Competitive Annealing Mediated Isothermal Amplification of Nucleic Acids

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■ Experiment section

Reagents and materials. A 200-bp DNA fragment of the MERS-orf1a genome (GenBank: KX108946.1) with a T7 promoter sequence and two extra restriction enzyme sites (we named as Mor1a gene, fig. S1) was cloned into a pUC57-simple vector was provided by BGI Biological Engineering Technology and Services Co. Ltd (Shenzhen, China). The artificial RNA of Mor1a gene was prepared by using RiboMax™ Large Scale RNA Production Systems following the recommended instruction. The copy number of the plasmid was determined using the DNA copy number calculator which is available at http://cels.uri.edu/gsc/cndna.html.

Primers and probes were provided by BGI Biological Engineering Technology and Services Co. Ltd (Shenzhen, China). The *Bst* 2.0 WarmStart DNA polymerase, AMV Reverse Transcriptase and 10× ThermoPol reaction buffer (including 200 mM Tris-HCl, 100 mM KCl, 100 mM $(NH_4)_2SO_4$, 20 mM $MgSO_4$, and 1% Triton X-100) were purchased from New England BioLabs (Ipswich, MA, USA). Deoxynucleotide triphosphates (dNTPs) and RiboMax™ Large Scale RNA Production Systems were from Promega (Madison, WI, USA). Eva Green and GelRed was obtained from Biotium (Hayward, CA, USA). Restricted enzymes were purchased from Thermo Fisher Scientific (Shanghai, China). DNA purification kit and Nuclease-free water were purchased from Tiangen Biotech Co., Ltd. (Beijing, China). Other reagents, unless specified, were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Instruments. The real-time fluorescence detection of nucleic acid was performed using StepOne™ Real-Time PCR detection system (The Applied Biosystems) at 1-min intervals. Gels were imaged by Tanon 4100 Gel Image Analysis System (Tanon Science & Technology Co., Ltd.). Nucleic acid concentrations were measured by Quawell UV-Vis Spectrophotometer Q5000 (Quawell Technology, Inc.).

Reaction mixture for CAMP and modified CAMP reaction. The assays were carried out in 25 μl reaction mixtures containing the following components: 8 U *Bst* 2.0 WarmStart DNA polymerase, 2.5 μl 10× ThermoPol reaction buffer, 1 M betaine, 6 mM $MgSO_4$, 1.4 mM of each dNTP, and an appropriate amount of nucleic acid

template. The amount of primers needed for CAMP reaction was 1.6 μM for NF and NR, with the addition of outer primers, loop primers and the combination primers system termed as O-CAMP, L-CAMP, and OL-CAMP, respectively. For O-CAMP, another for F2 (0.2 μM) and R2 (0.2 μM) were added; For L-CAMP, another NLF (0.8 μM) and NLR (0.8 μM) were added; and for OL-CAMP, another F2 (0.2 μM) and R2 (0.2 μM), 0.8 μM NLF (0.8 μM) and NLR (0.8 μM) were added. As to reverse transcription CAMP (RT-CAMP), just another 5 U AMV Reverse Transcriptase were added into reaction mixture. The reactions were incubated at 63°C and heating at 80°C for 10 min to terminate the reaction.

Reaction mixture for LAMP reaction. The assays were carried out in 25 μl reaction mixtures containing the following components: 8 U *Bst* 2.0 WarmStart DNA polymerase, 2.5 μl 10× ThermoPol reaction buffer, 1 M betaine, 6 mM $MgSO_4$, 1.4 mM of each dNTP, and an appropriate amount of nucleic acid template. The amount of primers needed for CAMP reaction was 1.6 μM for FIP and BIP, 0.2 μM for F3 and B3. As to reverse transcription LAMP (RT-LAMP), just another 5 U AMV Reverse Transcriptase were added into reaction mixture.

Gel electrophoresis of CAMP, O-CAMP, L-CAMP and OL-CAMP products. The amplification products were detected by fluorescence monitoring using a real-time PCR. After the amplification, part of the products was digested by *Xho*I, *Hind*III, and both respectively, then analyzed by 1% agarose gel in 1× TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0). The gels were stained with GelRed and visualized by UV transillumination using 4100 Gel Image Analysis System.

78 Table S1. Primer sequences of NF, NR, F2, R2, NLF and NLR targeting the Mor1a gene.

Primer	5′→3′
Mor1a-NF	CAGGCAACAAGAAAAGTGTC-ATTTGTGACTATGGCCTTCG
Mor1a-NR	GACACTTTTCTTGTTGCCTG-TGGGAGTAGTGGGCTCGTAGAC
Mor1a- F2	ATTCCCACACAGTTGTTC
Mor1a-R2	TGCAATCAGCGCTGACGAA
Mor1a-NLF	CGAGAAACACAAACACACC
Mor1a-NLR	TGGGAGTAGTGGGCTCGTAGAC

