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Supporting Information

A novel method to control carryover contamination in isothermal nucleic acid amplification

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Materials and methods:

Materials

All primers (**Supplementary Table S1**) were designed by NUPACK software (http://www.nupack.org/), synthesized by Sangon Biotech (Shanghai, China), and purified by HPLC. The *Bst* 2.0 WarmStart^{TM} DNA polymerase and ThermoPol buffer were purchased from New England Biolabs (Beijing, China). The *Gsu* I DNA restriction enzyme was purchased from Thermo Scientific (Waltham, Massachusetls, USA). 20×Eva Green was purchased from Bridgen (Beijing, China). dNTPs were purchased from TIANGEN BIOTECH (Beijing, China) and dUTP (100 mM) was purchased from Sangon Biotech (Shanghai, China). 2000 bp DNA Marker and the chemicals used to prepare electrophoresis were purchased from Dalian Takara Company (China).

The model DNA "contaminants" were produced by normal LAMP reaction using PE-primers. The model DNA "contaminants" were serial 10-fold diluted from10^{1} to 10^{12}-fold.

METHODS

The normal LAMP reaction was performed in a total 10 μL mixture containing 0.2 μM each F3 and B3, 1.6 μM each FIP and BIP, 1.6 mM dNTPs, 0.25×Eva Green, 1.6 U *Bst* 2.0 WarmStart^{TM} DNA polymerase, and 1×ThermoPol buffer (20 mM Tris-HCl, 10 mM KCl, 10 mM $(NH4)_2SO_4$, 2 mM $MgSO_4$, 0.1% Triton X-100, pH 8.8). The LAMP reaction was initiated by 1 μL targets and incubated at 65℃. The

LAMP reaction was performed in a CFX96 Real-Time PCR detection system (Bio-Rad) at 1-min intervals.

The PE-LAMP reaction was performed in 10 μL containing 0.2 μM each F3 and B3, 1.8 μM each PE-FIP and PE-BIP, 1.6 mM dNTPs, 0.25×Eva Green, 1.6 U *Bst* 2.0 WarmStart™ DNA polymerase, 0.5 U *Gsu* I DNA restriction enzymes and 1×FastDigest buffer. The reaction was firstly incubated at 37℃ for 10 min, followed by heating at 70℃ for 10 min, and isothermal cycles were performed at 65℃ for 60 min.

Supplementary Figures

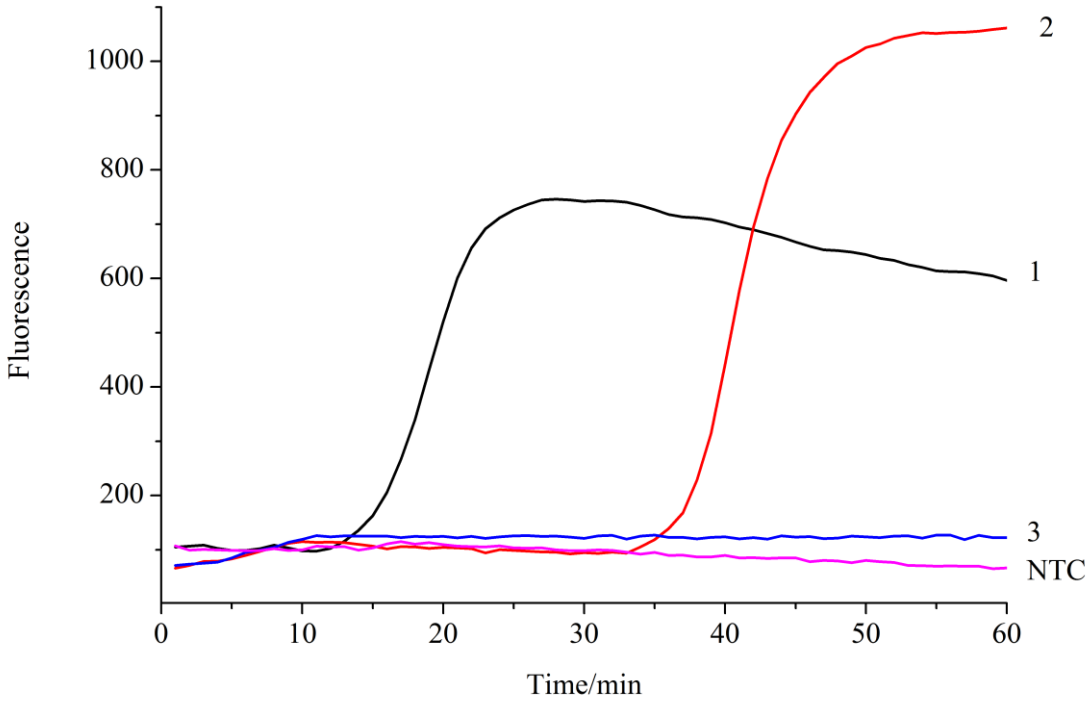

Figure S1. The detection limit of the model "contaminants".

