Supporting Information

Clustered Regularly Interspaced Short Palindromic Repeats/Cas9-Mediated Lateral Flow Nucleic Acid Assay

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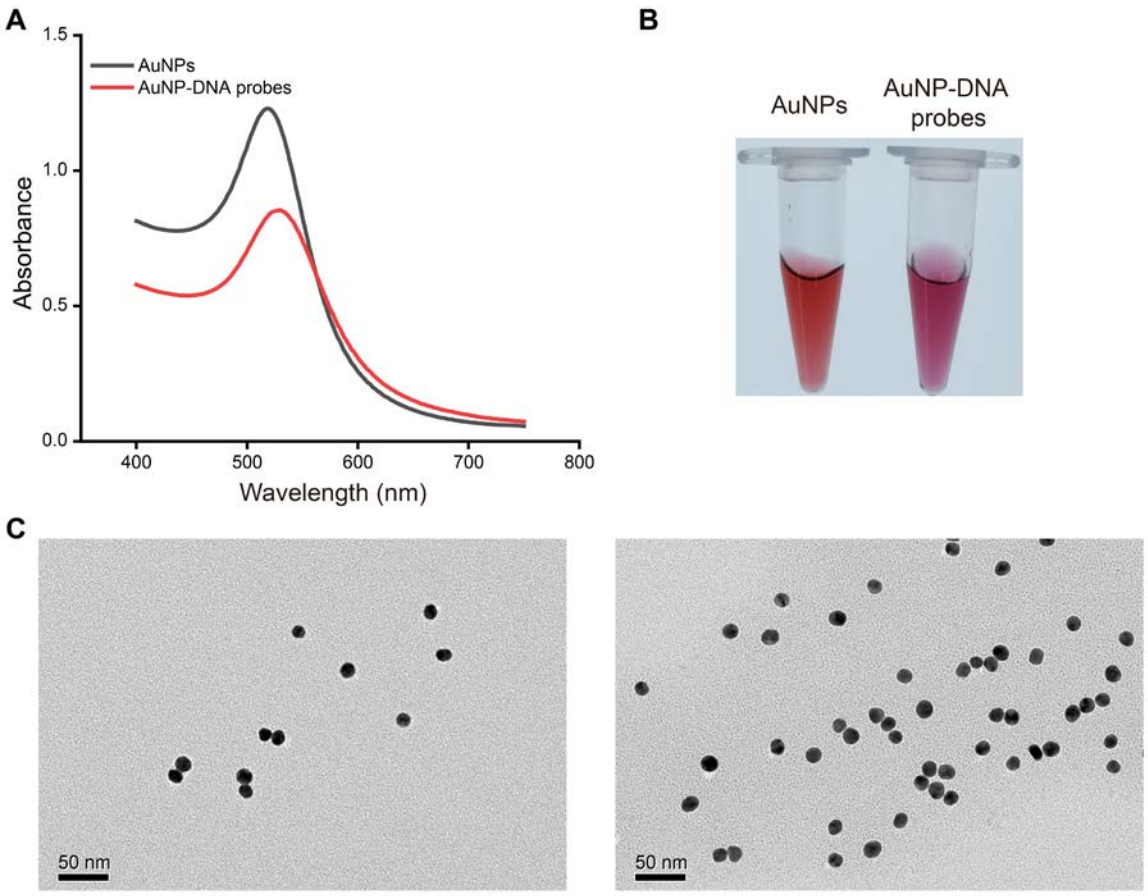

Figure S1. Characterization of AuNPs and AuNP-DNA probes. (A) UV-Vis spectra of synthesized AuNPs and AuNP-DNA probes. (B) Pictures of AuNPs and AuNP-DNA probes solution. (C) Transmission electron microscope images of AuNPs (left panel) and AuNP-DNA probes (right panel).

