Supplementary Information

Detection of Coronaviruses using RNA Toehold Switch Sensors

Soan Park and Jeong Wook Lee

Table S1. Sequences of toehold switch sensors and target regions.

Target region	Location in genome	Normalized ensemble defect (%)	Sensor name	Sensor sequence
AGUUUCCAGGAC GCUGCCUCUAAU AUCUUUGUUAU U	9756 (*orf1ab* gene)	24.8	M1	**TAATACGACTCACTATAGGG**AATAACAAAGA TATTAGAGGCAGCGTCCTGGAAACTGGACTTT AGAACAGAGGAGATAAAGATG***AGTTTCCAGG AC***AACCTGGCGGCAGCGCAAAAGATGCGTAA A
GCAAACAUAGUC UACGAGCCCACU ACUCCCAUUUCG	11178 (*orf1ab* gene)	19.3	M2	**TAATACGACTCACTATAGGG**CGAAATGGGAG TAGTGGGCTCGTAGACTATGTTTGCGGACTTTA GAACAGAGGAGATAAAGATG***GCAAACATAGTC*** AACCTGGCGGCAGCGCAAAAGATGCGTAAA
ACUUAUGCCAUC UUUGCUUACUCA CCACAGCUUACA	11511 (*orf1ab* gene)	19.3	M3	**TAATACGACTCACTATAGGG**TGTAAGCTGTGG TGAGTAAGCAAAGATGGCATAAGTGGACTTTA GAACAGAGGAGATAAAGATG***ACTTATGCCATC*** AACCTGGCGGCAGCGCAAAAGATGCGTAAA
ACUACUCCCAUU UCGUCAGCGCUG AUUGCAGUUGC A	11199 (*orf1ab* gene)	27.9	M4	**TAATACGACTCACTATAGGG**TGCAACTGCAAT CAGCGCTGACGAAATGGGAGTAGTGGACTTTA GAACAGAGGAGATAAAGATG***ACTACTCCCATT*** AACCTGGCGGCAGCGCAAAAGATGCGTAAA
AGUUCAUGUCU ACUCCCAAUGCC AUCUUUAUUUC UG	19953 (*orf1ab* gene)	17.2	M5	**TAATACGACTCACTATAGGG**CAGAAATAAAG ATGGCATTGGGAGTAGACATGAACTGGACTTT AGAACAGAGGAGATAAAGATG***AGTTCATGTCT G***AACCTGGCGGCAGCGCAAAAGATGCGTAAA
AAAUGGGCCGCU UCUUCAAUCAUA CUCUAGUUCUUU	21934 (*S* gene)	16.5	M6	**TAATACGACTCACTATAGGG**AAAGAACTAGA GTATGATTGAAGAAGCGGCCCATTTGGACTTTA GAACAGAGGAGATAAAGATG***AAATGGGCCGC A***AACCTGGCGGCAGCGCAAAAGATGCGTAAA
CUCGCUUAUCGU UUAAGCAGCUCU GCGCUACUAUGG	27499 (*orf5* gene)	27.0	M7	**TAATACGACTCACTATAGGG**CCATAGTAGCGC AGAGCTGCTTAAACGATAAGCGAGGGACTTTA GAACAGAGGAGATAAAGATG***CTCGCTTATCGA*** AACCTGGCGGCAGCGCAAAAGATGCGTAAA
ACACACCAAACC AUUAUUUAUUA GAAACUUCGAUC A	27280 (*orf5* gene)	14.0	M8	**TAATACGACTCACTATAGGG**TGATCGAAGTTT CTAATAAATAATGGTTTGGTGTGTGGACTTTAG AACAGAGGAGATAAAGATG***ACACACCAAACA*** AACCTGGCGGCAGCGCAAAAGATGCGTAAA
GAGCCAUUAAAC UUGACCCAAAGA AUCCCAACUACA	29563 (*N* gene)	19.8	M9	**TAATACGACTCACTATAGGG**TGTAGTTGGGAT TCTTTGGGTCAAGTTTAATGGCTCGGACTTTAG AACAGAGGAGATAAAGATG***GAGCCATTAAAC***A ACCTGGCGGCAGCGCAAAAGATGCGTAAA
GCCAUUAAACUU GACCCAAAGAAU CCCAACUACAAU	29565 (*N* gene)	19.2	M10	**TAATACGACTCACTATAGGG**ATTGTAGTTGGG ATTCTTTGGGTCAAGTTTAATGGCGGACTTTAG AACAGAGGAGATAAAGATG***GCCATTAAACTA***A ACCTGGCGGCAGCGCAAAAGATGCGTAAA
AUGUCAUAUCA ACAUCACAUAAA UUAGUCUUGUC UG	16334 (*orf1ab* gene)	23.1	S1	**TAATACGACTCACTATAGGG**CAGACAAGACT AATTTATGTGATGTTGATATGACATGGACTTTAG AACAGAGGAGATAAAGATG***ATGTCATATCAG***A ACCTGGCGGCAGCGCAAAAGATGCGTAAA

