Supplementary Information

Detection of four porcine enteric coronaviruses using CRISPR-Cas12a combined with multiplex reverse transcriptase loop-mediated isothermal amplification assay

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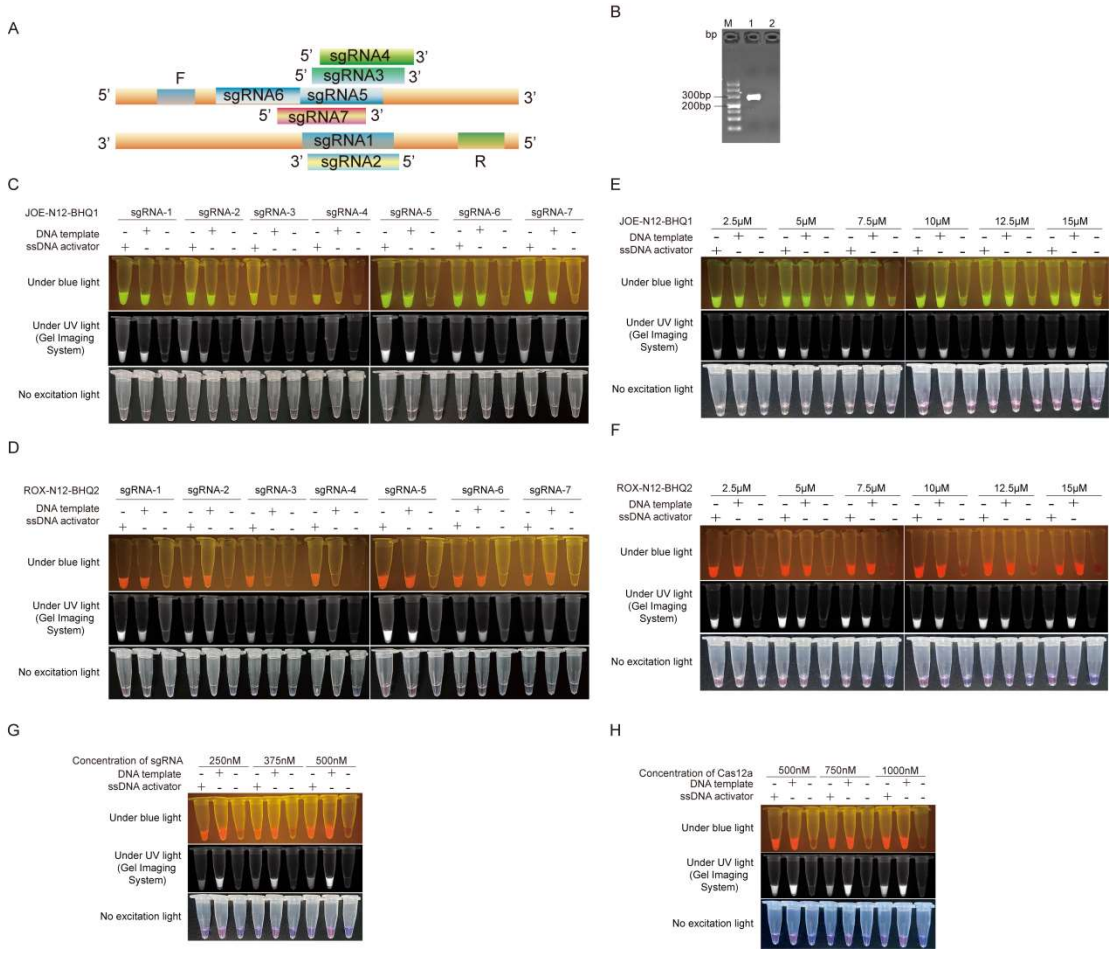


Fig S1. Screening of highly activity sgRNAs for detection of PEDV *ORF3* gene and optimum reaction conditions. (A) Schematic diagram of the PCR primers (F/R) and sgRNAs for detection of *ORF3* gene; **(B)** The *ORF3* amplicon was examined by agarose gel electrophoresis (265 bp); Lane M, DNA Ladder; bp, base pairs; Lane 1, ORF3-plasmid (PUC57-ORF3); Lane 2, non-template control (NTC). **(C)** Colorimetric/fluorescence Cas12a-based assay for detection of PEDV ORF3, JOE-dye ssDNA-FQ reporter was used; **(D)** Colorimetric/fluorescence Cas12a-based assay for detection of PEDV ORF3, ROX-dye ssDNA-FQ reporter was used; **(E)** Evaluation the optimum concentration of JOE-dye ssDNA-FQ reporter for CRISPR/Cas12a-based cleavage assay; **(F)** Evaluation the optimum concentration of ROX-dye ssDNA-FQ reporter for CRISPR/Cas12a-based cleavage assay; **(G)** Evaluation the optimum concentration of sgRNA for CRISPR/Cas12a-based cleavage assay; **(H)** Evaluation the optimum concentration of Cas12a protein for CRISPR/Cas12a-based cleavage assay.

