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Electronic Supplementary Material

Loop-Mediated Isothermal Amplification Combined with Lateral Flow Dipstick for On-Site Diagnosis of African Swine Fever Virus

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Supplementary Materials

Operation Details of PCR and LAMP Assays

The annealing temperatures for each pair of primers were optimized using gradient PCR and listed in Supplementary Table S1. Conventional PCR reaction mixture consisted of 12.5 μL 2 × M5 Pfu PCR MasterMix (Mei5, Beijing, China), 10 pmol of each primer, 1 μL template and double distilled water (ddH_2O) creating a final volume of 25 μL. The PCR parameters included an initial denaturation step for 5 min at 94 ℃ followed by 32 cycles of denaturation at 94 ℃ for 50 s, annealing for 50 s, extension at 72 ℃ for 10 - 30 s depending on the sizes of the products (60 s/kb) and a final extension step at 72 ℃ for 10 min.

nPCR involved two rounds of amplifications. The first round of amplification was carried out with the outer primers, subsequently, the product of the first amplification was used as templates in the second round of amplification. The reaction mixture and parameters of each round of amplification were the same as that of conventional PCR. The products of conventional PCR and nPCR were monitored by electrophoresis in 1% agarose gels.

qPCR assay was conducted with 10 μL 2 × SsoFastTM EvaGreen® Supermix (Bio-Rad, California, USA), 10 pmol of each primer, 1 μL template and ddH_2O making the final volume 20 μL. The parameters were: initial denaturation for 30 s at 95 ℃ followed by 39 cycles of denaturation at 95 ℃ for 5 s,

annealing/extension for 5 s, and a final melting curve at 65-95 ℃ with increment 0.5 ℃/5 s. Data was analyzed by Bio-Rad CFX Maestro 1.1 software (Bio-Rad, California, USA). Plate read was added during the extension and melting curve steps. To establish the standard curves, *pEASY*®-T1 vectors carrying the *B646L* gene of China/2018/AnhuiXCGQ strain were extracted using TIANprep Mini Plasmid Kit (TIANGEN, Beijing, China). Subsequently, the plasmids were 10-fold serial diluted from 1.3×10^8 to 1.3×10^3 copies/μL, and the diluted plasmids were used as standard samples in the qPCR standard curves. Slope and intercept of the standard curve, amplification efficiency (E) and corresponding correlation coefficient (R^2) were generated using Bio-Rad CFX Maestro 1.1 software.

The LAMP reaction was conducted under gradient temperatures (50℃−68 ℃) to determine the optimal reaction temperature. The concentration of $MgSO_4$ (4−10 mmol/L) and dosage of Bst 2.0 WarmStart DNA Polymerase (New England Biolabs, Massachusetts, USA) (1−8 U) were also optimized. The optimal reaction mixture contained 2.5 μL 10 × Isothermal Amplification Buffer (contains 2 mmol/L of $MgSO_4$), 2 mmol/L of $MgSO_4$ (4 mmol/L of $MgSO_4$ total), 1.4 mmol/L of each dNTP, 1.6 μmol/L of FIP/BIP, 0.2 μmol/L of F3/B3, 8 U of Bst 2.0 WarmStart DNA Polymerase, 1 μL template and ddH_2O creating a final volume of 25 μL. The mixture was incubated for 40 min. Products of LAMP were monitored by electrophoresis in 2% agarose gel.

The reaction mixture of LAMP-LFD was same as LAMP. After incubation for 40 min, 20 μL of the product and 80 μL of HybriDetect Assay Buffer (Milenia biotec, Gießen, Germany) were mixed in a new tube, subsequently an LFD strip (Milenia biotec, Gießen, Germany) was dipped into this mixture. After 3−5 minutes, test lines and control lines appeared in positive reactions.