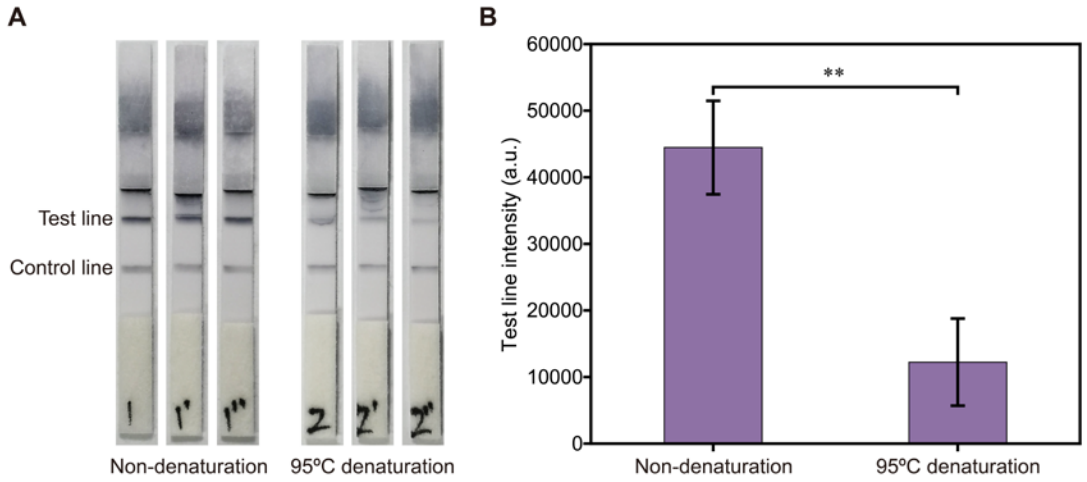

Figure S2. Verification of the use of Cas9 for CASLFA under the denaturing and non-denaturing conditions. (A) Photographs of lateral flow detection under nondenaturing and 95°C denaturing conditions (3 replicates). (B) Quantitation of test line intensities. (n = 3 technical replicates, two-tailed Student's t test; **, $p < 0.01$; error bars represent means ± SEM)

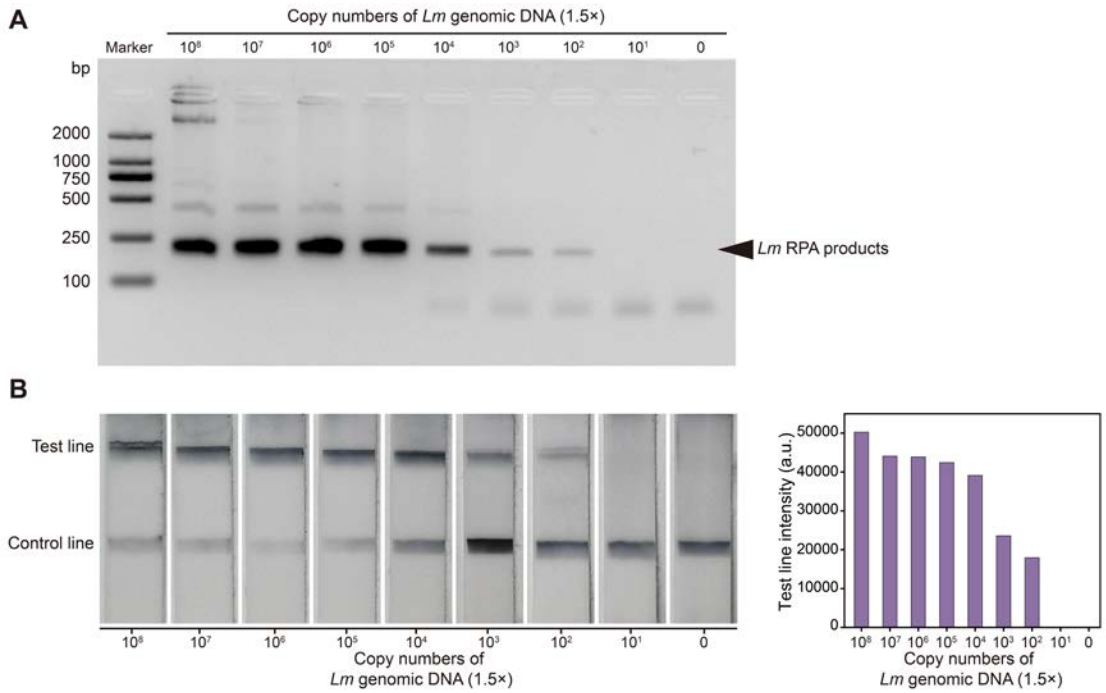

Figure S3. CASLFA detection of *Lm* genomic DNA based on the RPA method. (A) Agarose gel electrophoresis of *Lm* RPA products. (B) Photographs of strips used for *Lm* detection at various genomic DNA concentrations. The column graph shows the quantitation of test line intensities of strips shown on the left. *Lm*, *Listeria monocytogenes*.