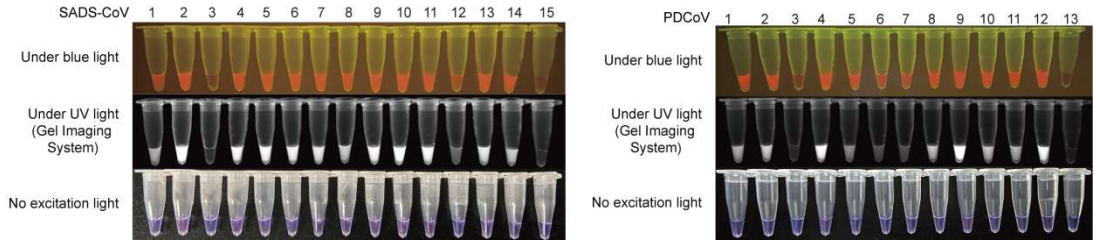
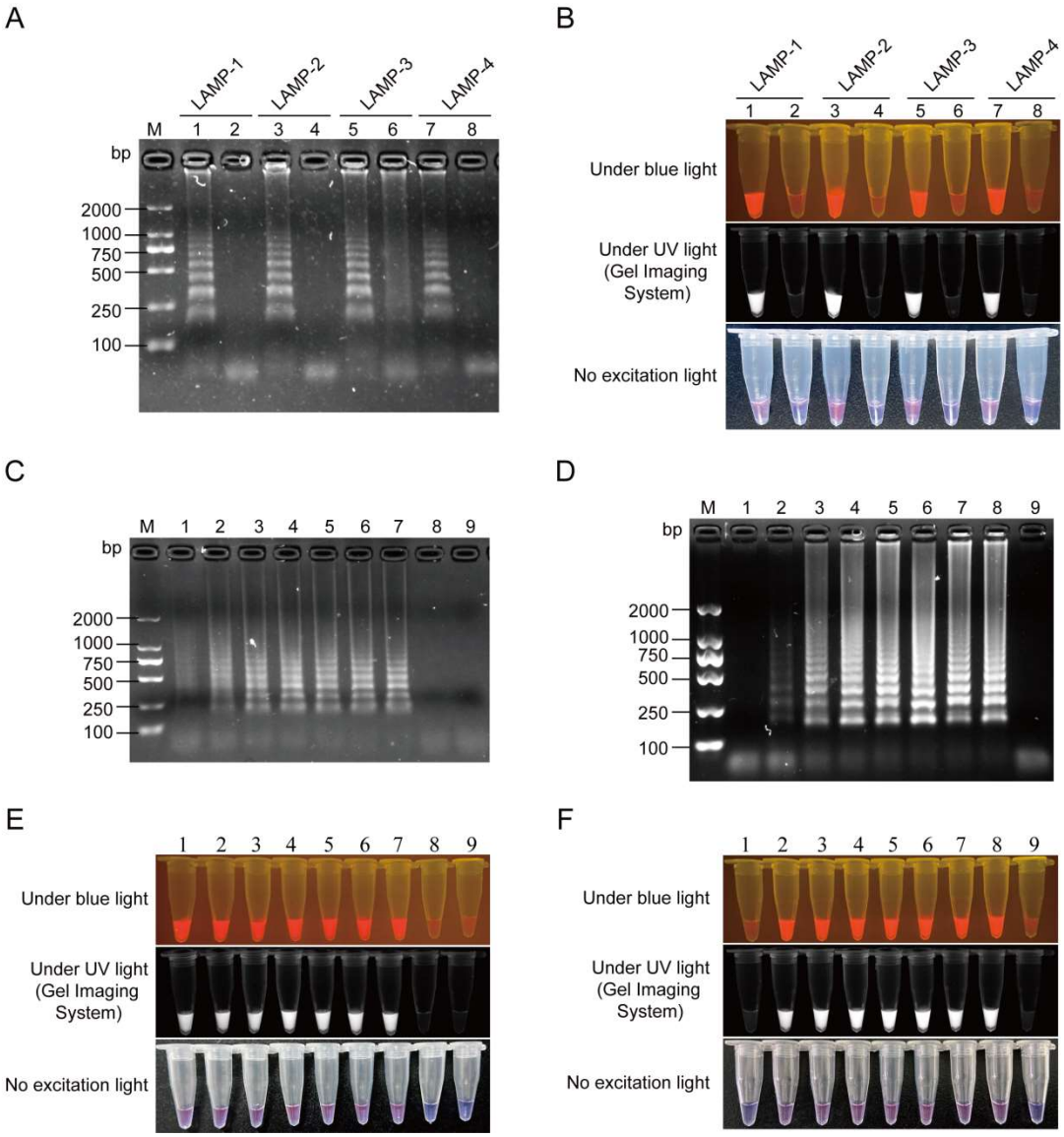


Fig S2. Screening of highly activity sgRNAs for detection of PDCoV *N* and SADS-Co*V N* gene. (A) Colorimetric/fluorescence CRISPR/Cas12a-based assay for detection of SADS-CoV *N* gene; 1-14 represents SADS-CoV *N* gene plasmid used as a template for testing the activity of from sgRNA1 to sgRNA14; 15, non-template control (NTC); **(B)** Colorimetric/fluorescence CRISPR/Cas12a-based assay for detection of PDCoV *N* gene; 1-12 represents PDCoV *N* gene plasmid used as a template for testing the activity of from sgRNA1 to sgRNA12; 13, non-template control (NTC).



FigS3. Optimization of the reaction conditions in LAMP for PEDV detection. (A) The amplification efficiencies of four LAMP primer pairs by electrophoresis analysis. **(B)** Detecting the fluorescence signal of PEDV *ORF3* gene under the blue (470 nM) and UV lights in CRISPR/Cas12a cleavage assay; Lane M: DNA ladder, bp: base pairs. Lanes or tubes: 1, 3, 5, 7: PEDV-ORF3 plasmid DNA; 2, 4, 6, 8: non-template control. **(C, E)** The effect of the reaction temperature on LAMP-Cas12a assay; Lane M: DNA Ladder 2000 Maker; Lanes or tubes: 1: 53 °C; 2: 55 °C; 3: 57.5 °C; 4: 60 °C; 5: 62.5 °C; 6: 65 °C; 7: 67.5 °C; 8: 71 °C; 9: non-template control (NTC; 65 ºC); **(D, F)** The effect of reaction time on LAMP-Cas12a assay; Lane M: DNA Ladder 2000 Maker; Lanes or tubes: 1: 5 min; Lane 2: 15 min; Lane 3: 20 min; Lane 4: 25 min; Lane 5: 30 min; Lane 6: 35 min; Lane 7: 40 min; Lane 8: 45 min; lane 9: non-template control (NTC; 45 min).