AUGACAAAGAU CCAAAUUUCAAA GAUCAAGUCAU UU	29292 (*N* gene)	26.3	S2	**TAATACGACTCACTATAGGG**AAATGACTTGAT CTTTGAAATTTGGATCTTTGTCATGGACTTTAG AACAGAGGAGATAAAGATG***ATGACAAAGATA***A ACCTGGCGGCAGCGCAAAAGATGCGTAAA

The sequence of sensors consists of **T7 promoter** + target region (a + b) + conserved domain sequence + ***a part of target region*** (complementary sequence of stem region, b′) + conserved domain sequence. The conserved domain sequence was from the series B sensor from the previous publication [1].

Table S2. List of RT-LAMP primer sequences

Sensor†	RT-LAMP primer	Sequence (5' to 3')
M6	F3	ATTAGCCCATCTACCAGC
	B3	TGGCAAAAGAAGTATAGGAATT
	FIP with T7 promoter (5′ F1c-T7 promoter-F2 3′)	ACCATCTGAGAAATTACCAACTGAATAATACGACTCACTATAG GGGCTACTATACGAAAAATTTACCCT
	FIP with T7 promoter complementary sequence (5′ F2c-T7 promoter-F1 3′)	ACCATCTGAGAAATTACCAACTGAACCCTATAGTGAGTCGTATT AGCTACTATACGAAAAATTTACCCT
	BIP with T7 terminator complementary sequence (5′ B2c-T7 terminator-B1 3′)	CCGATGGATGTGGCACTTTACCAAAAAACCCCTCAAGACCCGT TTAGAGGCCCCAAGGGGTTATGCTACA GGACAATGATTTCCAGAG
	BIP with T7 terminator (5′ B1c-T7 terminator-B2 3′)	CCGATGGATGTGGCACTTTACTAGCATAACCCCTTGGGGCCTCT AAACGGGTCTTGAGGGGTTTTTTGCAG GACAATGATTTCCAGAG
	BIP without T7 terminator	CCGATGGATGTGGCACTTTACCAGCAGGACAATGATTTCC
	Loop F‡	GAACCCAGCATAAAAGC
	Loop B	TTTATTGTATTCTAGAGCCTCGCTC
S1	F3	TGGGGCTTGTGTTCTTTG
	B3	CTAATGGGTGGTTTATGTGATT
	FIP with T7 promoter complementary sequence (5′ F2c-T7 promoter-F1 3′)	TGGTCGTAACAGCATTTACAACATACCCTATAGTGAGTCGTA TTAACAGACTTCATTAAGATGTGGT
	BIP without T7 terminator	TAATCCGTATGTTTGCAATGCTCCCATACCTCCTAAGTAAAGT TGAG
	Loop F‡	AGAATGGTCTACGTATGCAAGC
	Loop B	AGGTTGTGATGTCACAGATGTG
S2	F3	GTTCTTCGGAATGTCGCG
	B3	CGGTAAGGCTTGAGTTTCA
	FIP with T7 promoter complementary sequence (5′ F2c-T7 promoter-F1 3′)	CCAATTTGATGGCACCTGTGTCCCTATAGTGAGTCGTATTA ATTGGCATGGAAGTCACAC
	BIP without T7 terminator	AAGCATATTGACGCATACAAAACATGCCTTCTTCTTTTTG TCCTT
	Loop F	GGTCAACCACGTTCCCGA
	Loop B	TCCCACCAACAGAGCCTAAA

†Target region of each sensor is amplified by RT-LAMP.

‡Loop F primer of M6 and S1 was obtained by changing the parameter condition from the default setting, Tm 60 °C to 51 °C and dimer check -3.5 to -4.5.

