Electronic Supplementary Material (ESI) for Analytical Methods.

Support information

Multiplex real-time PCR using double strand displacing nucleic acids as primers and probes for the detection of nucleic acids

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Table S1 Comparison of different nucleic acid quantification technologies

	SYBR Green	Taqman	Molecular beacon	Double strand probe
Specificity	Medium	High	High	High
Multiplexing	No	Yes	Yes	Yes
design	-	medium	difficult	easy
Fluorescence feature	reversible	unreversible	reversible	reversible

Table S2 Sequences of the designed oligonucleotides

Name	Sequences(5′-3′)
ORF1ab-F(FAM)	6-FAM-CCCTGTGGGTTTTACACTTAA
ORF1ab-F(BHQ1)	TGTAAAACCCACAGGG-BHQ1
ORF1ab-R	ACGATTGTGCATCAGCTGA
N-F(HEX)	HEX-GGGGAACTTCTCCTGCTAGAAT
N-F(BHQ1)	AGCAGGAGAAGTTCCCC-BHQ1

N-R	CAGACATTTTGCTCTCAAGCTG
E_Sarbeco_F(ROX)	ROX-ACAGGTACGTTAATAGTTAATAGCGT
E_Sarbeco_F(Dabcyl)	TTAACTATTAACGTACCTGT-Dabcyl
E_Sarbeco_R	ATATTGCAGCAGTACGCACACA

Table S3 The fraction of SDR primers based melting process at different temperatures. The initial concentration of fluorophore and quencher labeled primers, template were set 200 nM, 300 nM and 1 nM.

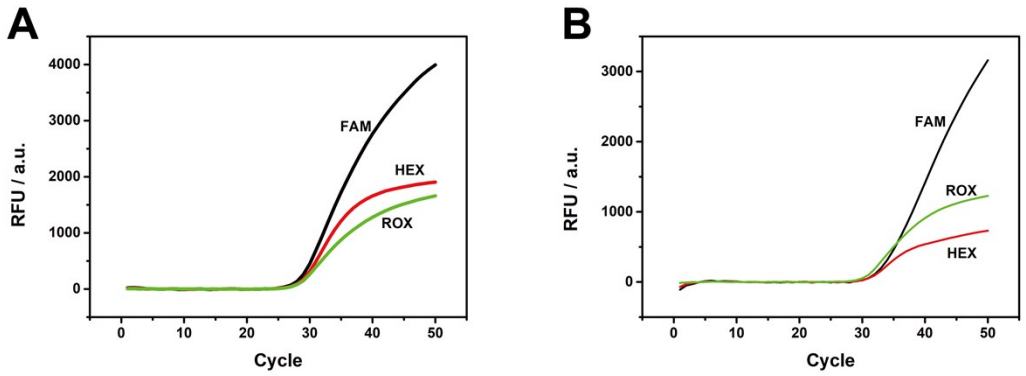
	57 °C	59 °C	61 °C	63 °C
ORF1ab-F(FAM)	26 nM	45 nM	70 nM	100 nM
ORF1ab-F(BHQ1)	130 nM	150 nM	170 nM	200 nM
ORF1ab-F(FAM)/ ORF1ab-F(BHQ1)	170 nM	150 nM	130 nM	99 nM
ORF1ab-F(FAM)/template	1 nM	1 nM	1 nM	1 nM
N-F(HEX)	1nM	2.6nM	6.3nM	14nM
N-F(BHQ1)	100nM	100nM	110nM	120nM
N-F(HEX)/ N-F(BHQ1)	200nM	200nM	190nM	180nM
N-F(HEX)/template	1nM	1nM	1nM	1nM
E_Sarbeco_F(ROX)	11nM	26nM	51nM	85nM
E_Sarbeco_F(Dabcyl)	110nM	130nM	150nM	190nM
E_Sarbeco_F(ROX)/ E_Sarbeco_F(Dabcyl)	190nM	170nM	150nM	110nM
E_Sarbeco_F(ROX)//template	1nM	1nM	1nM	1nM

Table S4 False signal ratio at different melting temperature. False signal ratio is

defined as the ration of free fluorophore labeled primer to total fluorophore labeled primer

	57 °C	59 °C	61 °C	63 °C
ORF1ab-F	13%	22.5%	35%	50%
N	0.5%	1.3%	3.2%	7%
E_Sarbeco	5.5%	13%	25.5%	42.5%

Fig. S1 RT-PCR results of pseudo virus (A) and RNAs extraced from throat swab sample (B).



Positive plasmid sequences for ORF and N gene (Color font representation amplification products)

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCG
GAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCC
GTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTAT
GCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAAT
ACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCAT
TCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTA
TTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGT
AACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAAT
TCGAGCTCGGTACCTCGCGAATGCATCTAGATATCGGATCCCGACGCTAA
TGACCCTGTGGGTTTTACACTTAAAAACACAGTCTGTACCGTCTGCGGTAT
GTGGAAAGGTTATGGCTGTAGTTGTGATCAACTCCGCGAACCCATGCTTC
AGTCAGCTGATGCACAATCGTTTTTAAACGGGTTTGCGGCAGCAGTAGGG
GAACTTCTCCTGCTAGAATGGCTGGCAATGGCGGTGATGCTGCTCTTGCTT
TGCTGCTGCTTGACAGATTGAACCAGCTTGAGAGCAAAATGTCTGGTAAA

GGCCAACAACGACGGGCCCGTCGACTGCAGAGGCCTGCATGCAAGCTTGG
CGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAA
TTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCC
TAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTC
CAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGC
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CTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAA
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CTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCA
AGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTA
TCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCC
ACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGC
GGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAG
AACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAA
GAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGT
TTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGA
AGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTC
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GCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAG
ATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGAT
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CAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCC
ATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTT
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TCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGA
GTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCT
CCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATG
GCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCT
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GCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGA
GCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACA
CGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTT
ATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAA
ATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGAC
GTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTAT
CACGAGGCCCTTTCGTC

Positive plasmid sequences for E gene (Color font representation amplification products)

GCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGA
GACTGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTC
AGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAATTATGCG
GCATCAGAGCAGATGGTAGACAGAGTGCACCAGATGCGGTGAGAAATAC
CGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCATTC
AGGCTGCGCAGCTGTTGGGAAGGGCGGTCGGTGCGGGCCTCTTCGCTATT
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CGCCAGGGTTTTCCCAGTCACGACGTTGCAGAACGACGGCCAGAGAGTTA
GAGGACGGAACCTCGCGAATACAACGAGATATCGGGTCCCACACACAGG
TACGTTAATAGTTAATAGCGTACTTCTTTTTCTTGCTTTCGTGGTATTCTTG
CTAGTTACACTAGCCATCCTTACTGCGCTTCGATTGTGTGCGTACTGCTGC
AATATGACACGGGCACGACGAGAACACAGGCCTGCGTGCAGAGATGGCG
TAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATT
CCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCT
AATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCC
AGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCG
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TTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAA
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