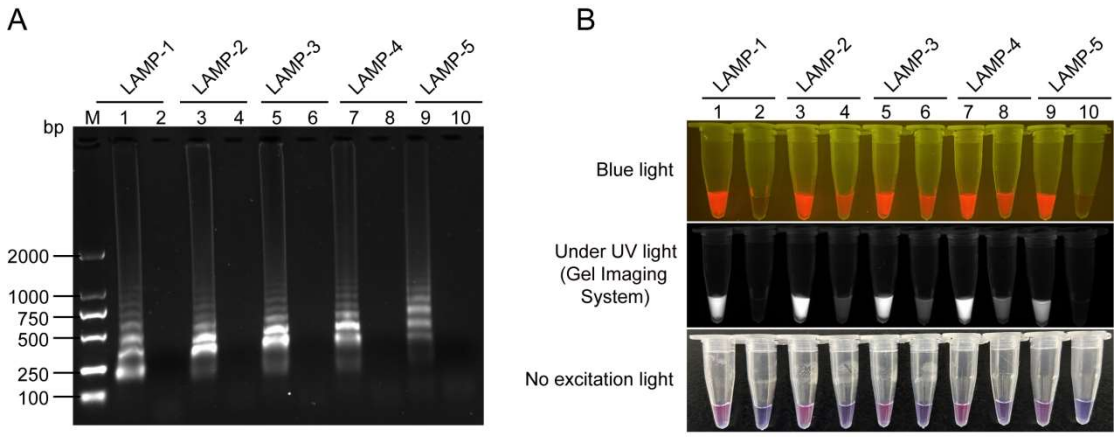


Fig S4. Screening of LAMP primer pairs for detection of TGEV *N* gene. (A) Agarose gel electrophoresis of LAMP products of TGEV *N* gene; **(B)** CRISPR/Cas12a cleavage assay to detection LAMP products of TGEV *N* gene. 1, 3, 5, 7, 9: TGEV-N plasmid DNA; 2, 4, 6, 8, 10: non-template control (NTC).

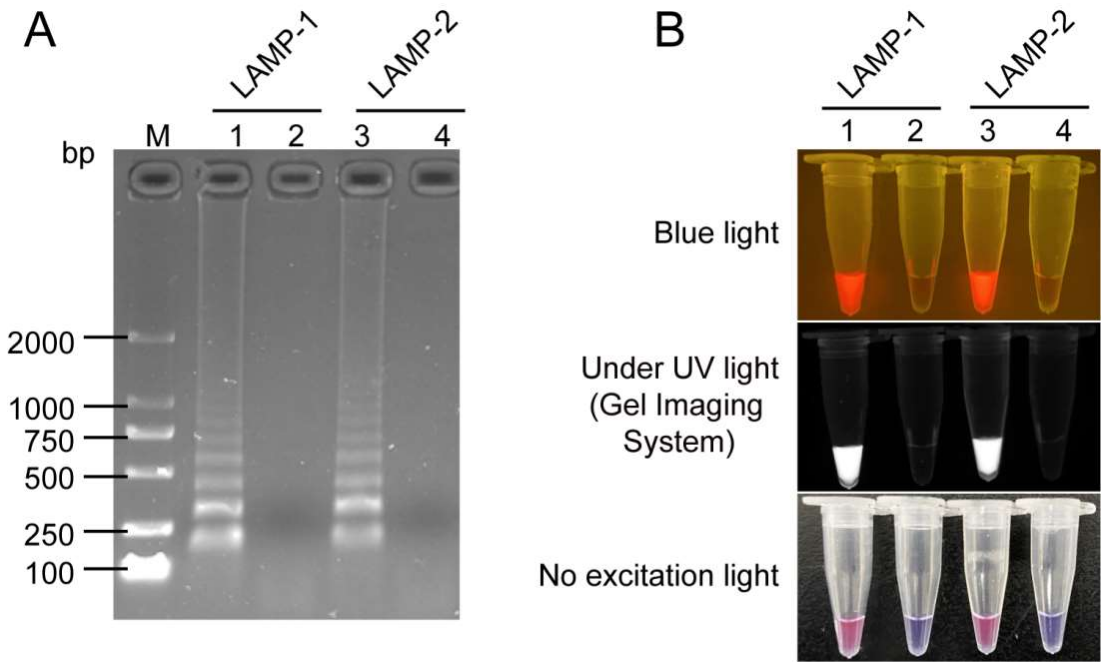


Fig S5. Screening of LAMP primer pairs for detection of PDCoV *N* gene. (A) Agarose gel electrophoresis of LAMP products of PDCoV *N* gene; **(B)** CRISPR/Cas12a cleavage assay to detection LAMP products of PDCoV *N* gene; 1, 3: PDCoV-N plasmid DNA was used as template; 2, 4: non-template control (NTC).

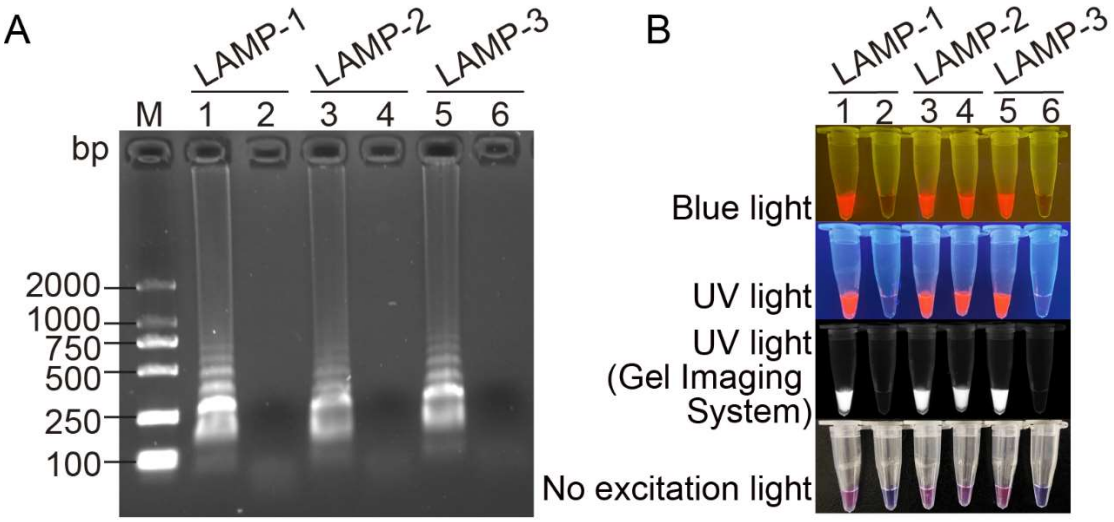


Fig S6. Screening of LAMP primer pairs for detection of SADS-CoV *N* gene. (A) Agarose gel electrophoresis of LAMP products of SADS-CoV *N* gene; **(B)** CRISPR/Cas12a cleavage assay to detection LAMP products of SADS-CoV *N* gene. 1, 3, 5: SADS-CoV-N plasmid DNA was used as template; 2, 4, 6: non-template control (NTC).