79 Figure S1. Localization of primers and restriction enzyme cutting sites in the nucleotide sequence of
80 modified MERS-orf1a (part).
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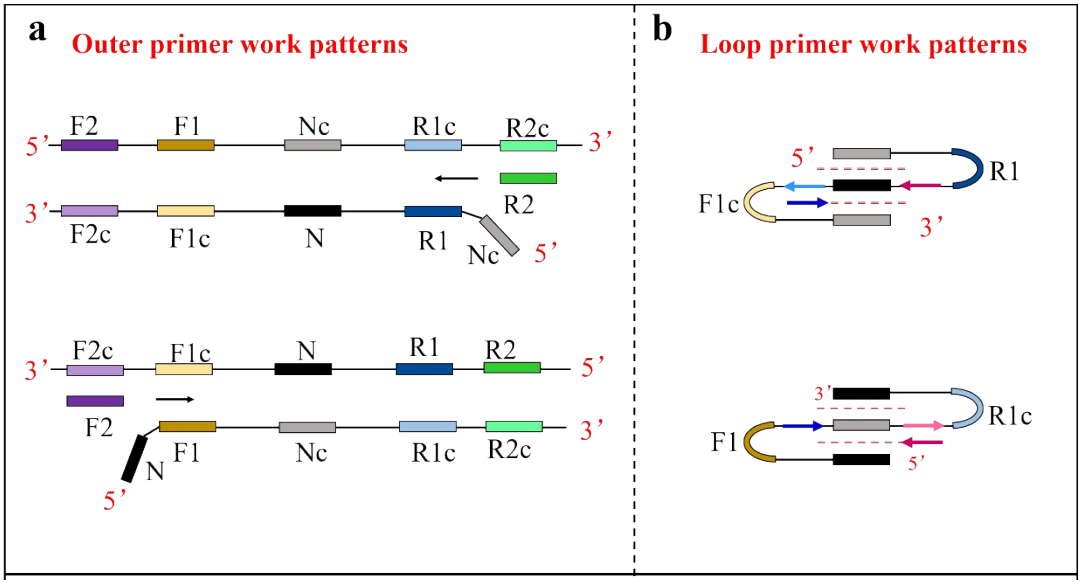

82 Figure S2. (a) Representative scheme for work pattern of the outer primers. (b) Representative scheme
83 for work pattern of the loop primers.
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Table S2. (a) Primer sequences of CAMP and LAMP targeting the H1N1 gene (GenBank: GQ290690.1).

	Primer	5′→3′
CAMP	H1N1-NF	CTCACTAGCATCAGGATAACAGGGTTGAATGCCCCTAATTACC
	H1N1-NR	CCTGTTATCCTGATGCTAGTGAGCGATTTGAACCATGCCAATTGTCC
	H1N1-F2	ATGGTCAGGCTTCATACAAG
	H1N1-R2	ATTCTGATTGAAGAACACCC
LAMP	H1N1-FIP	TAAAGCAAGAACCATTAATGCAGGCTTGGAGAAACAACATATTGAGG
	H1N1-BIP	CCATAATGACTGATGGACCAAGTAACTGATTTTACTACCTTTCCCTT
	H1N1-F3	GGCATAATAACAGATACTATCAAGA
	H1N1-B3	AGGAACACTCTTCATAGTGG

(b)Sequence of H1N1 gene (GQ290690.1, partially):

TCCGGCCCAGACAATGGGGCAGTGGCTGTATTGAAGTACAATGGCATAATAACAGATACT

ATCAAGAGTTGGAGAAACAACATATTGAGGACACAAGAATCTGAATGTGCCTGCATTAAT

GGTTCTTGCTTTACCATAATGACTGATGGACCAAGTAATGGTCAGGCTTCATACAAGATTT

TCAAAATAGAAAAGGGAAAGGTAGTAAAATCAGTTGAGTTGAATGCCCCTAATTACCACT

ATGAAGAGTGTTCCTGTTATCCTGATGCTAGTGAGGTGATGTGTGTATGCAGGGACAATTG

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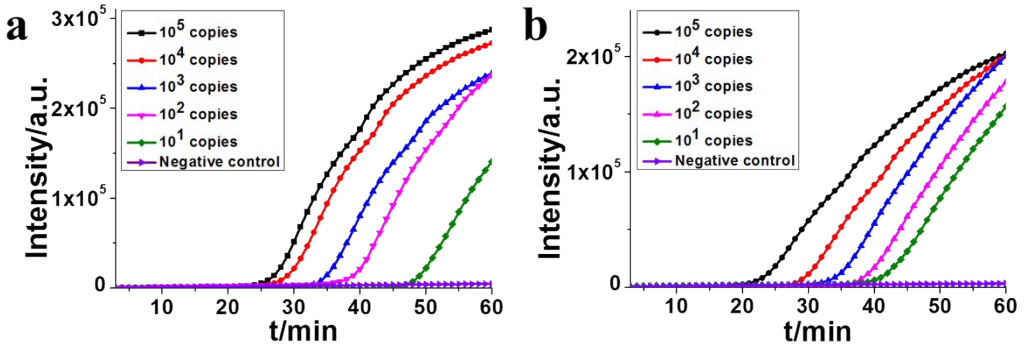

Figure S3. Comparison of the sensitivity of O-CAMP and LAMP through analysis of serially diluted H1N1 DNA. Reactions were performed at 63 °C for 60 min. The DNA concentrations were as follows: 10^5 copies, 10^4 copies, 10^3 copies, 10^2 copies, 10^1 copies and negative control (nuclease-free water). (a): Isothermal amplification plot of CAMP with outer primer. (b): Isothermal amplification plot of LAMP.

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