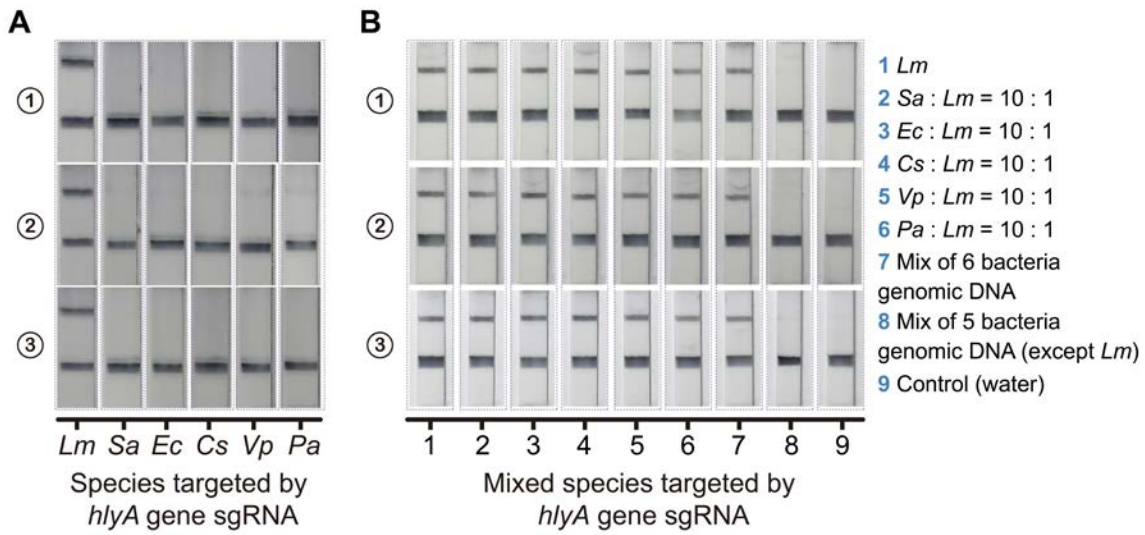

Figure S4. Evaluation of the selectivity and anti-interference ability of CASLFA. (A) CASLFA method can correctly identify *Lm* from other five food-borne pathogens. Each CASLFA detection system contains 3 ng bacterial genomic DNA. (B) CASLFA method could identify *Lm* from the mixed species. 1 ng of *Lm* genomic DNA (1); 10 ng of *Sa* genomic DNA plus 1 ng of *Lm* genomic DNA (2); 10 ng of *Ec* genomic DNA plus 1 ng of *Lm* genomic DNA (3); 10 ng of *Cs* genomic DNA plus 1 ng of *Lm* genomic DNA (4); 10 ng of *Vp* genomic DNA plus 1 ng of *Lm* genomic DNA (5); 10 ng of *Pa* genomic DNA plus 1 ng of *Lm* genomic DNA (6); a mixed genomic DNA sample (each 1 ng) containing *Lm* (7); a mixed genomic DNA sample (each 1 ng) excluding *Lm* (8); and genomic DNA free control (9). *Lm*, *Listeria monocytogenes*; *Sa*, *Staphylococcus aureus*; *Ec*, *Escherichia coli*; *Cs*, *Cronobacter sakazakii*; *Vp*, *Vibrio parahemolyticus*; *Pa*, *Pseudomonas aeruginosa*. Each experiment was repeated three times.

