

## **Supplementary Information**

# **Detection of Coronaviruses using RNA Toehold Switch Sensors**

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**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 ( <i>orf1ab</i> gene)	24.8	M1	<u>TAATACGACTCACTATAGGGAATAACAAAGA</u> <u>TATTAGAGGCAGCGTCCTGGAAACTGGACTTT</u> <u>AGAACAGAGGAGATAAAGATGAGTTTCCAGG</u> <u>ACAACCTGGCGGCAGCGCAAAAGATGCGTAA</u> A
GCAAACAUAGUC UACGAGCCCACU ACUCCCAUUUCG	11178 ( <i>orf1ab</i> gene)	19.3	M2	<u>TAATACGACTCACTATAGGGCGCAAATGGGAG</u> <u>TAGTGGGCTCGTAGACTATGTTTGCGGACTTTA</u> <u>GAACAGAGGAGATAAAGATGGCAAACATAGTC</u> <u>AACCTGGCGGCAGCGCAAAAGATGCGTAA</u>
ACUUAUGCCAUC UUUGCUUACUCA CCACAGCUUACA	11511 ( <i>orf1ab</i> gene)	19.3	M3	<u>TAATACGACTCACTATAGGGTGTAAGCTGTGG</u> <u>TGAGTAAGCAAAGATGGCATAAGTGGACTTTA</u> <u>GAACAGAGGAGATAAAGATGACTTATGCCATC</u> <u>AACCTGGCGGCAGCGCAAAAGATGCGTAA</u>
ACUACUCCCAUU UCGUCAGCGCUG AUUGCAGUUGC A	11199 ( <i>orf1ab</i> gene)	27.9	M4	<u>TAATACGACTCACTATAGGGTGCAACTGCAAT</u> <u>CAGCGCTGACGAAATGGGAGTAGTGGACTTTA</u> <u>GAACAGAGGAGATAAAGATGACTACTCCATT</u> <u>AACCTGGCGGCAGCGCAAAAGATGCGTAA</u>
AGUUCAUGUCU ACUCCCAAUGCC AUCUUUAUUUC UG	19953 ( <i>orf1ab</i> gene)	17.2	M5	<u>TAATACGACTCACTATAGGGCAGAAATAAAG</u> <u>ATGGCATTGGGAGTAGACATGAACTGGACTTT</u> <u>AGAACAGAGGAGATAAAGATGAGTTCATGTCT</u> <u>GAACCTGGCGGCAGCGCAAAAGATGCGTAA</u>
AAAUGGGCCGCU UCUUCAAUCAUA CUCUAGUUCUUU	21934 ( <i>S</i> gene)	16.5	M6	<u>TAATACGACTCACTATAGGGGAAAGAAGCTAGA</u> <u>GTATGATTGAAGAAGCGGCCCATTTGGACTTTA</u> <u>GAACAGAGGAGATAAAGATGAAATGGGCCGC</u> <u>AAACCTGGCGGCAGCGCAAAAGATGCGTAA</u>
CUCGCUUAUCGU UUAAGCAGCUCU GCGCUACUAUGG	27499 ( <i>orf5</i> gene)	27.0	M7	<u>TAATACGACTCACTATAGGGCCATAGTAGCGC</u> <u>AGAGCTGCTTAAACGATAAGCGAGGGACTTTA</u> <u>GAACAGAGGAGATAAAGATGCTCGCTTATCGA</u> <u>AACCTGGCGGCAGCGCAAAAGATGCGTAA</u>
ACACACCAAACC AUUAUUUAUUA GAAACUUCGAUC A	27280 ( <i>orf5</i> gene)	14.0	M8	<u>TAATACGACTCACTATAGGGTGATCGAAGTTT</u> <u>CTAATAAATAATGGTTTTGGTGTGTGGACTTTAG</u> <u>AACAGAGGAGATAAAGATGACACACCAAACA</u> <u>AACCTGGCGGCAGCGCAAAAGATGCGTAA</u>
GAGCCAUUAAAC UUGACCCAAAGA AUCCCAACUACA	29563 ( <i>N</i> gene)	19.8	M9	<u>TAATACGACTCACTATAGGGTGTAGTTGGGAT</u> <u>TCTTTGGGTCAAGTTTAATGGCTCGGACTTTAG</u> <u>AACAGAGGAGATAAAGATGGAGCCATTAAACA</u> <u>ACCTGGCGGCAGCGCAAAAGATGCGTAA</u>
GCCAUUAAACUU GACCCAAAGAAU CCCAACUACAAU	29565 ( <i>N</i> gene)	19.2	M10	<u>TAATACGACTCACTATAGGGATTGTAGTTGGG</u> <u>ATTCTTTGGGTCAAGTTTAATGGCGGACTTTAG</u> <u>AACAGAGGAGATAAAGATGGCCATTAAACTAA</u> <u>ACCTGGCGGCAGCGCAAAAGATGCGTAA</u>
AUGUCAUAUCA ACAUCACAUAAA UUAGUCUUGUC UG	16334 ( <i>orf1ab</i> gene)	23.1	S1	<u>TAATACGACTCACTATAGGGCAGACAAGACT</u> <u>AATTTATGTGATGTTGATATGACATGGACTTTAG</u> <u>AACAGAGGAGATAAAGATGATGTCATATCAGA</u> <u>ACCTGGCGGCAGCGCAAAAGATGCGTAA</u>