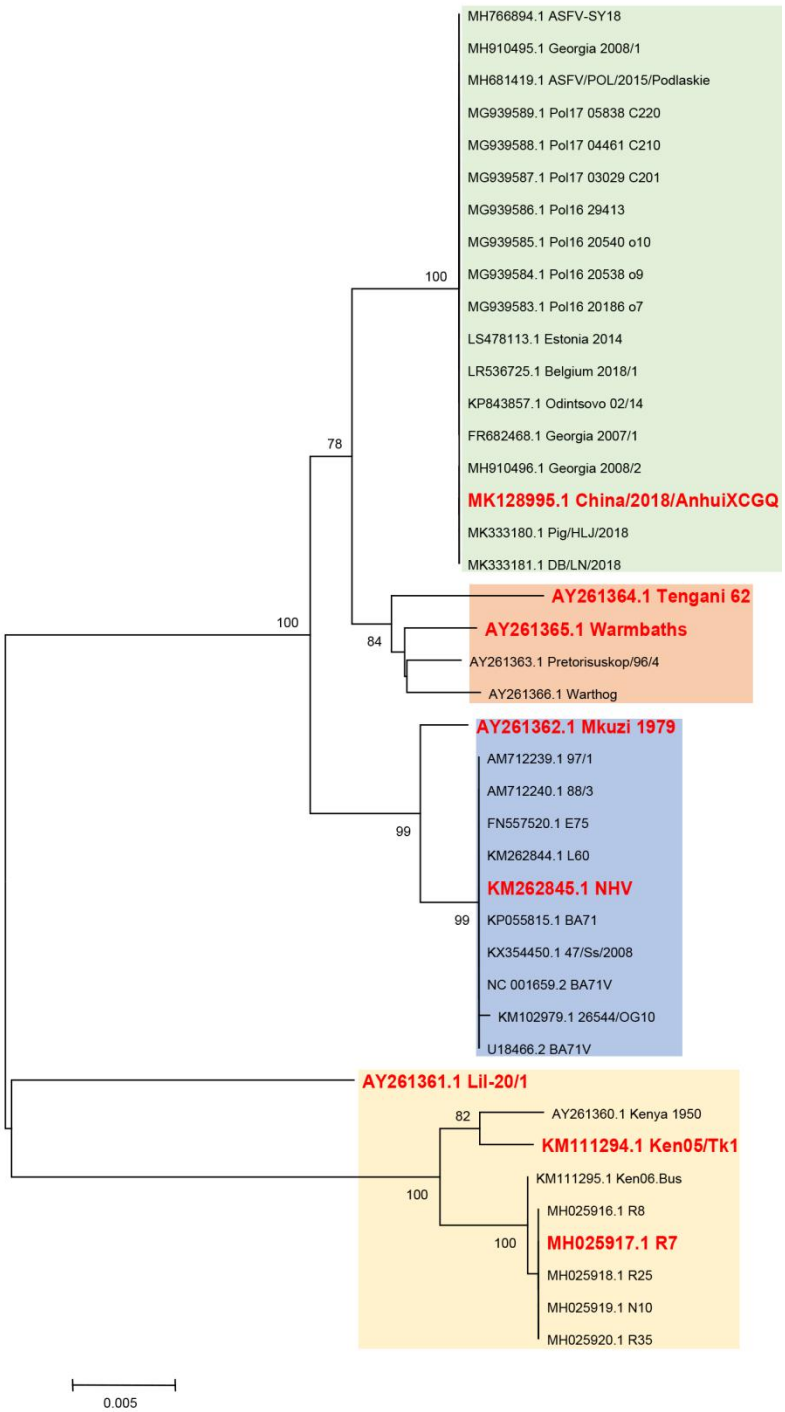

Fig. S1. Phylogenetic analyses based on *B646L* gene of ASFV. 42 ASFV strains were grouped into 4 clades, and the sequences used in this study distributed in all 4 clades. The strains picked up for specificity study are highlighted in red font.

Table S1. Details of PCR and LAMP primers.

Assay	Primer	Primer sequence (5'-3')	Length of production	Reaction or anealing temprature
qPCR	qF[a] qR[a]	GTCCGTGATAGGAGTAATATCT CGTGTAGCCATACCAACC	101 bp	56 ℃
nPCR	nF[b] nR[b]	AGCAAGGTTCACGTTCTC ACTGGATATAAGCACTTGGT	369 bp	52 ℃
LAMP	FIP	AAGACGTAATGTTCATTACAGCT GT-ACTTAATCCAGAGCGCAAG	NA	65 ℃
	BIP	CGTCCGTGATAGGAGTAATATCTT G-TTCACAGCATTTTCCCGAG		
	F3[c]	GTTCTCATTAAACCAAAAGCG		
	B3[c]	GCCATACCAACCCGAAAT		
LAMP-LFD	Bio-FIP[d]	Bio- AAGACGTAATGTTCATTACAGCT GT-ACTTAATCCAGAGCGCAAG	NA	65 ℃
	FITC-BIP[d]	FITC- CGTCCGTGATAGGAGTAATATCTT G-TTCACAGCATTTTCCCGAG		
OIE-PCR	primer 1 primer 2	ATGGATACCGAGGGAATAGC CTTACCGATGAAAATGATAC	278 bp	53 ℃

[a] Primers of qPCR were also used in conventional PCR and nPCR (act as inner primers).

[b] nF and nR were outer primers.

[c] F3 and B3 of LAMP assay were also used in LAMP-LFD assay.

[d] In LAMP-LFD assay, FIP and BIP were individually 5'-modified by biotin (Bio) and fluorescein isothiocyanate (FITC).

NA, not applicable.

Table S2. Strains used in the specificity study.

Specie	Strain	GenBank no.	Specie	Strain	GenBank no.
CSFV	Thiverval	EU490425.1	Delt-CoV	CH/SCMS/2017	NA
RVA	NX	NA	PCV	MiSD-1	KP282147.1
PEDV	CV777	NA	PRV	Bartha	JF797217.1
TGEV	HUA	NA	PPV	N	HM989009.1
PRRSV	SCcd17	MG914067			

NA, not applicable.