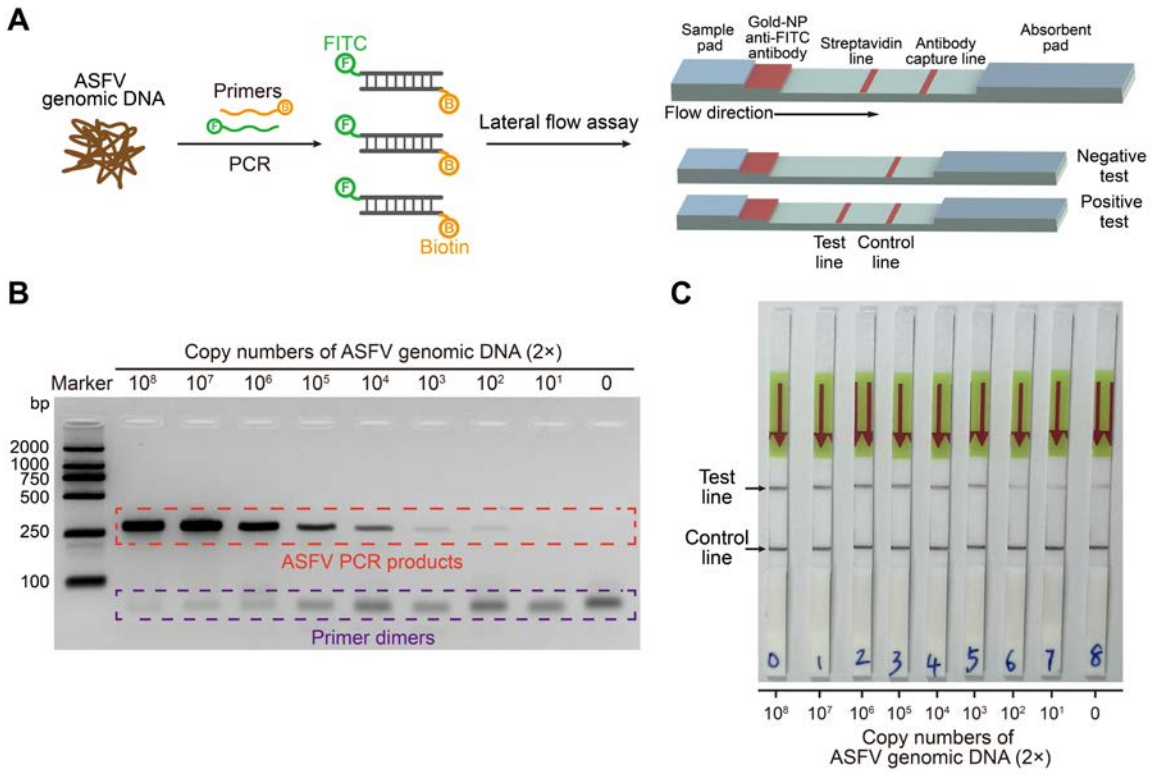

Figure S5. Traditional LFAs based on direct detection of amplification products. (A) Detection principle of commercial test strips (HybriDetect lateral flow strips from Milenia Biotec GmbH). (B) Agarose gel electrophoresis analysis of African swine fever PCR products. (C) Detection of ASFV using HybriDetect lateral flow strips (Milenia Biotec GmbH).

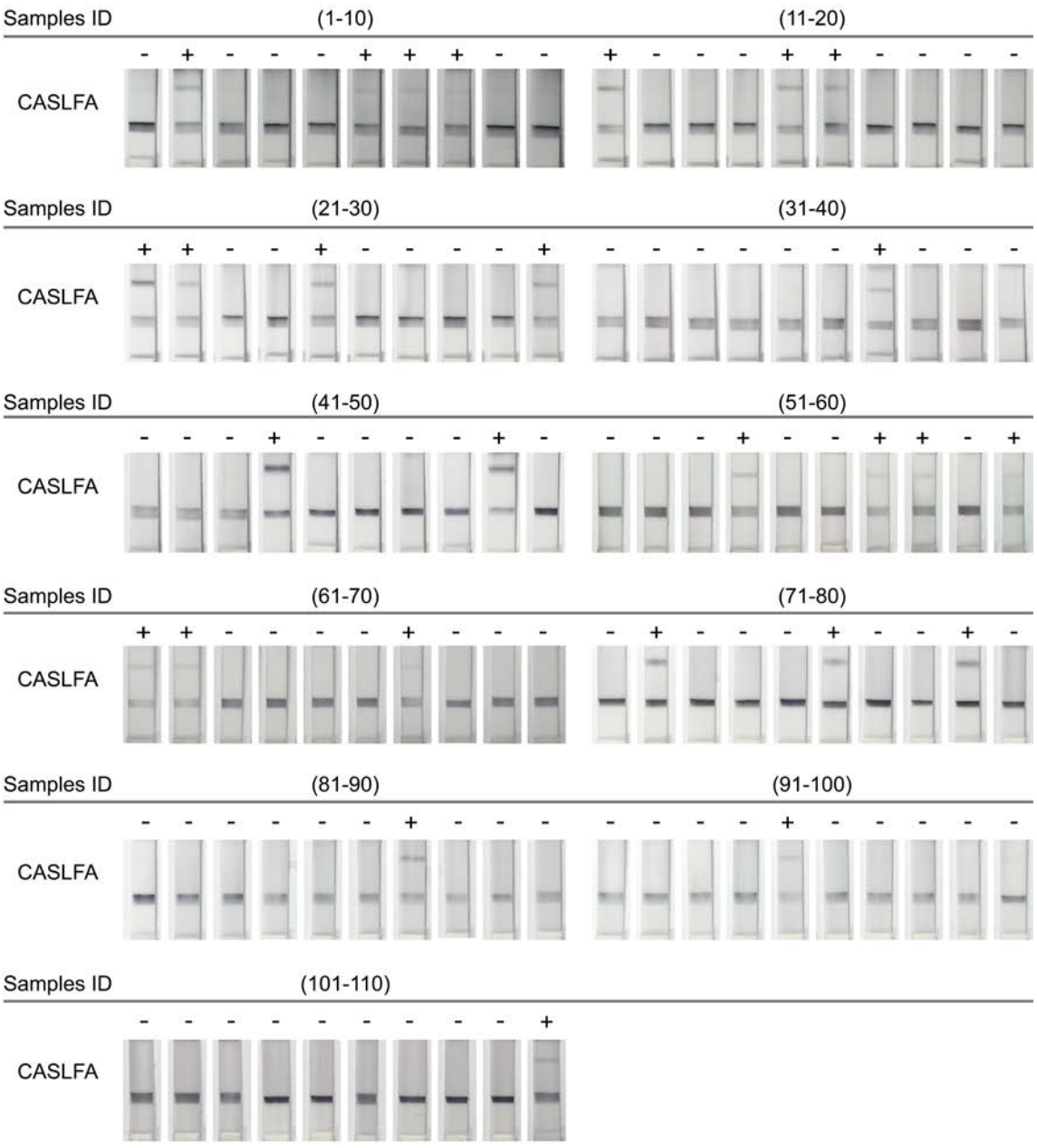

Figure S6. Image of ASFV detection in 110 suspected swine serum samples using CASLFA method. The "+" represents the positive sample confirmed by CASLFA, the "–" represents the negative sample confirmed by CASLFA.