AUGACAAAGAU				<b>TAATACGACTCACTATAGGGAAATGACTTGAT</b>
CCAAUUUCAA	29292		S2	<b>CTTTGAAATTTGGATCTTTGTCATGGACTTTAG</b>
GAUCAAGUCAU	(N gene)	26.3		<b>AACAGAGGAGATAAAGATGATGACAAAGATAA</b>
UU				<b>ACCTGGCGGCAGCGCAAAGATGCGTAAA</b>

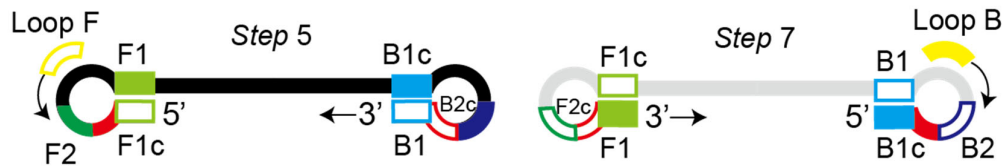
The sequence of sensors consists of **T7 promoter** + **target region (a + b)** + conserved domain\_sequence + **a part of target region** (complementary sequence of stem region, b') + conserved domain sequence. The conserved domain sequence was from the series B sensor from the previous publication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

**Table S2.** List of RT-LAMP primer sequences

Sensor <sup>†</sup>	RT-LAMP primer	Sequence (5' to 3')
M6	F3	ATTAGCCCATCTACCAGC
	B3	TGGCAAAGAAGTATAGGAATT
	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 TTAGAGGCCCAAGGGGTTATGCTACA GGACAATGATTCCAGAG
	BIP with T7 terminator (5' B1c-T7 terminator-B2 3')	CCGATGGATGTGGCACTTTACTAGCATAACCCCTTGGGGCCTCT AAACGGGTCTTGAGGGGTTTTTGCAG GACAATGATTCCAGAG
	BIP without T7 terminator	CCGATGGATGTGGCACTTTACCAGCAGGACAATGATTCC
	Loop F <sup>‡</sup>	GAACCCAGCATAAAAGC
	Loop B	TTTATTGTATTCTAGAGCCTCGCTC
	S1	F3
B3		CTAATGGGTGGTTTATGTGATT
FIP with T7 promoter complementary sequence (5' F2c-T7 promoter-F1 3')		TGGTCGTAACAGCATTTACAACATACCCTATAGTGAGTCGTA TTAACAGACTTCATTAAGATGTGGT
BIP without T7 terminator		TAATCCGTATGTTTGCAATGCTCCCATACCTCCTAAGTAAAGT TGAG
Loop F <sup>‡</sup>		AGAATGGTCTACGTATGCAAGC
Loop B		AGGTTGTGATGTCACAGATGTG
S2	F3	GTTCTTCGGAATGTCCGG
	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

<sup>†</sup>Target region of each sensor is amplified by RT-LAMP.

