Electronic Supplementary Material (ESI) for Analytical Methods.
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Supplementary information

Thermal cycling optimization

Since the reaction temperature slightly lower than primers' T_m value is the both beneficial for combination of primers to target and the enzymes maintaining high activity, therefore 61 ℃ was first fixed as T_{min} for T_{max} optimization. As shown in Fig.S1A, the T_{max} of 74 ℃ exhibited the lowest T_t value. Then 74 ℃ was fixed as T_{max} for T_{min}, the result of which was shown in Fig.S1B. As expected, the reaction with 61 ℃ as T_{min} showed the lowest T_t value. In summary, the thermal cycling at the range of 74 ℃ to 61 ℃ was most beneficial for ASEA reaction, thus 74 ℃ and 61 ℃ were employed as T_{max} and T_{min} in this work.

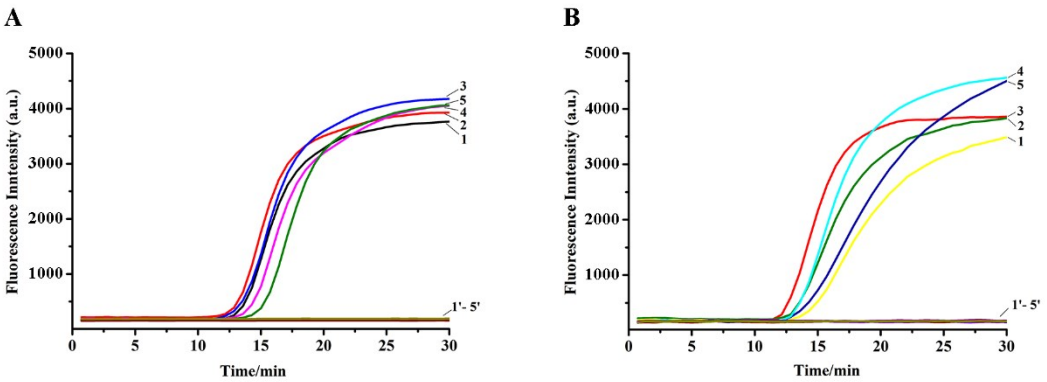

Fig.S1. T_{max} and T_{min} optimization of ASEA for CPV detection. (A) T_{max} optimization at T_{min} of 61 ℃. ASEA reactions were carried out at the T_{max} of (1) 75 ℃, (2) 74 ℃, (3) 73 ℃, (4) 72 ℃ and (5) 71 ℃, respectively. 1'-5' represented corresponding NTC. (b) T_{min} optimization at the T_{max} of 72 ℃. ASEA reactions were carried out at the T_{min} of (1) 63 ℃, (2) 62 ℃, (3) 61 ℃, (4) 60 ℃ and (5) 59 ℃, respectively. 1'-5' represented corresponding NTC. The CPV genomic DNA at the concentration of 1.0×10^{-13} M was employed for thermal cycling optimization.

Determination of ASEA's LOD

In order to confirm the LOD of this ASEA, a series of CPV genomic DNA of the concentration between 1.0×10^{-17} M to 1.0×10^{-18}, specifically, 1.0×10^{-17} M, 8.0×10^{-18} M, 6.0×10^{-18} M, 4.0×10^{-18} M, 2.0×10^{-18} M and 1.0×10^{-18} M, was employed as template for ASEA detection. The result showed fluorescence accumulated in the reaction with 1.0×10^{-17} M and 8.0×10^{-18} M CPV genomic DNA, but no fluorescence signals were detected in the reaction with CPV genomic DNA lower than 8.0×10^{-18} M (Fig.S2), demonstrated the LOD of ASEA was approximately 8.0×10^{-18} M (equivalent to 4.8 copies/μL).

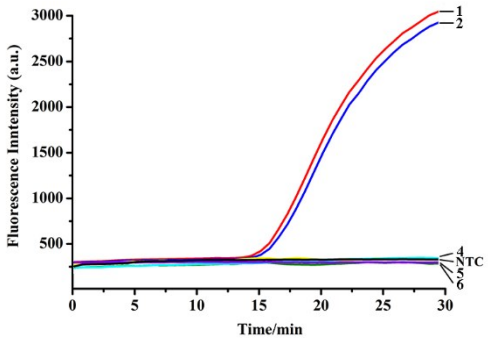

Fig.S2. The fluorescence curves of ASEA reactions with the template of CPV genomic DNA at the concentration of (1) 1.0×10^{-17} M, (2) 8.0×10^{-18} M, (3) 6.0×10^{-18} M, (4) 4.0×10^{-18} M, (5) 2.0×10^{-18} M, and (6) 1.0×10^{-18} M. NTC represented no target control.