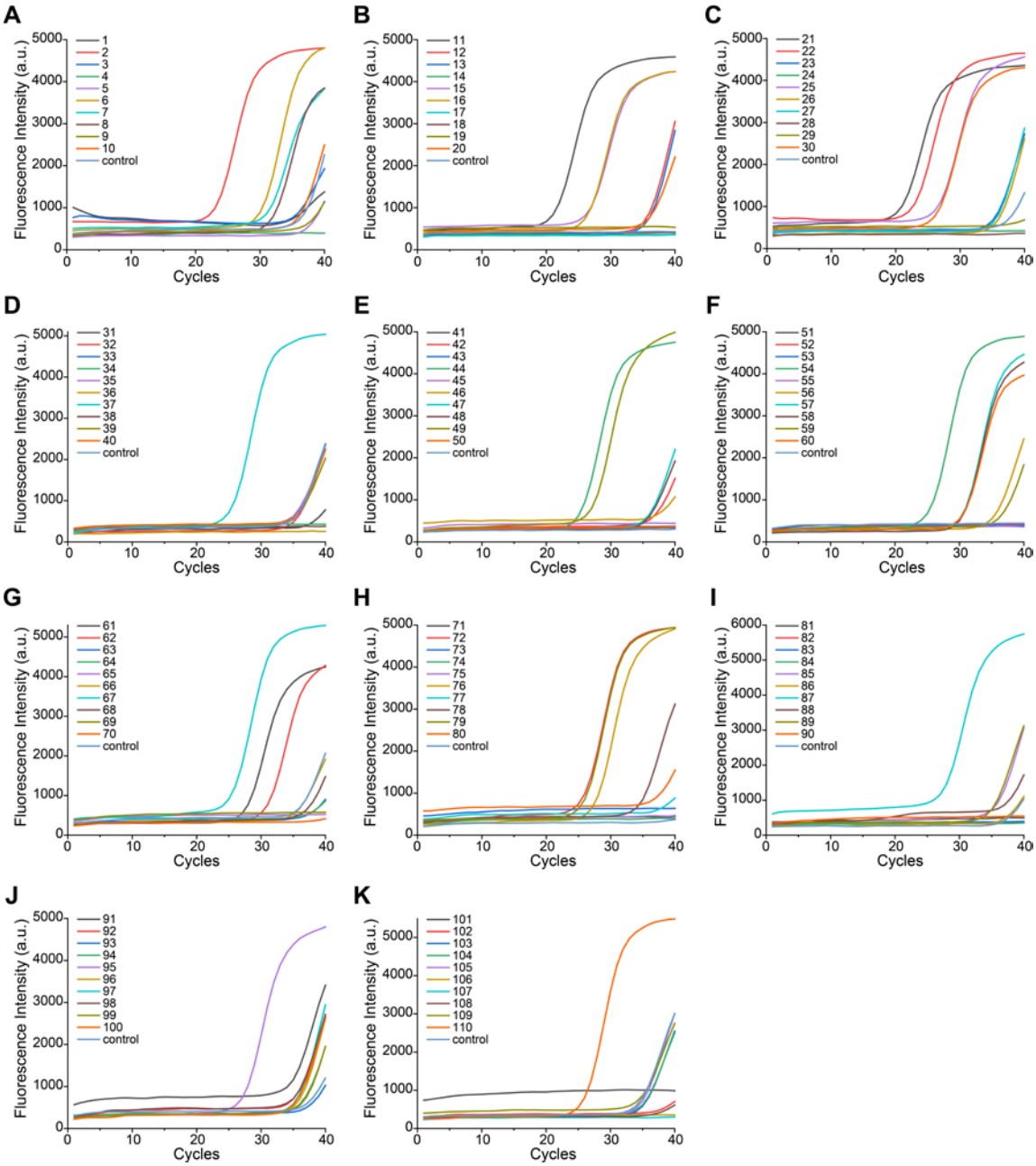

Figure S7. ASFV detection in 110 suspected swine serum samples by real time PCR (RT-PCR) analysis. (A) Samples 1-10 and negative control (water). (B) Samples 11-20 and negative control (water). (C) Samples 21-30 and negative control (water). (D) Samples 31-40 and negative control (water). (E) Samples 41-50 and negative control (water). (F) Samples 51-60 and negative control (water). (G) Samples 61-70 and negative control

(water). (H) Samples 71-80 and negative control (water). (I) Samples 81-90 and negative control (water). (J) Samples 91-100 and negative control (water). (K) Samples 101-110 and negative control (water).