<sup>‡</sup>Loop F primer of M6 and S1 was obtained by changing the parameter condition from the default setting, T<sub>m</sub> 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

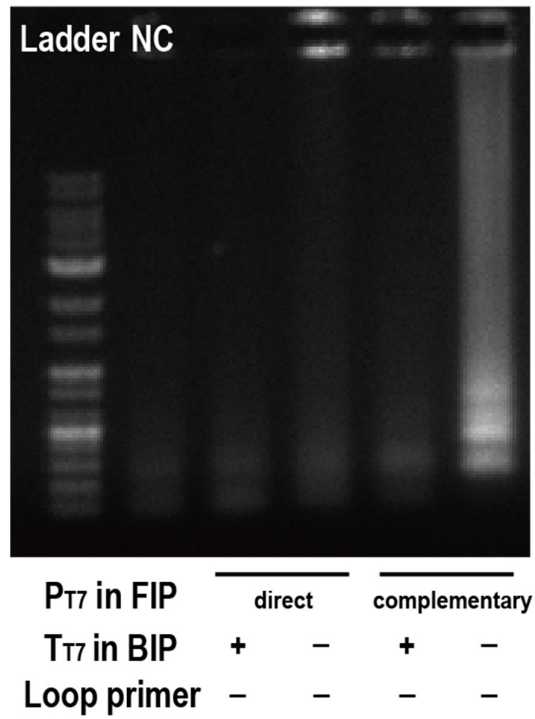
MERS-CoV	1	- TTGCTAATGGGTTT	GTCGTCGGTATAGGAGCAGCTGCCAA-TTCCA	CTGT	
SARS-CoV	1	- TTGTCCGTGGTTGG	GTTTTTGGTTCTACCATGAACAACA--AGTCA	CACAG	
SARS-CoV-2	1	- TAATAAGAGGCTGG	ATTTTTGGTACTACTTTAGATTCGA--AGACC	CAG	
HCoV-OC43	1	- TTATTAAGATCGT	GTAATGTATAGTGAGTTCCTGCTAT-AACTA	TATAG	
HCoV-NL63	1	AACACTACTTTTGAT	TTTTTAAGTAATGCTTCTAGTTCTTTGACT	GAT	
HCoV-HKU1	1	- TGTATGTTAATAAA	ACTTTGTATAGTGAGTTTAGTACTAT-AGTTA	TATAG	
MERS-CoV	51	GC	ACTG-TTATTATTAGCCCATCTACCAG-CGCTA	CTATACGAAAAATTT	
SARS-CoV	51	TC	GGTGATTATTATTAACAATTCTACTAA-TGTT	GTTATACGAGCATGTA	
SARS-CoV-2	51	TC	CCTACTTATTGTTAATAACGCTACTAA-TGTT	GTTATTAAGTCTGTG	
HCoV-OC43	51	GT	AGTACTT-TTGTAATACATCCTATAG-TGTGG	TAGTACAACCACGTA	
HCoV-NL63	51	AG	TTAATTTGTTATTTACAGAACAGTTAGGTGC	GCCTTTGGGCATAACTA	
HCoV-HKU1	51	GT	AGTGTTTT-TATTAACAACCTCTTATAC-TATT	GTTGTTCAACCTCATA	
MERS-CoV	101	AC	CCTGCTTTT-----ATGCTGG-----	-----GTTCTTCAG	
SARS-CoV	101	AC	TTTTGA-----ATTGTG-----TGACA	ACCCTTTCT----	
SARS-CoV-2	101	AA	TTTCA-----ATTTG-----TAATG	ATCCATTT-----	
HCoV-OC43	101	CA	ATCAATTAACACAGGATGGTGATAATAAATT	ACAAGGTCTTTTAGAG	
HCoV-NL63	101	TA	TCTGGTGAA-----ACTGTG-----	-----CGTCTGCATTTATAT	
HCoV-HKU1	101	-----	-----ATGGTG-----	-----TTTTGGAG	
MERS-CoV	151	TT	GGTAATTTCTCAGATGGTA	AAATGGGCCGCTTCTTCAATCATACTCTA	
SARS-CoV	151	-TT	GCTGTTTCTAAAC--CCA-TGGGTA-CACAG	AC--ACATACTATGA	
SARS-CoV-2	151	-T	GGGTGTTTATTACC--ACA-AAAACAACAA	AAGTTGGATGGAAAGTGA	
HCoV-OC43	151	GT	CTCTGTTTGCCAGT--ATAATATGTGCGAG	TACCCACAACGATTTGT	
HCoV-NL63	151	AA	TGTAACCTCGT-ACT--TTTTATGTGCCAG	CAGCTT-ATAACTTACT	