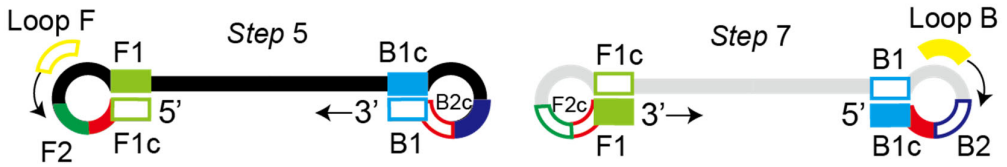

Figure S1. Schematic representation of Loop F and Loop B primers in the LAMP dumbbell-like structure

```
MERS-CoV      1  -TTGCTAATGGGTTTGTCGTCCGTATAGGAGCAGCTGCCAA-TTCCACTG
SARS-CoV      1  -TTGTCCGTGGTTGGGTTTTTGGTTCTACCATGAACAACA--AGTCACAG
SARS-CoV-2    1  -TAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTCGA--AGACCCAG
HCoV-OC43     1  -TTATTAAAGATCGTGTAATGTATAGTGAGTTCCCTGCTAT-AACTATAG
HCoV-NL63     1  AACACTACTTTTGATTTTTTAAGTAATGCTTCTAGTTCTTTTGACTGTAT
HCoV-HKU1     1  -TGTATGTTAATAAAACTTTGTATAGTGAGTTTAGTACTAT-AGTTATAG

MERS-CoV     51  GCACTG-TTATTATTAGCCCATCTACCAG-CGCTACTATACGAAAAATTT
SARS-CoV     51  TCGGTGATTATTATTAACAATTCTACTAA-TGTTGTTATACGAGCATGTA
SARS-CoV-2   51  TCCCTACTTATTGTTAATAACGCTACTAA-TGTTGTTATTAAAGTCTGTG
HCoV-OC43    51  GTAGTACTT-TTGTAAATACATCCTATAG-TGTGGTAGTACAACCACGTA
HCoV-NL63    51  AGTTAATTTGTTATTTACAGAACAGTTAGGTGCGCCTTTGGGCATAACTA
HCoV-HKU1    51  GTAGTGTTT-TTATTAACAACTCTTATAC-TATTGTTGTTCAACCTCATA

MERS-CoV    101  ACCCTGCTTTT-------ATGCTGG----------------GTTCTTCAG
SARS-CoV    101  ACTTTGA----------ATTGTG--------TGACAACCCTTTCT----
SARS-CoV-2  101  AATTTCA----------ATTTTG--------TAATGATCCATTTT----
HCoV-OC43   101  CAATCAATTCAACACAGGATGGTGATAATAAATTACAAGGTCTTTTAGAG
HCoV-NL63   101  TATCTGGTGAA-------ACTGTG----------CGTCTGCATTTATAT
HCoV-HKU1   101  ----------------ATGGTG----------------TTTTGGAG

MERS-CoV    151  TTGGTAATTTCTCAGATGGTAAAATGGGCCGCTTCTTCAATCATACTCTA
SARS-CoV    151  -TTGCTGTTTCTAAAC--CCA-TGGGTA-CACAGAC---ACATACTATGA
SARS-CoV-2  151  -TGGGTGTTTATTACC--ACA-AAAACAACAAAAGTTGGATGGAAAGTGA
HCoV-OC43   151  GTCTCTGTTTGCCAGT--ATAATATGTGCGAGTACCCACAAACGATTTGT
HCoV-NL63   151  AATGTAACTCGT-ACT---TTTTATGTGCCAGCAGCTT-ATAAACTTACT
HCoV-HKU1   151  ATTACAGCTTGTCAAT--ACACTATGTGTGAGTATCCTCATACTATTTGT

MERS-CoV    201  GTTCTTTTGCCCGATGGATGTGGCACTTTACTTAGAGCTTTTTATTGTAT
SARS-CoV    201  TATTCGA----------TAATGCA-TTTAATTGCA-CTTTCGAGTACAT
SARS-CoV-2  201  GTTCAGAG---TTTATTCTAGTGCG-AATAATTGCA-CTTTTGAATATGT
HCoV-OC43   201  CATCCTAACCTGGGTAATCATCGCA-AAGAACTATGGCATTTGGATACAG
HCoV-NL63   201  AAACTTAGTGTTAAATGTTACTTTA-ACTATTCCTGTGTTTTTAGTGT-T
HCoV-HKU1   201  AAATCTAA---AGGTAGTTCTCGTA-ATGAATCTTGGCATTTTGATAAAT

MERS-CoV    251  -TCTAGAGCCTCGCTCTGGAAATCATTGTCCTGCTGGCAATTCCTATACT
SARS-CoV    251  ATCTGATGCCTTTTCGCTTGATGTTTCAGAAAA-GTCAGGTAATTTTAAA
SARS-CoV-2  251  CTCTCAGCCTTTTCTTATGGACCTTGAAGGAAA-ACAGGGTAATTTCAAA
HCoV-OC43   251  GTGTTGTTTCCTGTTTATATAAGCGTAATTTCACATATGATGTGAATGCT
HCoV-NL63   251  GTCAACGCCACCGTTACTGTGAATGTCACCACA--CATAATGGCCGTGTA
HCoV-HKU1   251  CTGAACCTTTGTGTCTGTTCAAGAAAAATTTTACTTATAATGTTTCTACA

MERS-CoV    301  TCTTTTGCCACTTATCACACTCCTGCAACAGATTGTTCTGATGGCAATTA
SARS-CoV    301  CACTTACGAGAGTTTGTGTTTAAAAATAAAGATGGGTTTCTCTATGTTTA
SARS-CoV-2  301  AATCTTAGGGAATTTGTGTTTAAGAATATTGATGGTTATTTTAAAATATA
HCoV-OC43   301  GATTATTTGTATTTTCATTTTTATCAAGAAGGTGGTACTTTTTATGCATA
HCoV-NL63   301  GTTAACTACACTGTTTGTGATGATTGTAATGGTTATACTGATAACATATT
HCoV-HKU1   301  GATTGGTTGTATTTTCATTTTTATCAAGAACGTGGCACTTTTTATGCTTA

MERS-CoV    351  CAATCGTAATGCCAGTCTGAACTCTTTTAAGGAGTATTTTAATTTACGTA
SARS-CoV    351  TAAGGGCTATCA------A---CCTATAGATGTAGTTCGTGATCTACCTT
SARS-CoV-2  351  TTCTAAGCACAC------G---CCTATTAATTTAGTGCGTGATCTCCCTC
HCoV-OC43   351  TTTTACAGACACTGGTGTT---GTTACTAAGTTTTTGTTTAATGTTTATT
HCoV-NL63   351  TTCTGTTCAACAGGATGGCCGCATTCCTAATGGTTTCCCT--TTTAATAA
HCoV-HKU1   351  TTATGCTGATTCTGGCATG---CCTACTACTTTTTTATTTAGTTTGTATC
```

