

Table S1. Sequences of oligonucleotides used in this study.

Name	sequence (5'-3')	nt
EX-F-558	ACTCAACCAAGTCATTCTGAGA	22
EX-R-558	TCTGATGCCGCATAGTTAAGC	21
F-558	AATAAGGGCGACACGGAAAT	20
R-558	TGGATCTCAACAGCGGTAAG	20
Ta	ACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCG	38
Tb	GACGGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGT	43
558-5-5N	CCGTCGCCGC	10
558-5-5P	AAGAGGCGGC	10
558-5-4N	CCGTCGCTGC	10
558-5-4P	AAGAGGCAGC	10
558-5-3N	CCGTCGTCAC	10
558-5-3P	AAGAGGTGAC	10
558-5-2N	CCGTCGTTAC	10
558-5-2P	AAGAGGTAAC	10
558-6-6N	CCGTCGCGCCG	11
558-6-6P	AAGAGCGGCGC	11
558-7-7N	CCGTCCGCGCGC	12
558-7-7P	AAGAGCGCGGCG	12
605-5-5N	TAGTTGCCGC	10
605-5-5P	CGCTAGCGGC	10
FIP	TCGTTCCATTTGTACATTGGACGTACAATAAGTACGACTCTAGC	45
BIP	CTATGCGTACTATTTTTGAAGCTGGCACTGTTAGCACCACCGAAGT CA	48

Note: a) **EX-F-558** and **EX-R-558** are the primers to prepare the target used in target cyclization research; b) **F-558** and **R-558** are the primers to detect the target cyclization yield by qPCR; c) **Ta** and **Tb** form the short duplex to mimic the target in Figure 1A; d) **558-5-5N** to **558-7-7P** are the sequences of dynamic adapters in target cyclization research. The sequence **N** is un-phosphorylated strand and the sequence **P** is phosphorylated strand. The bold parts are the complimentary parts of the adapters; e) **605-5-5N** and **605-5-5P** are the sequences of the adapter used in the detection of *V. parahaemolyticus*; f) **FIP** and **BIP** are the primers for simplified LAMP; g) The numbers of 558 and 605 indicate the length of the DNA target fragments.

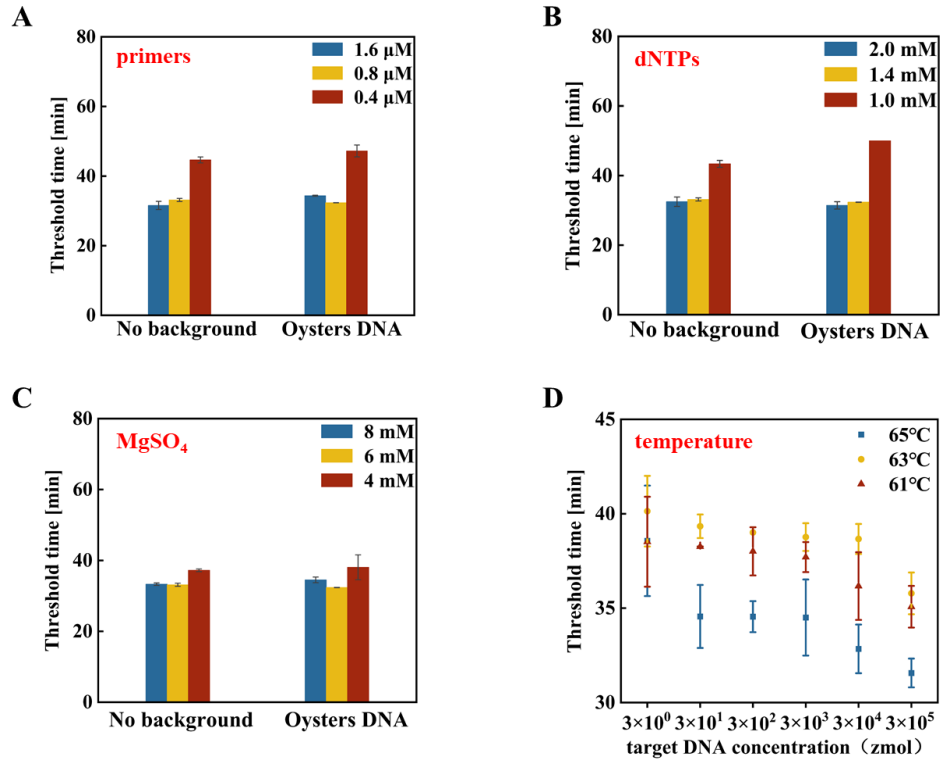


Figure S1. Optimization of reaction conditions for tRCA-lamp. (A) Concentration of primer.