HCoV-HKU1	151	AT	TACAGCTTGTC AAT--ACA	CTATGTGTGAGTATCCTCATACTATTTGT	
MERS-CoV	201	GT	TCTTTTGCCCGATGGATGTGGCACTTTACTT	AGAGCTTTTTTATTGTA	
SARS-CoV	201	TAT	TTCGA-----TAATGCA-TTTAATTGCA-	CTTCGAGTACAT	
SARS-CoV-2	201	GT	TCAGAGG-----TTTATTCTAGTGCG-AATA	AATTGCA-CTTTGAAATATGT	
HCoV-OC43	201	CA	TCTAACCTGGGTAATCATCGCA-AAGA	ACTATGGCATTTTGGATACAG	
HCoV-NL63	201	AA	ACTTA	GTGTTAAATGTTACTTTA-ACTATTCCTGT	TTTTTAGTGT-T
HCoV-HKU1	201	AA	ATCTAA	-----AGGTAGTTCTCGTA-ATGAATCTT	GGCATTTTGATAAAT
MERS-CoV	251	-T	CTAGAGCCTCGCTCTGGAAATCATTTGCTC	TGCTGGCAATTCCTATA	
SARS-CoV	251	AT	CTGATGCCTTTTCGCTTGATGTTTCAGAAA	A--GTCAGGTAATTTTAA	
SARS-CoV-2	251	C	TCTCAGCCTTTTCTTATGGACCTTGAAGG	AAA--ACAGGTAATTTCAA	
HCoV-OC43	251	GT	GTTGTTTCTGTTTATATAAGCGTAATTT	CACATATGATGTGAATGCT	
HCoV-NL63	251	GT	CAACGCCACCGTTACTGTGAATGTCACCA	CA--CATAATGGCCGTGTA	
HCoV-HKU1	251	CT	GAACTTTGTGTCTGTTCAAGAAAAATTTT	ACTTATAATGTTTCTACA	
MERS-CoV	301	T	CTTTTGCCACTTATCACACTCCTGCAACAG	ATTGTTCTGATGGCAATTA	
SARS-CoV	301	CA	CTTACGAGAGTTTGTGTTTAAAAATAAAG	ATGGGTTTCTCTATGTTTA	
SARS-CoV-2	301	AA	TCTTAGGGAATTTGTGTTAAGAATATTG	ATGGTTATTTTAAATATA	
HCoV-OC43	301	G	ATTATTTGTATTTTCAATTTTATCAAGA	AAGGTGGTACTTTTTATGCATA	
HCoV-NL63	301	G	TAACTACACTGTTTGTGATGATTGTAAT	GGTTATACTGATAACATATT	
HCoV-HKU1	301	G	ATTGGTTGATTTTCAATTTTATCAAGA	ACGTTGGCACTTTTTATGCTTA	
MERS-CoV	351	CA	ATCGTAATG	CCAGTCTGA	ACTCTTTTAAAGGAGTATTTTAAATTTACGTA
SARS-CoV	351	TA	AGGGCTATCA	-----A-----CCTATAGATGTAGTT	CGTGATCTACCTT
SARS-CoV-2	351	TT	CTAAGCACA	C-----G-----CCTATTAATTTAGT	GGTGTATCTCCCTC
HCoV-OC43	351	TT	TACAGACA	CTGGTGT--GTTACTAAGTTTTTTGT	TTAATGTTTATT
HCoV-NL63	351	TT	CTGTTCAAC	AGGATGGCCGCATTCCTAATGGTTTTCC	CT--TTAATAA
HCoV-HKU1	351	TT	ATGCTGATT	CTGGCATG--CCTACTACTTTTTTATTT	AGTTTGTATC

