reaction mixture containing 1×Isothermal Amplification Buffer, 0.8 mM dNTP and 0.5 μL Evagreen. The polymerase concentrations varied from 0.16 U/μL to 0.28 U/μL and final concentrations of each primer varied between 1.5 μM to 3.0 μM. The reaction efficiency of different conditions was determined by threshold time values of fluorescence curves.

3. DNA detection of L. monocytogenes by ASEA

TIANamp Bacteria DNA Kit (Tiangen Biotech Co., Ltd, Beijing, China) was used to extract the bacterial genomic DNA of *L. monocytogenes*. The quality and concentration of extracted genomic DNA were determined using the NanoDrop™ One (Thermo Fisher Scientific Inc., US). The resulting genomic DNA and 50-nt synthesized DNA fragments of *L. monocytogenes* were prepared as the targets to evaluate the feasibility of the ASEA on detecting DNA. Then, 10-fold serial dilutions of the 50-nt synthesized DNA were used to test the sensitivity of the ASEA method, with concentrations varied from 1.2×10^2 copies-1.2×10^5 copies in the final reaction

mixture. A non-target control (NTC) was conducted with DNase-free water as the target. All above ASEA reactions were monitored with the CFX Connect™ Real-Time System (BioRad, CA).

4. ASEA products analysis

Melting curves of ASEA products, which used genomic DNA and 50-nt synthesized DNA of *L. monocytogenes* as targets, were analyzed with a high temperature of 95℃ for 0.2 s and a low temperature of 50℃ for 0.2 s. Then, the appropriate melting temperature for each target, also known as the melting peak, was obtained at 0.2℃/s over the temperature range of 50-90℃. ASEA products were also analyzed with 12.5% polyacrylamide gel electrophoresis (PAGE) (Bio-Rad, CA) and sequenced in Sangon Biotech (Shanghai, China).

5. Specificity of the ASEA

The specificity of primers (P1 and P2) was tested according to its mismatch tolerance in the ASEA assay. All sequences used were shown in Table S1. All ASEA reactions in the specificity assay were conducted with 50-nt synthesized DNA fragments as the targets, and each group had its NTC with DNase-free water as targets. All reactions were monitored with the CFX Connect™ Real-Time System (BioRad, CA) and ASEA products were also analyzed with 12.5% PAGE (Bio-Rad, CA).

6. RNA detection by ASEA

The capacity of ASEA detection on different RNA targets, such as synthesized 50-nt RNA and the total RNA of *L. monocytogenes*, was also investigated. The sequence of synthesized 50-nt RNA was shown in Table S1. The total RNA of *L. monocytogenes* was extracted using the *TransZol* Up Plus RNA Kit (TransGen Biotech Co., Ltd, Beijing, China), whose quality and concentration were also determined with the NanoDrop™ One (Thermo Fisher Scientific Inc., US). All reactions were monitored with the CFX Connect™ Real-Time System (BioRad, CA) and ASEA products were also analyzed with 12.5% PAGE (Bio-Rad, CA). The ASEA conditions for RNA

detection were subsequently optimized by the adding an initial incubation time.