Figure S2. Sensor M6's target RNA and its upstream and downstream flanking sequences in the *S* gene of MERS-CoV. Homology regions to this sequence from other coronaviruses including SARS-CoV, SARS-CoV-2, HCoV-OC43, HCoV-NL63, and HCoV-HKU1 were aligned for sequence comparison. The red box indicates the homologous sequences of the target region of sensor M6. The blue box denotes the homologous sequences of its

upstream and downstream flanking sequences that were synthesized for the cross-reactivity test. HCoV-229E sequence was excluded from the alignment; it showed many mismatches and gaps.

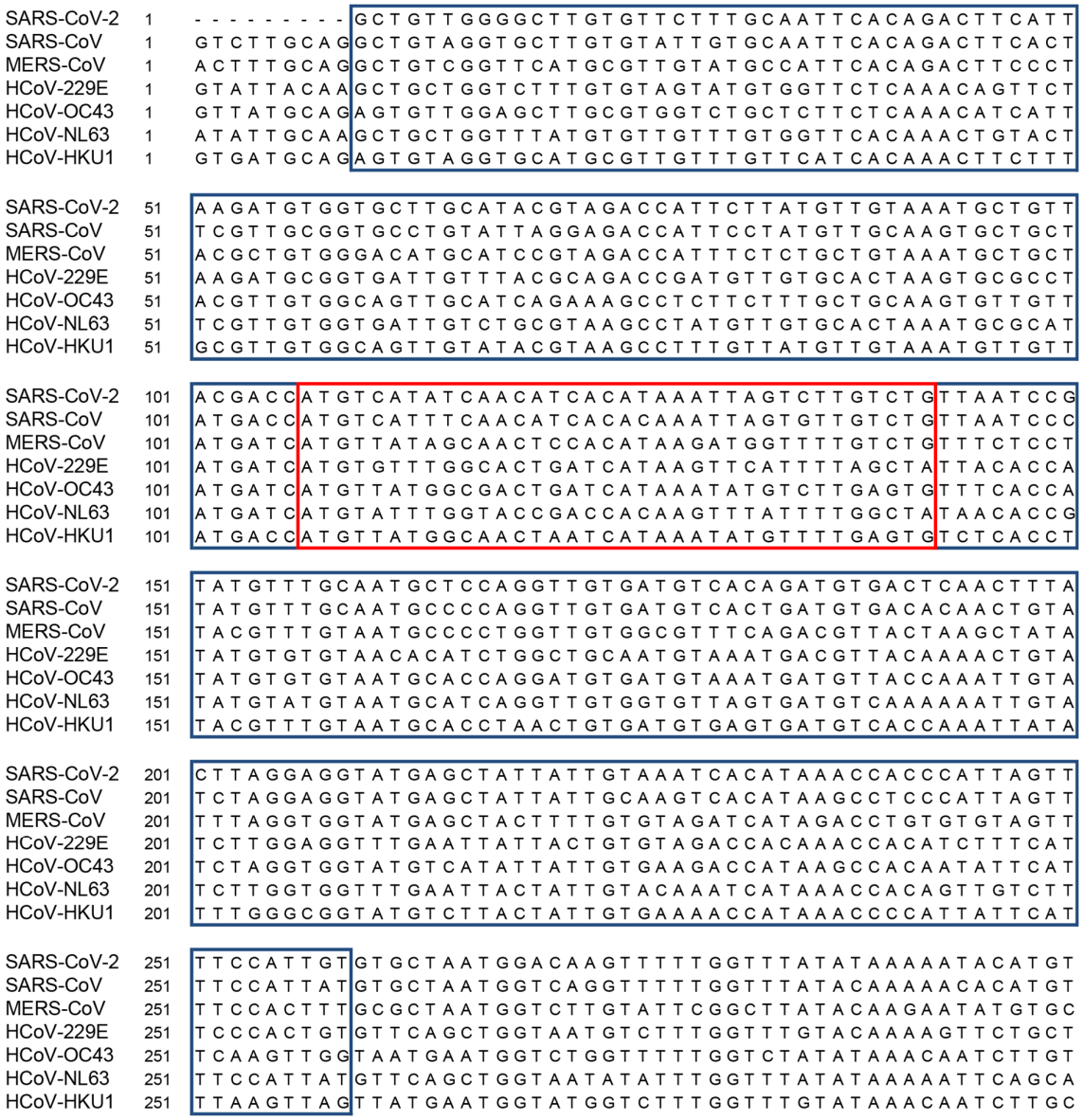

Figure S3. Sensor S1's target RNA and its upstream and downstream flanking sequences in the *orf1ab* gene of SARS-CoV-2. Homology regions to this sequence from other coronaviruses including SARS-CoV, MERS-CoV, HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1. The red box indicates the homologous