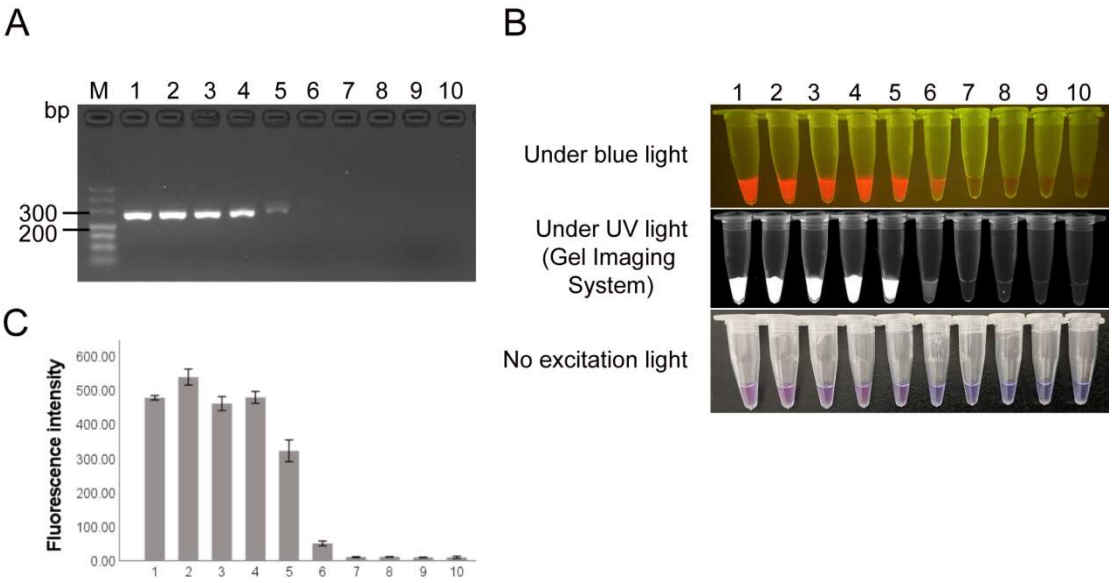


Fig S7. Sensitivity of the PCR-Cas12a assay for detection of *ORF3* gene using serially diluted ORF3-plasmid (8×10^7 to 8×10^0 copies/μL). (A) Products of the PCR reaction by using agarose gel electrophoresis analysis; **(B)** Colorimetric/fluorescence signals from a series of 10-fold dilutions of PUC57-ORF3 plasmid DNA using PCR-Cas12a assay; **(C)** Sensitivity of the PCR-Cas12a assay in a multi-functional microplate reader (n=3); Lane M, DNA Ladder; bp, base pairs; 1, 8×10^7 copies/μL; 2, 8×10^6 copies/μL; 3, 8×10^5 copies/μL; 4, 8×10^4 copies/μL; 5, 8×10^3 copies/μL; 6, 8×10^2 copies/μL; 7, 8×10^1 copies/μL; 8, 8×10^0 copies/μL; 9, 1×10^0 copies/μL; 10, non-template control (NTC).