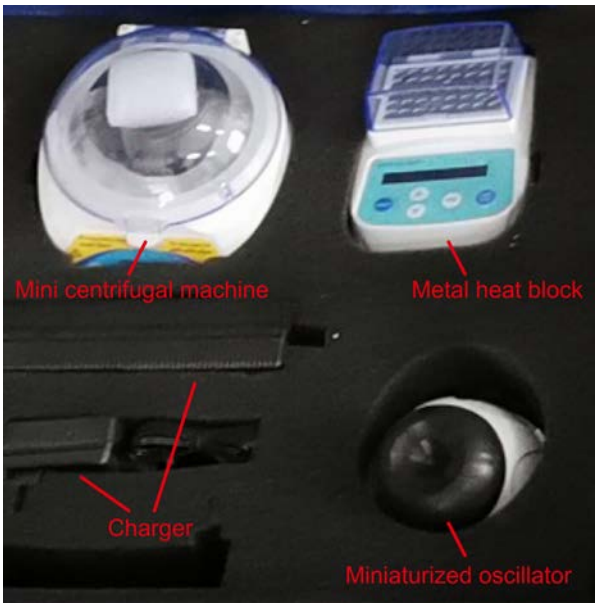

Figure S8. A tool kit used for on-site detection of ASFV. The tool kit contains a mini centrifugal machine, a metal heat block, and a miniaturized oscillator.

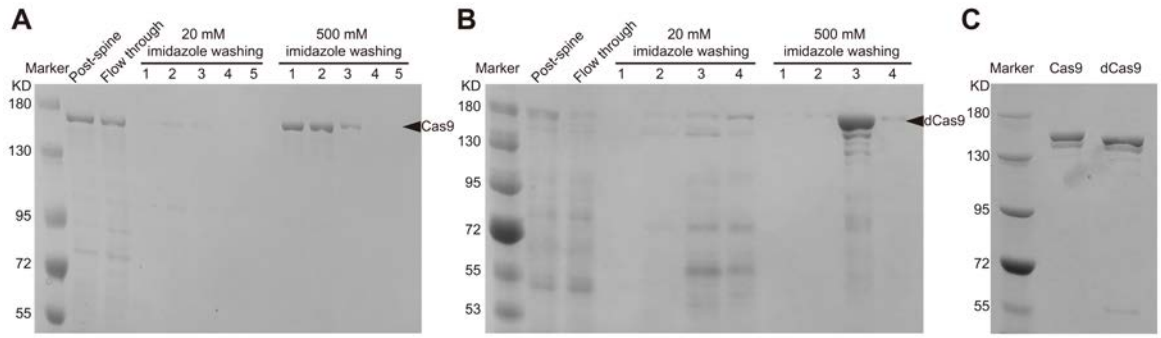

Figure S9. SDS-PAGE analysis of the purified Cas9 and dCas9 proteins. (A) SDS-PAGE analysis of Cas9 purified with a Ni-NTA column. (B) SDS-PAGE analysis of dCas9 purified with a Ni-NTA column. (C) SDS-PAGE analysis of purified Cas9 and dCas9. Black triangles indicate the positions of Cas9 and dCas9 proteins in the electropherograms.

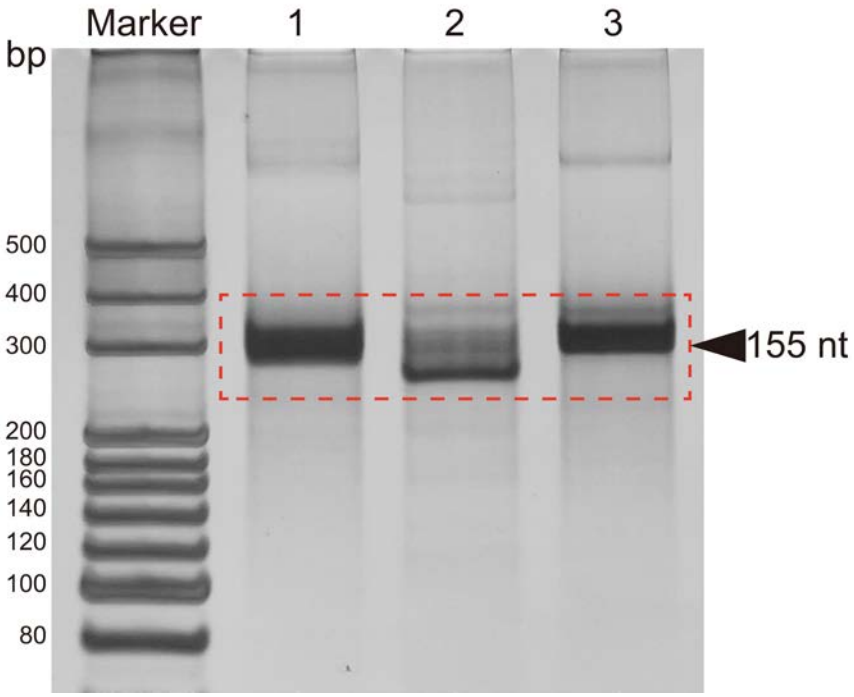

Figure S10. PAGE analysis of the synthesized sgRNAs. Lane 1: sgRNA used to detect *Lm*; Lane 2: sgRNA used to detect transgenic rice; Lane 3: sgRNA used to detect African swine fever virus. The bands in the red dashed box are the synthesized sgRNAs with a length of 155 nt.