1-3 respectively represented that 10^4, 10^8, 10^{12}-fold diluted model DNA "contaminants" were used as the targets for normal LMAP reaction. The 10 μL reaction system contained 2.0×10^{-7} M F3 and B3, 1.6×10^{-6} M PE-FIP and PE-BIP, 1.6 mM dNTPs, 0.25×Eva Green, 1.6 U *Bst* 2.0 WarmStart™ DNA polymerase and 1×FastDigest Buffer. NTC was no target control.

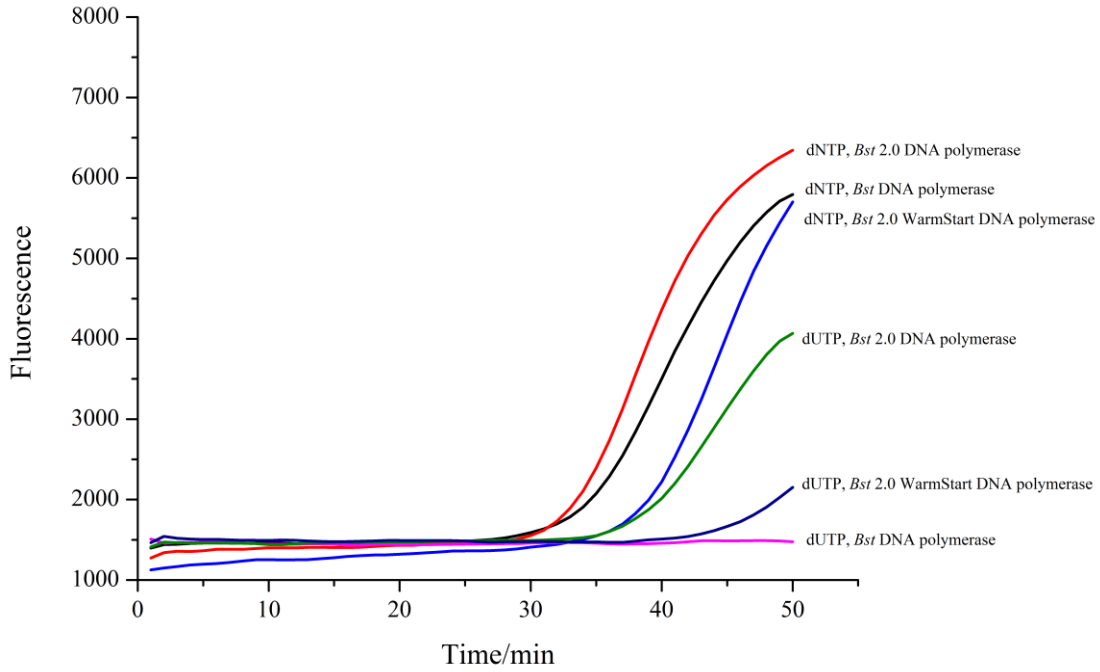

Figure S2. The LAMP reactions were catalyzed by three DNA polymerases with and without 1.6 mM dUTPs.

The reaction mixture contained 0.2 μM each F3 and B3, 1.6 μM each FIP and BIP, 1.6 mM dNTPs, 0.25×Eva Green, 1.6 U *Bst*, *Bst* 2.0 or *Bst* 2.0 WarmStart™ DNA polymerase, and 1×ThermoPol buffer. The LAMP reaction was initiated by 1 μL culture fluid of *V. Parahemolyticus* diluted 100-fold at 65℃ for 60 min.

Supplementary Table

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

Name	Sequence (from 5′ to 3′)
V.	GCGCAAGGTTACAACATCACGTTGTTTGATACTCACGCCTTGTTCGAGACGCTAA
Parahemolyticus	CTTCTGCGCCCGAAGAGCACGGTTTCGTGAACGCGAGCGATCCTTGTTTGGACAT
（[a]M36437.1	CAACCGCTCATCGTCTGTCGATTACATGTACACCCACGCATTGCG**CTCTGAGTGT**
[b]1093-1300)	**GCAGCGTC**TGGTGCTGAGAAGTTTGTGTTCTGGAATGTCACGC **(208 nt)**
F3	GCGCAAGGTTACAACATCAC
B3	GCGTGACATTCCAGAACACA
Normal FIP	CGCGTTCACGAAACCGTGCTGATACTCACGCCTTGTTCGA
Normal BIP	TTGGACATCAACCGCTCATCGT**GACGCTGCACACTCAGAG**
PE-FIP	CGCGTTCACGAAACCGTGCCTGGAGTGATACTCACGCCTTGTTCGA
PE-BIP	TTGGACATCAACCGCTCATCGTCTGGAG**GACGCTGCACACTCAGAG**

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

The dotted sequence of the target was complementary with primer B3. The underlined portion was the same as primer F3. The bold portion of the target and primer BIP were complementary. The boxed portion of the target and primer FIP were the same. The red portions of PE-FIP and PE-BIP were recognition sites for the endonuclease *Gsu* I.