[target DNA] = 3 amol, [MgSO₄] = 8 mmol/L, [dNTP] = 1.4 mmol/L and 3.2 U Bst2.0 DNA polymerase at 65°C for 1 h. **(B)** Concentration of dNTP. [target DNA] = 3 amol, [primers] = 0.8 μ mol/L, [MgSO₄] = 8 mmol/L and 3.2 U Bst2.0 DNA polymerase at 65°C for 1 h. **(C)** Concentration of MgSO₄. [target DNA] = 3 amol, [primers] = 0.8 μ mol/L, [dNTP] = 1.4 mmol/L and 3.2 U Bst2.0 DNA polymerase at 65°C for 1 h. **(D)** Reaction temperature. [target DNA] = 3 amol, [primers] = 0.8 μ mol/L, [MgSO₄] = 8 mmol/L, [dNTP] = 1.4 mmol/L and 3.2 U Bst2.0 DNA polymerase for 1 h.

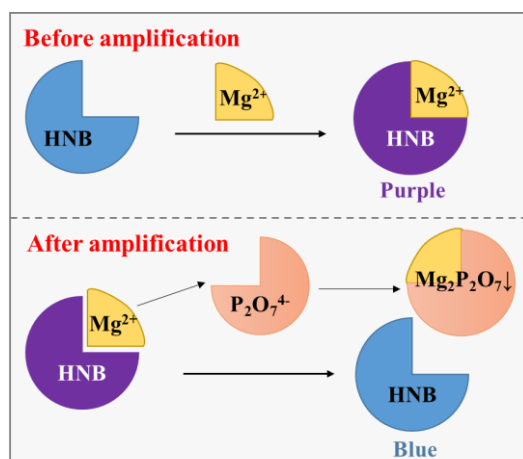


Figure S2. Principle of the colour changes of HNB before and after the amplification. Before the amplification, HNB is purple due to its binding with Mg²⁺. With the proceeding of amplification, Mg²⁺ forms sediment gradually with the pyrophosphates so that HNB becomes blue, its original colour.