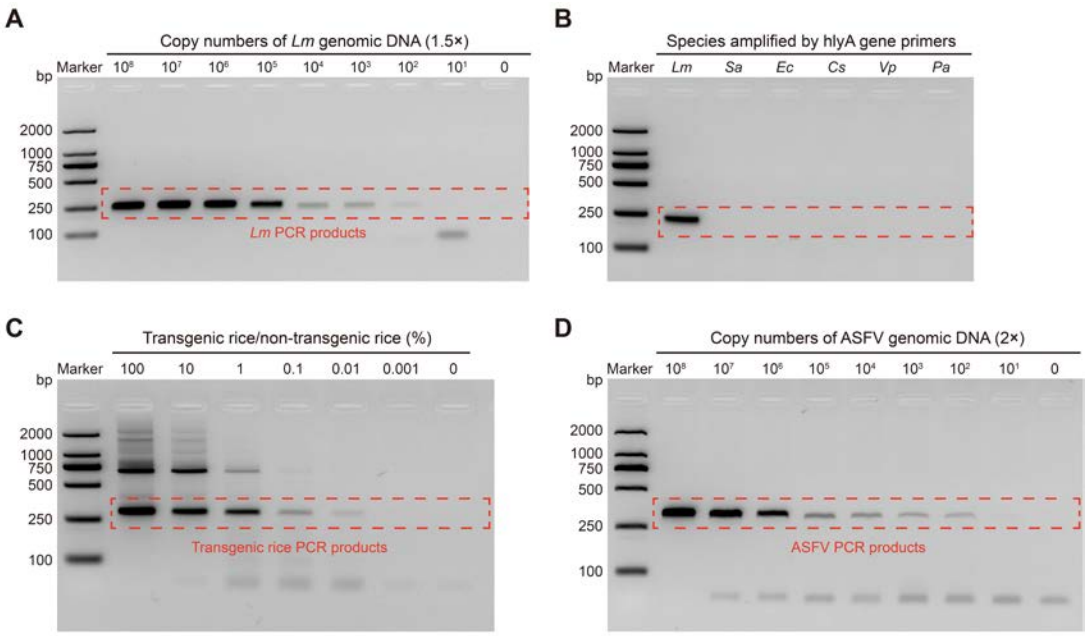

Figure S11. Agarose gel electrophoresis analysis of PCR products. (A) Agarose gel electrophoresis of *Lm* PCR products. (B) Evaluation of the selectivity of amplification using *Lm* primers by agarose gel electrophoresis. *Lm*, *Listeria monocytogenes*; *Sa*, *Staphylococcus aureus*; *Ec*, *Escherichia coli*; *Cs*, *Cronobacter sakazakii*; *Vp*, *Vibrio parahemolyticus*; *Pa*, *Pseudomonas aeruginosa*. (C) Agarose gel electrophoresis of transgenic rice PCR products. (D) Agarose gel electrophoresis of African swine fever PCR products.

Table S1. Related parameters of Figure 4E.

Methods	AUC	Cutoff value	Sensitivity	Specificity	95% CI
CASLFA	1.000	>3223 (Test line intensity)	100%	100%	1.000-1.000

Table S2. Comparison of CASLFA method with current NALFA (nucleic acid lateral flow assay) methods.

Types of reaction principles	Methods	Detection limit	Analytical specificity	Test strip universality (Yes/No)	Temperature requirement	Equipment requirement	Test duration (Sample→Result)	Readouts
Direct detection of amplicons based on primer tags	LAMP (loop-mediated isothermal amplification) -based NALFA[1]	<1000 copies (*P. falciparum* DNA)	Medium (aerosol contamination and nonspecific amplification)	Yes	Isothermal	Few (a hot plate)	<50 min	Visual
	Paper-based RNA extraction, *In Situ* isothermal amplification, and lateral flow detection[2]	500 copies (virus RNA)	Medium (aerosol contamination and nonspecific amplification)	Yes	Isothermal	Few (a heat block)	45 min	Visual
	Multiplexed RPA-based NALFA[3]	368 copies (synthetic *Entamoeba* DNA)	Medium (primer dimers)	Yes	Isothermal	Medium (a heat block and a flatbed scanner)	45 min (not include sample preparation time)	Visual
	Direct detection of tailed amplicons by lateral flow assay[4]	8.67×10^5 copies (synthetic DNA)	Medium (primer dimers)	Yes	Isothermal	Few	~15 min (not include sample preparation time)	Visual
Detection of amplicons based on hybridization with tags modified probes	Fluorescent probe-based lateral flow assay[5]	50 copies-500 copies (plasmid DNA)	High	No	Thermal cycling	High (thermocycler and fluorescent reader)	>89 min	Fluorescence
	Padlock probes and lateral flow nucleic acid biosensors[6]	3 ng (~6×10^5 copies) (genomic DNA)	High	No	Isothermal	Few	75 min	Visual
	One step visual detection of PCR products with gold nanoparticles and a NALF device[7]	1000 copies (synthetic DNA)	High	No	Thermal cycling	High (thermocycler)	90 min	Visual
Indirect detection of amplicons based on Cas12/13 recognition and *trans*-cleavage	SHERLOCK technologies combined with lateral flow assays[8]	<10 copies (synthetic zika virus RNA)	Medium (uncontrollable liquid flow rate)	Yes	Isothermal	Few	>1 h (from crude sample to detection)	Visual
Direct detection of amplicons based on CRISPR/Cas9 recognition	CASLFA (this work)	150 copies (genomic DNA)	High	Yes	Isothermal	Few (a metal heat block)	~38 min (from crude sample to detection)	Visual