Supplementary Figures and Tables

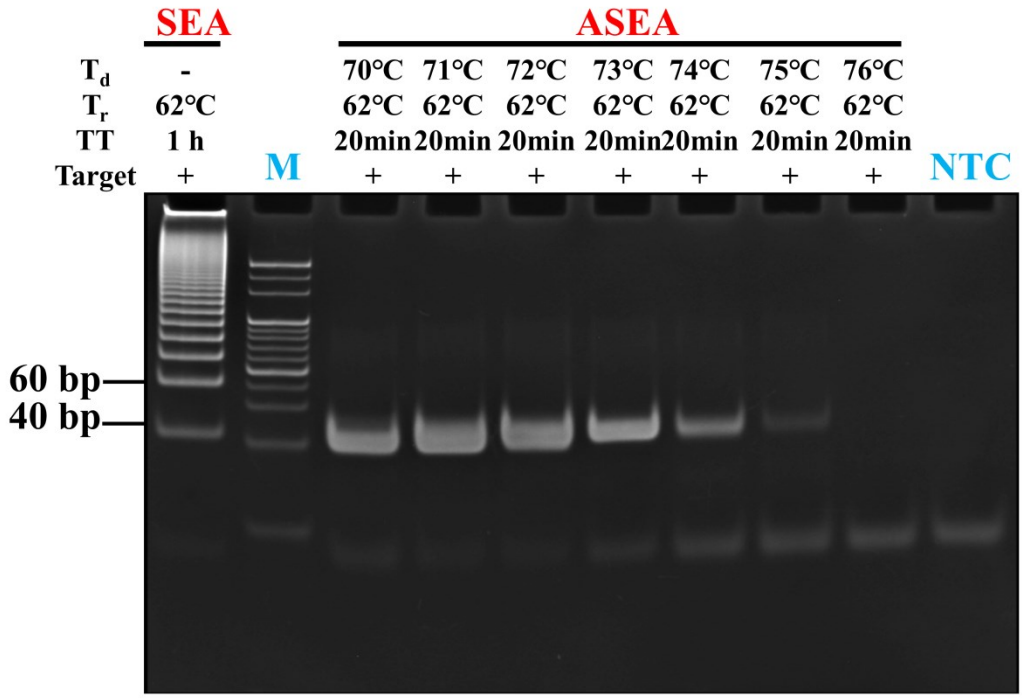

Figure S1. PAGE image of SEA or ASEA products after amplification with different treatments. Different treatments were displayed on the top of PAGE image. M meant 20-bp DNA ladder. T_d, T_r and TT meant the denaturation temperature, renaturation temperature and total reaction time, respectively. NTC meant non-targeted control, which was conducted with DNase-free water as the target.

To figure out the influence of the narrow-thermal-cycling on amplicons, ASEA products with different T_d values from 70℃ to 76℃ were compared with the SEA products under the isothermal condition (62℃). As shown in Figure S1, ASEA products amplified with different T_d values all showed one main single band and SEA products indeed had many ladder bands. Thus, the narrow-thermal-cycling could be an efficient approach to produce such single bands in ASEA and eliminate cascaded products.

Table S2 Comparison among four different nucleic acid amplification methods[1-7]

	qPCR/qRT-PCR	ASEA	LAMP/RT-LAMP	SEA
Reaction temperature	55-95℃	**62-74℃**	**65℃**	**62℃**
Primers	**2**	**2**	4-6	2
Products	**Specific products,** primer dimer	**Specific products**, primer dimer	Cascaded products, primer dimer	Cascaded products, primer dimer
Target length requirement	**>40 nt/bp**	**>40 nt/bp**	>200 nt/bp	**>40 nt/bp**
Instrument	Thermal cycler	Thermal cycler	Water or metal bath	Water or metal bath
DNA detection				
Sensitivity	**10^1-10^2 copies**	**60-120 copies**[a]	**10^1-10^2 copies**	**10^4 copies**
Reaction time	1 h	**Within 15 min**	Around 30 min	Around 45 min
RNA detection				
Enzyme	Reverse transcriptase and *Taq* DNA polymerase	***Bst* DNA polymerase**	Reverse transcriptase and *Bst* DNA polymerase	***Bst* DNA polymerase**
Additional reverse transcription step	Yes	**No**	Yes	**No**
Sensitivity	**~10^2 copies**	6×10^4-1.2×10^5 copies[b]	**~10^2 copies**	**100 amol 16S rRNA**
Reaction time	1.5 h	**Within 20 min**	1 h	1 h

a, b. The detection limit of DNA/RNA fragments in ASEA was 10 aM (DNA)/10 fM (RNA) in either10 μL or 20 μL reaction system, and the copy number should be 60 copies (DNA)/ 6×10^4 copies (RNA) in 10 μL reaction system and be 120 copies (DNA)/ 1.2×10^5 copies (RNA) in 20 μL reaction system.

Reference:

1. Coudray-Meunier, C., Fraisse, A., Martin-Latil, S., Guillier, L., Delannoy, S., Fach, P. and Perelle, S. (2015) A comparative study of digital RT-PCR and RT-qPCR for quantification of Hepatitis A virus and Norovirus in lettuce and water samples. *International journal of food microbiology*, 201, 17-26.

2. Williams, J.M., Trope, M., Caplan, D.J. and Shugars, D.C. (2006) Detection and quantitation of *E. faecalis* by real-time PCR (qPCR), reverse transcription-PCR (RT-PCR), and cultivation during endodontic treatment. *Journal of Endodontics*, 32, 715-721.

3. Li, H., Li, K., Bi, Z., Gu, J., Song, D., Lei, D., Luo, S., Huang, D., Wu, Q. and Ding, Z. et al.. (2019) Development of a reverse transcription-loop-mediated isothermal amplification (RT-LAMP) assay for the detection of porcine pegivirus. *Journal of Virological Methods*, 270, 59-65.

4. Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N. and Hase, T. (2000) Loop-mediated isothermal amplification of DNA. *Nucleic Acids Reaserch*, 28, e63.

5. Nagamine, K., Hase, T. and Notomi, T. (2002) Accelerated reaction by loop-mediated isothermal amplification using loop primers. *Molecular and Cellular Probes*, 16, 223-229.

6. Shi, C., Shang, F., Zhou, M., Zhang, P., Wang, Y. and Ma, C. (2016) Triggered isothermal PCR by denaturation bubble-mediated strand exchange amplification. Chemical Communications, 52, 11551-11554.

7. Shi, W., Wei, M., Wang, Q., Wang, H., Ma, C. and Shi, C. (2019) Rapid diagnosis of Mycoplasma pneumonia infection by denaturation bubble-mediated strand exchange amplification: comparison with LAMP and real-time PCR. *Scientific Reports-UK*, 9.