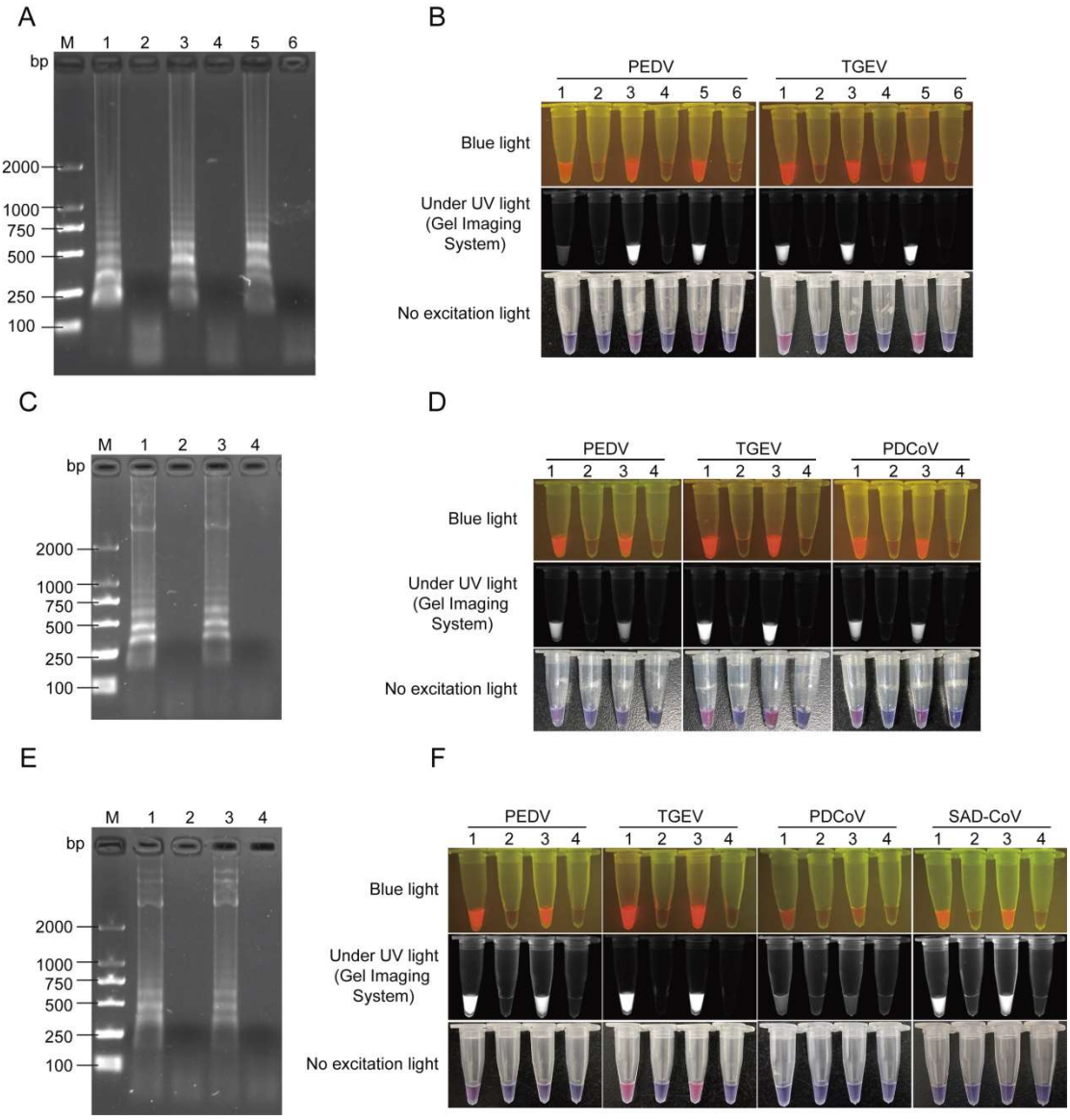


Fig S8. Establishment of multiple LAMP-Cas12a assay for porcine diarrhea coronavirus. (A) The agarose gel electrophoresis for detection of double amplification of PEDV and TGEV; **(B)** The fluorescence signal of Cas12a-based assay for detection of double amplification of PEDV and TGEV; 1, 2: original primer concentration; 3, 4: 1/2 dilution of the original primer concentration; 5, 6: 1/3 dilution of the original primer concentration; 1, 3, 5: plasmid DNA; 2, 4, 6: non-template control (NTC); **(C)** The agarose gel electrophoresis for detection of triple amplification of PEDV, TGEV and PDCoV; **(D)** The fluorescence signal of Cas12a-based assay for detection of triple amplification of PEDV, TGEV and PDCoV; 1, 2: 1/3 dilution of original primer concentration; 3, 4: 1/4 dilution of original primer concentration; **(E)** The agarose gel electrophoresis for detection of quadruple amplification of PEDV, TGEV, PDCoV and SADS-CoV; **(F)** The fluorescence signal of Cas12a-based assay for detection of quadruple

amplification of PEDV, TGEV, PDCoV and SADS-CoV; 1, 2: 1/3 dilution of original primer concentration; 3,4: 1/4 dilution of original primer concentration.

Table S1. Synthetic insert cloned into pUC57 and pMD18T vector.

Oligonucleotides Name	Sequence (5'-3')	Size of oligonucleotides (bp)
TGEV-N	AACTTATGTCCGAGAGACTTTGTACCCAAAG GAATAGGTAACAGGGATCAGCAGATTGGTT ATTGGAATAGACAAACTCGCTATCGCATGGT GAAGGGCCAACGTAAAGAGCTTCCTGAAAG GTGGTTCTTCTACTACTTAGGTACTGGACCTC ATGCAGATGCCAAATTTAAAGATAAATTAGA TGGAGTTGTCTGGGTTGCCAAGGATGGTGCC ATGAACAAACCAACCACGCTTGGTAGTCGTG GTGCTAATAATGAATCCAAAGCTTTGAAATT CGATGGTAAAGTGCCAGGCGAATTTCAACTT GAAGTTAACCAGTCAAGGGACAATTCAAGG TCACGCTCTCAATCTAGATCTCGGTCTAGAA ACAGATCTCAATCTAGAGGCAGGCAACAAT CCAATAACAAGAAGGATGACAGTGTAGAAC AAGCTGTTCTTGCCGCACTTAAAAAGTTAGG TGTTGACACAGAAAAACAACAGCAACGTTC TCGTTCTAAATCTAAAGAACGTAGTAACTCT AAAACAAGAGATACTACGCCTAAGAATGAA AACAAACACACCTGGAAGAGAACTGCAGGT AAAGGTGATGTGACAAGATTTTATGGAGCTA GAAGCAGTTCAGCCAATTTTGGTGACAGTGA CCTCGTTGCCAATGGGAGCAGTGCCAAGCAT TACCCACAATTGGCTGAATGTGTTCCATCTGT GTCTAGCATTTTGTTTGGAAGCTATTGGACTT CAAAGGAAGAT	**750**

pUC57-T7-sgRNA (partial fragment)	GGATCCCTAATACGACTCACTATAGGGAATT TCTACTGTTGTAGATTGAGACCGAGAGAGGG TCTCATTTTTTAAAGGGCCCGTCGACTGCAG AGGCCTGCATGCAAGCTT	**111**
PDCoV-N	GAAAACCATGGCTACTGGCTGCGTTACACCA GACAAAAGCCAGGTGGTACTCCGATTCCTCC ATCCTTTGCCTTTTATTATACTGGCACAGGTC CCAGAGGAAATCTTAAGTATGGTGAACTCCC TCCTAATGATACCCCAGCAACCACTCGTGTT ACTTGGGTTAAGGGTTCGGGAGCTGACACTT CTATTAAACCTCATGTTGCCAAACGCAACCC CAACAATCCTAAACATCAGCTGCTACCTCTC CGATTCCCAACCGGAGATGGCCCAGCTCAA GGTTTCAGAGTTGACCCCTTCAACGCTAGAG GAAGACCTCAGGAGCGTGGAAGTGGCCCAA GATCTCAATCTGTTAACTCCAGAGGCACAGG CAATCAGCCCAGGAAACGCGACCAATCTGC ACCAGCTGCGGTACGTCGTAAGACCCAGCAT CAAGCTCCCAAGCGGACTTTACCCAAGGGTG AAACCATTTCTCAGGTATTTGGCAACCGGTC TCGTACTGGTGCCAATGTCGGCTCTGCAGAC ACTGAGAAGACGGGTATGGCTGATCCTCGCA TCATGGCTCTAGCCGGACATGTGCCTGGTGT TCAGGAAATGTTTTTCGCTGGCCACCTTGAG AGCAACTTTCAGGCGGGGGCAATTACCCTTA CCTTCTCCTACTCAATCACAGTCAAGGAGGG TTTTCCTGACTATGGGAGACTTAAGGATGCG CTCAATACGGTCGTTAACCAGACCTATGAGC CACCTACCAAACCAACTAAGGACAAGAAGC	**900**