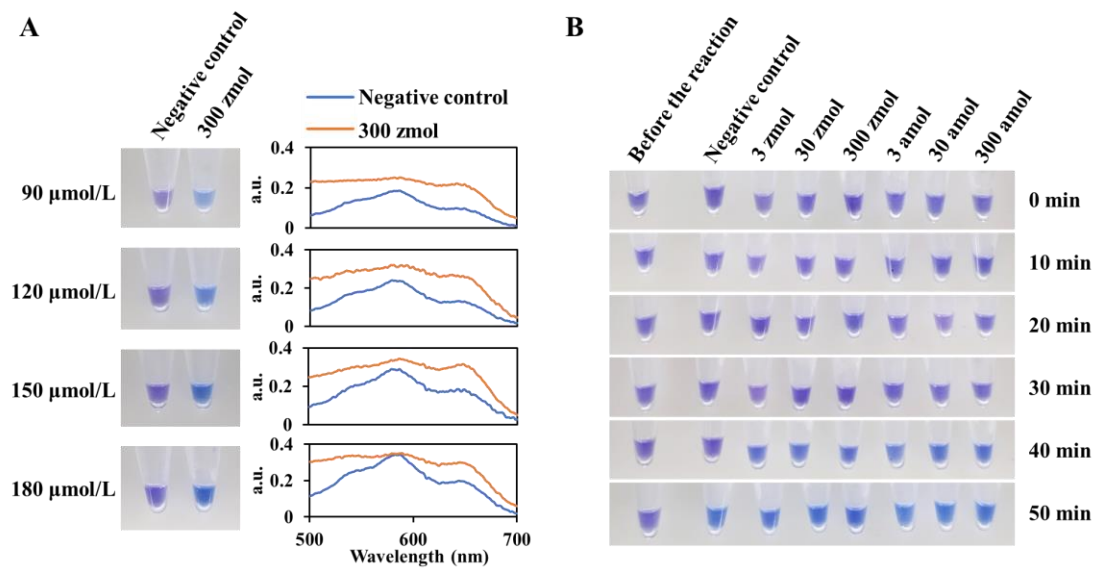


Figure S3. Optimization of the visual detection by HNB. (A) Colour changes under various concentrations of HNB. The corresponding spectra are also shown. Amplification time is 40 min. (B) Colour changes under different amplification time. [HNB]=120 $\mu\text{mol/L}$.

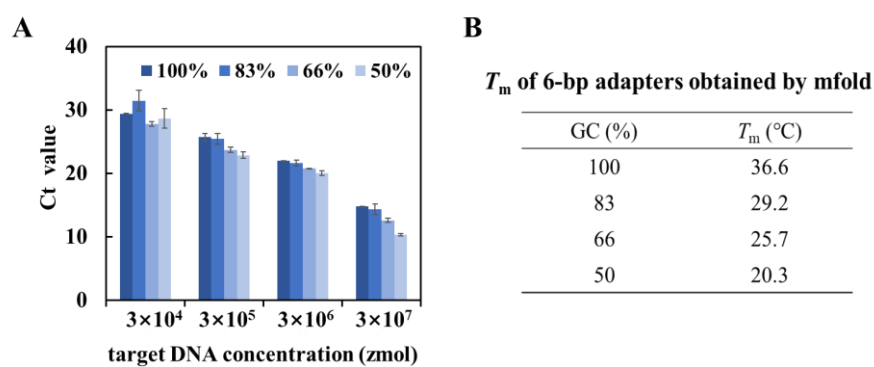


Figure S4. Target cyclization by 6-bp adapters with GC contents of duplex part from 50% to 100%. (A) Ligation reactions: [adapter] = 10 nmol/L, 2.5 U T4 DNA Ligase at 25°C for 2 h; qPCR reactions: [primers] = 0.4 μ mol/L, 1 μ L ligation products, the Ct values were the average of three individual experiments. **(B)** *T_m* values of adapters simulated by Mfold under the conditions of 10 mmol/L Na⁺ and 10 mmol/L Mg²⁺.

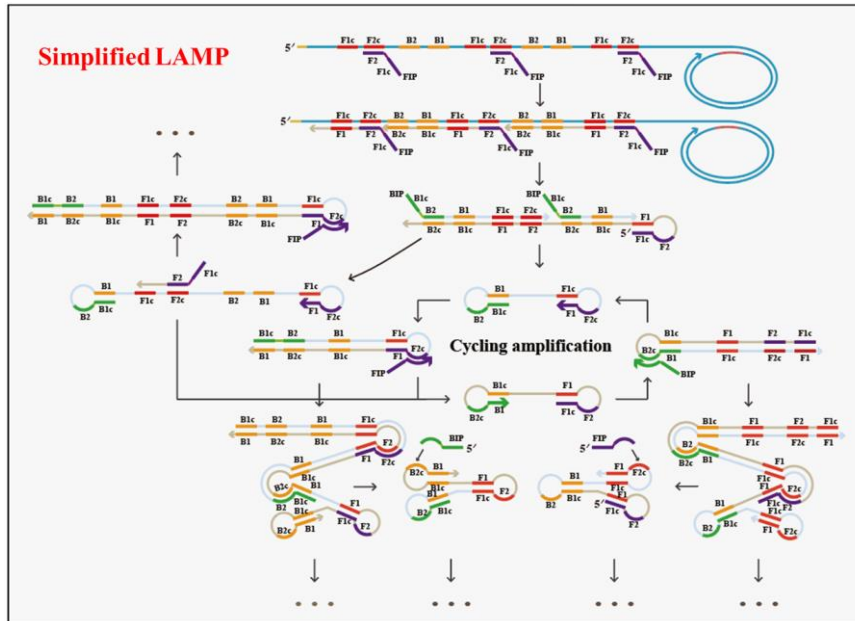


Figure S5. Detailed principle of simplified LAMP.