sequences of the target region of sensor S1. The blue box denotes the homologous sequences of its upstream and downstream flanking sequences that were synthesized for the cross-reactivity test.

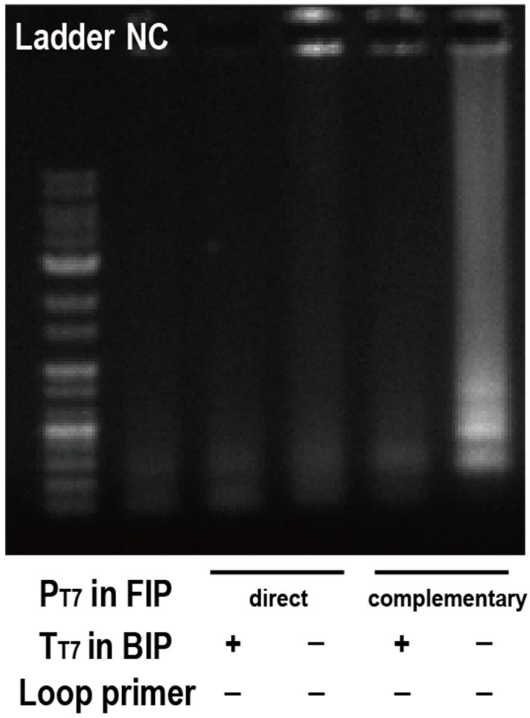

Fig S4. Gel electrophoresis of the modified RT-LAMP products. The ladder-like gel bands are the typical amplification pattern of LAMP. T7 promoter (P_{T7}) was inserted in the FIP primer with a direct or complementary sequence. In the presence of T7 terminator (T_{T7}) in the BIP primer, the RT-LAMP exhibited less amplification. Of note, we used RT-LAMP primers of sensor M6.

Reference

1. Pardee, K.; Green, A. A.; Takahashi, M. K.; Braff, D.; Lambert, G.; Lee, J. W.; Ferrante, T.; Ma, D.; Donghia, N.; Fan, M.; Daringer, N. M.; Bosch, I.; Dudley, D. M.; O'Connor, D. H.; Gehrke, L.; Collins, J. J. Rapid, Low-Cost Detection of Zika Virus Using Programmable Biomolecular Components. Cell 2016, 165, 1255–1266.