	CTGACAAACAAGTCCAGTCTGCTAAACCCA AACAGCAGAAGAAACCTAAAAAGGTAACTC TGCCAGCAGACAAACAGGATTGGGAGTGGG ATGATGCTTTTGAGATAAAGCAGGAATCAGC AGCGTAG	
SAD-CoV-N	CCAACGTAAAGATCAGCCTTCTAACTGGCACT TTTATTACCTTGGTACTGGTCCTCACGCAGAT GCTCCTTTCAGGAAACGGATTCAGGGTGTGCA TTGGGTCGCTGTTGACGGTGCTAAAACTAGCC CCACAGGTCTTGGTGTTCGCAATCGTAACAAA GAACCTGCTACACCTCAGTTTGGGTTTCAATT ACCACCAGACCTGACTGTTGTTGAGGTTACTT CTAGAAGTGCTTCACGTTCACAGTCTCGTTCT CGCAATCAAAGTCAAAGCCGCAGTGGTGCTC AGACACCTCGTGCTCAACAGCCGTCACAGTCT GTTGACATTGTTGCTGCAGTTAAACAAGCTTT GGCAGACTTGGGCATAGCTTCTAGCCAGTCCA GGCCTCAAAGTGGTAAAAATACACCCAAACC AAGAAGCAGAGCTGTCTCACCTGCACCTGCC CCTAAACCGGCTCGTAAGCAGATGGACAAAC CTGAATGGAAGCGTGTTCCTAATTCTGAGGAG GACGTGCGTAAATGCTTTGGTCCTCGCTCAGT TTCTAGAAATTTTGGTGACAGTGACCTCGTTC AGCACGGTGTTGAAGCTAAGCACTTTCCAAC AATTGCTGAGTTGCTTCCGACACAAGCTGCAC TAGCCTTTGGTAGTGAAATCACAACCAAAGA GTCTGGTGAATTTGTAGAAGTCACCTATCACT ATGTAATGAAGGTCCCCAAGACTGATAAAAA TCTACCCAGATTTCTTGAGCA	**750**

PEDV-ORF3	AGACAAGCTTCAAATGTGACGGGTTTTCTTTT CACCAGTGTTTTTATCTACTTCTTTGCACTGTT TAAAGCGTCTTCTTTGAGGCGCAATTATATTA TGTTGGCAGCGCGTTTTGCTGTCATTGTTCTTT ATTGCCCACTTTTATATTATTGTGGTGCATTTT TAGATGCAACTATTATTTGTTGCACACTTATTG GCAGGCTTTGTTTAGTCTGCTTTTACTCCTGGC GCTATAAAAATGCGCTCTTTATTATCTTTAATA CTACGACACTTTCTTTCCTCAATGGTAAAGCA GCTTATTATGACGGCAAATCCATTGT	**320**

Table S2. Primers and oligos used in this study.

Application	Oligonucleotides Name	Primer sequence (5'-3')	Target ID	Production Length(bp)
PCR primers	SADS-N-T7-F	TAATACGACTCACTATAGGCAGGAAACG GATTCAGGG	MG775253.1	**478**
	SADS-N-R	GGACCAAAGCATTTACGC		
	PEDV-ORF3-F	GAGGAAAGAAAGTGTCGTAG	KJ642645.1	**265**
	PEDV-ORF3-R	GACGGGTTTTCTTTTCAC		
sgRNA (For In Vitro synthesis)	T7-crRNA-F	TCGCTATTACGCCAGCTGGC		
	PEDV-sgR1-R	AGTCTGCTTTTACTCCTGGCGCTAATCTAC AACAGTAGAAAT	PEDV-sgRNA1	
	PEDV-sgR2-R	TCTGCTTTTACTCCTGGCGCTATAATCTAC AACAGTAGAAAT	PEDV-sgRNA2	
	PEDV-sgR3-R	GCGCATTTTTATAGCGCCAGGAGTATCTAC AACAGTAGAAAT	PEDV-sgRNA3	
	PEDV-sgR4-R	AGCGCATTTTTATAGCGCCAGGAGATCTA CAACAGTAGAAAT	PEDV-sgRNA4	
	PEDV-sgR5-R	ATAGCGCCAGGAGTAAAAGCAGACATCT ACAACAGTAGAAAT	PEDV-sgRNA5	
	PEDV-sgR6-R	CAAAGCCTGCCAATAAGTGTGCAAATCTA CAACAGTAGAAAT	PEDV-sgRNA6	