Table S3. Nucleic acids sequences used in this work (the bolded sequence is the T7 promoter sequence).

Nucleic acids ID	Sequences (5'-3')
AuNP-DNA probe 1	AAAAAAAAAATTTTTCATTGCTAGAGCAGGAGGATGTTGCCGTCCTCCTT
AuNP-DNA probe 2	AAAAAAAAAATTTTTCATTGCTAGAGCAGGGGTATTCCACTTGCA
Control line probe	biotin-CCTGCTCTAGCAATG
EGFP PCR primer1-F	ATGGTGAGCAAGGGCGAG
EGFP PCR primer1-R	TTACTTGTACAGCTCGTCCATGC
EGFP PCR primer2-F	biotin-TCCAGGAGCGCACCATCTTC
EGFP PCR primer2-F (no biotin)	TCCAGGAGCGCACCATCTTC
EGFP PCR primer2-R	TGCCGTTCTTCTGCTTGTCG
EGFP sgDNA-F (normal)	CCTC**TAATACGACTCACTATA**GGAAGGAGGACGGCAACATCCTGTTTAAG AGCTATGCTGGAAACAGCA
EGFP sgDNA-R (normal)	AAGAAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACT AGCCTTATTTAAACTTGCTATGCTGTTTCCAGCATAGCTC
EGFP sgDNA-F	CCTC**TAATACGACTCACTATA**GGAAGGAGGACGGCAACATCCTGTTTAAG AGCTATGCTGGAAAAAAGAAAAATGCAAGTGGAATACCAAAAAGA
Universal sgDNA-R	AAGAAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACT AGCCTTATTTAAACTTGCTATGCTGTTTTTTTCTTTTTGGTATTCC
Listeria monocytogenes hlyA gene PCR-F	biotin-CCGTAAGTGGGAAATCTG
Listeria monocytogenes hlyA gene PCR-R	TTGTTGTATAGGCAATGGG
Listeria monocytogenes hlyA gene RPA-F	biotin-GTAAGTGGGAAATCTGTCTCAGGTGATGTAG
Listeria monocytogenes hlyA gene RPA-R	ACTCCTGGTGTTTCCCGGTTAAAAGTAGCA
Listeria monocytogenes hlyA gene sgDNA-F	CCTC**TAATACGACTCACTATA**GGGATGAAGTTCAAATCATCGAGTTTAAG AGCTATGCTGGAAAAAAGAAAAATGCAAGTGGAATACCAAAAAGA
Transgenic rice 35S promotor-PCR-F	biotin-CCTCCTCGGATTCCATTG
Transgenic rice 35S promotor-PCR-R	GGATTGTGCGTCATCCCT
Transgenic rice 35S promotor sgDNA-F	CCTC**TAATACGACTCACTATA**GGAAATGCCATCATTGCGATAAGTTTAAGAG CTATGCTGGAAAAAAGAAAAATGCAAGTGGAATACCAAAAAGA
African swine fever p72 gene PCR-F	biotin-ATGGATACCGAGGGAATAGC
African swine fever p72 gene PCR-R	CTTACCGATGAAAATGATAC
African swine fever p72 gene PCR-R1	FITC-CTTACCGATGAAAATGATAC
African swine fever p72 gene RPA-F	biotin-GCCGAAGGGAATGGATACTGAGGGAATAGCAA
African swine fever p72 gene RPA-R	TCCCGAGAACTCTCACAATATCCAAACAGCAG
African swine fever p72 gene sgDNA-F	CCTC**TAATACGACTCACTATA**GGTGATAGTATTTAGGGGTTTGGTTTAAG AGCTATGCTGGAAAAAAGAAAAATGCAAGTGGAATACCAAAAAGA

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