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An ultra-fast, one-step RNA amplification method for the detection of *Salmonella* in seafood

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The optimization of renaturation temperature (Tr) and denaturation temperature (Td)

The ASEA reaction was performed in a 25 μL system in a PCR tube containing 2.5 μL of target DNA, 1×Isothermal Amplification Buffer, 0.8 mM dNTP, 1.5 μM each of primer P1 and P2, 0.625 μL Evagreen, and 3.75 U of Bst 2.0 Warmstart DNA Polymerase.

The 39-nt synthesized DNA of *Salmonella* was used for faster amplification by the enzyme. Because of the temperature in the previous SEA assay was below 65℃, renaturation temperature (Tr) was optimized from 57 to 61℃ in ASEA. The fluorescence signal of isothermal amplification reaction was detected by CFX Connect™ Real-Time PCR System (Bio-Rad, CA, USA) at 1-min intervals with the target concentrations of 1.0×10^{-9} M. Then, a denaturation temperature (Td) was introduced to develop thermal cycles within a narrow temperature range. Each thermal cycle consisted of incubating the reaction mixture at Td for 1 s and immediately reducing the temperature to Tr for another 1 s. That is, Tr and Td duration were 1 sec each. As Bst DNA polymerase could be inactivated at 80℃ for some time, the Td was optimized below 80℃ (from 74 to 78℃) using the CFX Connect™ RealTime PCR System (Bio-Rad, CA) to determine the reaction efficiency.

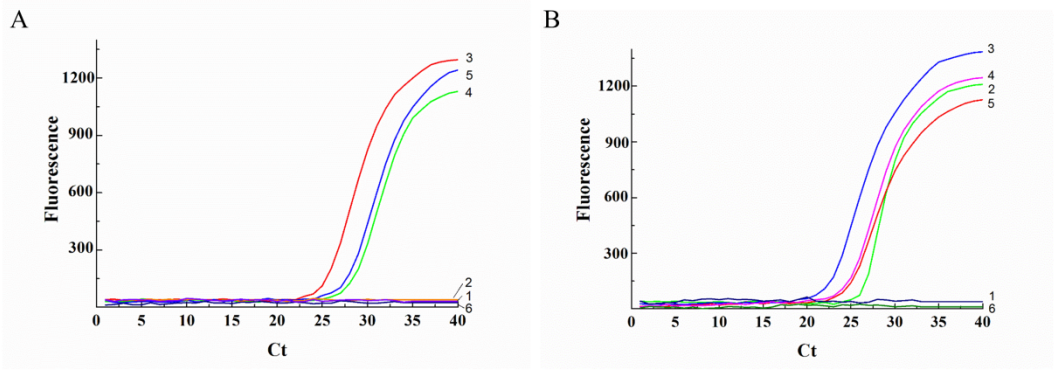

Fig. S1. The optimization of ASEA reactions for *Salmonella* detection. (A) 1-5 represented Real-time fluorescence curves of the renaturation temperature (Tr) values of the ASEA reaction, which are 57℃、58℃、59℃、60℃ and 61℃, respectively, 6 represented Non-targeted control (NTC). (B) 1-5 represented Real-time fluorescence curves of denaturation temperature (Td) values of the ASEA reaction, which are 74℃、75℃、76℃、77℃ and 78℃, respectively, 6 represented Non-targeted control (NTC).

Optimization of primer concentration and amount of enzyme

Next, the concentrations of primers and Bst DNA polymerase in the ASEA assay were optimized in a total 25 μL reaction mixture.The polymerase concentrations varied from 0.05 U/μL to 0.15 U/μL and final concentrations of each primer varied between 0.5 μM to 1.5 μM. The reaction efficiency of different conditions was determined by threshold time values of fluorescence curves.

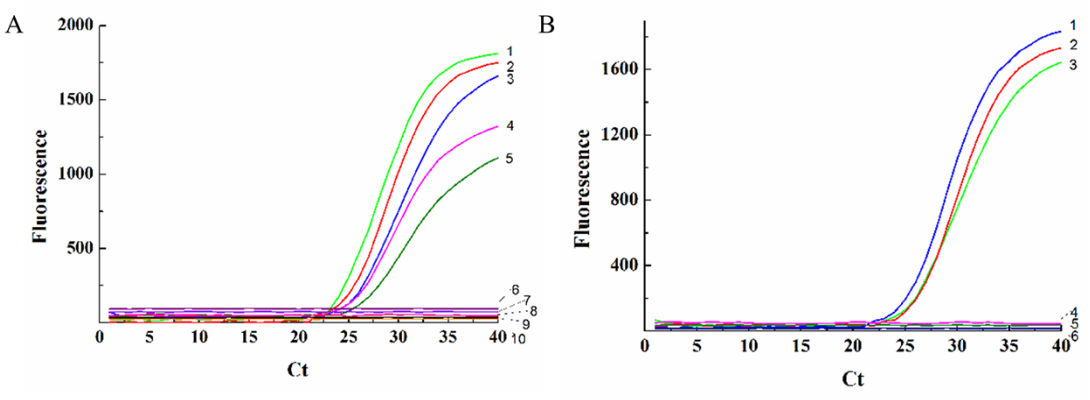

Fig. S2. The optimization of ASEA reactions for *Salmonella* detection . (A) 1–5 respectively represented the concentration of primers were 1.5 μM、1.25 μM、1 μM、0.75 μM、0.5 μM, 6-10 respectively represented corresponding Non-targeted control (NTC). (B) 1–3 respectively represented the concentration of enzyme were 0.15 U/μL，0.1 U/μL，0.05 U/μL, 4-6 respectively represented corresponding Non-targeted control (NTC), That is, DNase/RNase-free water was added to the ASEA reaction system as the target.

Table S1 ASEA and real-time PCR of 102 samples

Kind of samples*	Sample Number*	Cycle of threshold (Ct)	
		Specific ASEA for *Salmonella*	Real-time PCR for *Salmonella*
Oysters	1	35.22	32.07
	2	31.65	29.14
	3-17	ND	ND
	18-26	ND	ND
Scallops	27	23.84	25.32
	28	28:88	30:01
	29-33	ND	ND
Shrimp	34	36.44	31.43
	35-51	ND	ND
Fish	52-54	ND	ND
	55	ND	33.72
Chicken	56-68	ND	ND
	69-80	ND	ND
Eggs	81	29.35	30.67
	82-85	ND	ND
Eggs	86-102	ND	ND
DNase/ RNase-free	-	ND	ND

DNase/ RNase-free	-	ND	ND

ND: not detected.

*The 102 samples came from 17 oysters, 17 scallops, 17 shrimp, 17 fish, 17 chicken, and 17 eggs, and were sorted and numbered according to 1-102.

[#]**PCR reaction system includes:** 2 μL of Template DNA, 2 μL of 10 × Taq Buffer(Mg2+ plus), 0.4 μL of 10 mM dNTPs mix, 0.2 μL of Taq DNA polymerase (5 U/μL), 0.4 μL of 10 μM forward and reverse primer, 0.5 μL of 20 × Evagreen, and DNase/ RNase-free water were added to reach a final reaction volume of 20 μL.The CFX Connect™ Real-Time PCR System(BioRad, CA, USA) was used for thermal cycling: the whole system was pre-denatured at 95℃ for 5 min, followed by 35 cycles of denaturation at 95℃ for 30s, annealing at 56℃ for 30s and extension at 72℃ for 1 min.