	PEDV-sgR7-R	CGCCAGGAGTAAAAGCAGACTAAAATCT ACAACAGTAGAAAT	PEDV-sgRNA7
	TGEV-N-sgR1-R	GCTCTCAATCTAGATCTCGGTCTAATCTAC AACAGTAGAAAT	TGEV-sgRNA1
	TGEV-N-sgR2-R	AGAACAAGCTGTTCTTGCCGCACTATCTA CAACAGTAGAAAT	TGEV-sgRNA2
	TGEV-N-sgR3-R	AAAACAACAGCAACGTTCTCGTTCATCTA CAACAGTAGAAAT	TGEV-sgRNA3
	TGEV-N-sgR4-R	TAAATCTAAAGAACGTAGTAACTCATCTA CAACAGTAGAAAT	TGEV-sgRNA4
	TGEV-N-sgR5-R	CTTAAAAAGTTAGGTGTTGACACAATCTA CAACAGTAGAAAT	TGEV-sgRNA5
	TGEV-N-sgR6-R	CCCTTGACTGGTTAACTTCAAGTTATCTAC AACAGTAGAAAT	TGEV-sgRNA6
	SADS-sg1-R	GTCTGTTGACATTGTTGCTGCAGTATCTAC AACAGTAGAAAT	SADSCoV-sgRNA1
	SADS-sg2-R	AGCTGTCTCACCTGCACCTGCCCCATCTA CAACAGTAGAAAT	SADSCoV-sgRNA2
	SADS-sg3-R	CGTGCTGAACGAGGTCACTGTCACATCTA CAACAGTAGAAAT	SADSCoV-sgRNA3
	SADS-sg4-R	TCCTAATTCTGAGGAGGACGTGCGATCTA CAACAGTAGAAAT	SADSCoV-sgRNA4
	SADS-sg5-R	GCTAGAAGCTATGCCCAAGTCTGCATCTA CAACAGTAGAAAT	SADSCoV-sgRNA5
	SADS-sg6-R	TAGCCAGTCCAGGCCTCAAAGTGGATCTA CAACAGTAGAAAT	SADSCoV-sgRNA6
sgRNA (For In Vitro synthesis)	SADS-sg7-R	ATAGCTTCTAGCCAGTCCAGGCCTATCTA CAACAGTAGAAAT	SADSCoV-sgRNA7
	SADS-sg8-R	GCTTTGGTCCTCGCTCAGTTTCTAATCTAC AACAGTAGAAAT	SADSCoV-sgRNA8
	SADS-sg9-R	AATTTCTAGAAACTGAGCGAGGACATCTA CAACAGTAGAAAT	SADSCoV-sgRNA9
	SADS-sg10-R	TAAACCGGCTCGTAAGCAGATGGAATCTA CAACAGTAGAAAT	SADSCoV-sgRNA10
	SADS-sg11-R	TCAGGTCTGGTGGTAATTGAAACCATCTA CAACAGTAGAAAT	SADSCoV-sgRNA11
	SADS-sg12-R	CAACAGTCAGGTCTGGTGGTAATTATCTA CAACAGTAGAAAT	SADSCoV-sgRNA12

	SADS-sg13-R	CGTTCACAGTCTCGTTCTCGCAATATCTAC AACAGTAGAAAT	SADSCoV-sgRNA13
	SADS-sg14-R	CAGTCTCGTTCTCGCAATCAAAGTATCTA CAACAGTAGAAAT	SADSCoV-sgRNA14
	PDCoV-sg1-R	TCTAGCGTTGAAGGGGTCAACTCTATCTA CAACAGTAGAAAT	PDCoV-sgRNA1
	PDCoV-sg2-R	GCCGGACATGTGCCTGGTGTTCAGATCTA CAACAGTAGAAAT	PDCoV-sgRNA2
	PDCoV-sg3-R	CTGAGAAATGGTTTCACCCTTGGGATCTA CAACAGTAGAAAT	PDCoV-sgRNA3
	PDCoV-sg4-R	CAGAGGCACAGGCAATCAGCCCAGATCT ACAACAGTAGAAAT	PDCoV-sgRNA4
	PDCoV-sg5-R	CACTTCTATTAAACCTCATGTTGCATCTAC AACAGTAGAAAT	PDCoV-sgRNA5
	PDCoV-sg6-R	ATCCTTAAGTCTCCCATAGTCAGGATCTAC AACAGTAGAAAT	PDCoV-sgRNA6
sgRNA (For In Vitro synthesis)	PDCoV-sg7-R	CATCCTTAAGTCTCCCATAGTCAGATCTAC AACAGTAGAAAT	PDCoV-sgRNA7
	PDCoV-sg8-R	GGGTTCGGGAGCTGACACTTCTATATCTA CAACAGTAGAAAT	PDCoV-sgRNA8
	PDCoV-sg9-R	TGGCACCAGTACGAGACCGGTTGCATCTA CAACAGTAGAAAT	PDCoV-sgRNA9
	PDCoV-sg10-R	CGAGACCGGTTGCCAAATACCTGAATCTA CAACAGTAGAAAT	PDCoV-sgRNA10
	PDCoV-sg11-R	AGTTGCTCTCAAGGTGGCCAGCGAATCTA CAACAGTAGAAAT	PDCoV-sgRNA11
	PDCoV-sg12-R	AAGTTGCTCTCAAGGTGGCCAGCGATCTA CAACAGTAGAAAT	PDCoV-sgRNA12
	PEDV-L1-F3	GCACTGTTTAAAGCGTCTT	
	PEDV-L1-B3	AGGAAAGAAAGTGTCGTAGT	
	PEDV-L1-FIP	GCACCACAATAATATAAAAGTGGGCGGC GCAATTATATTATGTTGG	
LAMP primers	PEDV-L1-BIP	TTGTTGCACACTTATTGGCAGAAGAGCGC ATTTTTATAGCG	
	PEDV-L1-LF	AAGAACAATGACAGCAAAACGC	
	PEDV-L1-LB	TGTTTAGTCTGCTTTTACTCCTGG	
	PEDV-L2-F3	GCACTGTTTAAAGCGTCTT	

	PEDV-L2-B3	GAGGAAAGAAAGTGTCGTAGT
	PEDV-L2-FIP	GCACCACAATAATATAAAAGTGGGCGGC GCAATTATATTATGTTGG
	PEDV-L2-BIP	TTGTTGCACACTTATTGGCAGAAGAGCGC ATTTTTATAGCG
	PEDV-L2-LF	AAGAACAATGACAGCAAAACGC
	PEDV-L2-LB	TGTTTAGTCTGCTTTTACTCCTGG
	PEDV-L3-F3	GTTTAAAGCGTCTTCTTTGAG
	PEDV-L3-B3	AGGAAAGAAAGTGTCGTAGT
LAMP primers	PEDV-L3-FIP	GCACCACAATAATATAAAAGTGGGCGCG CAATTATATTATGTTGGC
	PEDV-L3-BIP	TTGTTGCACACTTATTGGCAGAGAGCGCA TTTTTATAGCG
	PEDV-L3-LF	AAGAACAATGACAGCAAAACGC
	PEDV-L3-LB	TGTTTAGTCTGCTTTTACTCCTGG
	PEDV-L4-F3	GTTTAAAGCGTCTTCTTTGAG
	PEDV-L4-B3	GAGGAAAGAAAGTGTCGTAGT
	PEDV-L4-FIP	GCACCACAATAATATAAAAGTGGGCGCG CAATTATATTATGTTGGC
	PEDV-L4-BIP	TTGTTGCACACTTATTGGCAGAAGAGCGC ATTTTTATAGCG
	PEDV-L4-LF	AAGAACAATGACAGCAAAACGC
	PEDV-L4-LB	TGTTTAGTCTGCTTTTACTCCTGG
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	TGEV-L1-B3	TGAACTGCTTCTAGCTCCA
	TGEV-L1-FIP	GTTGCTGTTGTTTTTCTGTGTCAGATGACA GTGTAGAACAAGC
	TGEV-L1-BIP	ACTCTAAAACAAGAGATACTACGCCCAC ATCACCTTTACCTGCA
	TGEV-L1-LF	ACCTAACTTTTTAAGTGCGGCAAG
	TGEV-L1-LB	GAATGAAAACAAACACACCTGGAAG
	TGEV-L2-F3	AATTTCAACTTGAAGTTAACCAG
	TGEV-L2-B3	AACGAGAACGTTGCTGTT

	TGEV-L2-FIP	TGCCTCTAGATTGAGATCTGTTTCTTCAAG GGACAATTCAAGGT
	TGEV-L2-BIP	AGAAGGATGACAGTGTAGAACAAGCGTT TTTCTGTGTCAACACCTA
	TGEV-L2-LF	CGAGATCTAGATTGAGAGCGTG
	TGEV-L2-LB	GTTCTTGCCGCACTTAA
	TGEV-L3-F3	CCAGGCGAATTTCAACTTG
	TGEV-L3-B3	GAACGAGAACGTTGCTGT
	TGEV-L3-FIP	TGCCTCTAGATTGAGATCTGTTTCTTCAAG GGACAATTCAAGGTC
	TGEV-L3-BIP	AGAAGGATGACAGTGTAGAACAAGCTGT TTTTCTGTGTCAACACC
	TGEV-L3-LF	GACCGAGATCTAGATTGAGAGCG
	TGEV-L3-LB	TGTTCTTGCCGCACTTAAAAAGTT
	TGEV-L4-F3	GGCGAATTTCAACTTGAAGT
	TGEV-L4-B3	GAACGAGAACGTTGCTGT
	TGEV-L4-FIP	GCCTCTAGATTGAGATCTGTTTCTGTCAA GGGACAATTCAAGGT
	TGEV-L4-BIP	GAAGGATGACAGTGTAGAACAAGCTGTT TTTCTGTGTCAACACC
	TGEV-L4-LF	CCGAGATCTAGATTGAGAGCGTG
	TGEV-L4-LB	TGTTCTTGCCGCACTTAAAAAGT
	TGEV-L5-F3	TCAAGGTCACGCTCTCAA
LAMP primers	TGEV-L5-B3	GTTTGTTTTCATTCTTAGGCG
	TGEV-L5-FIP	GCTTGTTCTACACTGTCATCCTTCT ATCTCGGTCTAGAAACAGATC
	TGEV-L5-BIP	GTTAGGTGTTGACACAGAAAAACAAATC TCTTGTTTTAGAGTTACTACGT
	TGEV-L5-LF	TGTTGCCTGCCTCTAGATTGA
	TGEV-L5-LB	CAGCAACGTTCTCGTTCTAAATCT
	SADSCOV-L1-F3	ACAAGCTTTGGCAGACTTGG
	SADSCOV-L1-B3	GAGCGAGGACCAAAGCATT
	SADSCOV-L1-FIP	TGCAGGTGAGACAGCTCTGCAGCTTCTAG CCAGTCCAGG

	SADSCOV-L1-BIP	GCCCCTAAACCGGCTCGTAAGCGCACGT CCTCCTCAGAA
	SADSCOV-L1-LF	CTTCTTGGTTTGGGTGTAT
	SADSCOV-L1-LB	ACAAACCTGAATGGAAGCGTGT
	SADSCOV-L2-F3	GCTCAGACACCTCGTGCT
	SADSCOV-L2-B3	CCATCTGCTTACGAGCCG
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	SADSCOV-L2-LB	AAATACACCCAAACCAAGAAGC
	SADSCOV-L3-F3	ACAAGCTTTGGCAGACTTGG
	SADSCOV-L3-B3	AGAAACTGAGCGAGGACCAA
	SADSCOV-L3-FIP	TGCAGGTGAGACAGCTCTGCTTAGCTTCT AGCCAGTCCAGG
	SADSCOV-L3-BIP	TGCCCCTAAACCGGCTCGTAACGCACGTC CTCCTCAGAA
	SADSCOV-L3-LF	TCTTGGTTTGGGTGTAT
	SADSCOV-L3-LB	CAAACCTGAATGGAAGCGTG
	PDCOV-L1-F3	GCAATCAGCCCAGGAAACG
	PDCOV-L1-B3	AACACCAGGCACATGTCC
LAMP primers	PDCOV-L1-FIP	TCACCCTTGGGTAAAGTCCGCCAGCTGCG GTACGTCGTA
	PDCOV-L1-BIP	ATGTCGGCTCTGCAGACACTGGCTAGAGC CATGATGCGAG
	PDCOV-L1-LF	TGGGAGCTTGATGCTGG
	PDCOV-L1-LB	AGACGGGTATGGCTGATC
	PDCOV-L2-F3	GCAATCAGCCCAGGAAACG
	PDCOV-L2-B3	TCCTGAACACCAGGCACAT
	PDCOV-L2-FIP	TCACCCTTGGGTAAAGTCCGCCAGCTGCG GTACGTCGTA
	PDCOV-L2-BIP	ATGTCGGCTCTGCAGACACTGGTCCGGCT AGAGCCATGA

	PDCOV-L2-LF	TGGGAGCTTGATGCTGG
	PDCOV-L2-LB	GGTATGGCTGATCCTCGC
ssDNA-FQ reporters	**ROX-N12-BHQ2**	/5′-**ROX**/GTATCCAGTGCG/3′**BHQ2**/
	JOE-N12-BHQ1	/5′-J**OE**/GTATCCAGTGCG/3′**BHQ1**/