**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.

SARS-CoV-2	1	- - - - -	GCTGTTGGGGCTTGTGTTCTTTGCAATTCACAGACTTCATT
SARS-CoV	1	GTCTTGCAG	GCTGTAGGTGCTTGTGTATTGTGCAATTCACAGACTTCACT
MERS-CoV	1	ACTTTGCAG	GCTGTGCGTTCATGCGTGTATGCCATTCACAGACTTCCCT
HCoV-229E	1	GTATTACAA	GCTGCTGGTCTTTGTGTAGTATGTGGTTCTCAAACAGTTCT
HCoV-OC43	1	GTTATGCAG	AGTGTGGAGCTTGCCTGGTCTGCTCTTCTCAAACATCATT
HCoV-NL63	1	ATATTGCAA	GCTGCTGGTTTATGTGTTGTTTGTGGTTCACAACTGTACT
HCoV-HKU1	1	GTGATGCAG	AGTGTAGGTGCATGCGTGTGTTGTTCATCACAACTTCTTT
SARS-CoV-2	51		AAGATGTGGTGCCTTGCATACGTAGACCATTCTTATGTTGTAATGCTGTT
SARS-CoV	51		TCGTTGCGGTGCCTGTATTAGGAGACCATTCTATGTTGCAAGTGCTGCT
MERS-CoV	51		ACGCTGTGGGACATGCATCCGTAGACCATTTCTCTGCTGTAATGCTGCT
HCoV-229E	51		AAGATGCGGTGATTGTTTACGCAAGCCGATGTTGTGCACTAAGTGCGCT
HCoV-OC43	51		ACGTTGTGGCAGTTGCATCAGAAAGCCTCTTCTTGTGCAAGTGTTGTT
HCoV-NL63	51		TCGTTGTGGTATTGTTGCTGCGTAAGCCTATGTTGTGCACTAAATGCGCAT
HCoV-HKU1	51		GCGTTGTGGCAGTTGTATACGTAAGCCTTTGTTATGTTGTAATGTTGTT
SARS-CoV-2	101		ACGACCATGTCATATCAACATCACATAAATTAGTCTTGTCTGTTAATCCG
SARS-CoV	101		ATGACCATGTCATTTCAACATCACACAAATTAGTGTGCTGTTAATCCC
MERS-CoV	101		ATGATCATGTTATAGCAACTCCACATAAGATGGTTTTGTCTGTTTCTCCT
HCoV-229E	101		ATGATCATGTTGTTTGGCACTGATCATAAGTTCAATTTAGCTATTACACCA
HCoV-OC43	101		ATGATCATGTTATGGCGACTGATCATAAATATGCTTGGAGTGTTCACCA
HCoV-NL63	101		ATGATCATGTTATTTGGTACCGACCACAAGTTTATTTTGGCTATAACACCG
HCoV-HKU1	101		ATGACCATGTTATGGCAACTAATCATAAATATGTTTTGAGTGTCTCACCT
SARS-CoV-2	151		TATGTTTGC AATGCTCCAGGTTGTGATGTCACAGATGTGACTCAACTTTA
SARS-CoV	151		TATGTTTGC AATGCCCAAGGTTGTGATGTCAGTGTGACACAACCTGTA
MERS-CoV	151		TACGTTTGT AATGCCCTGGTTGTGGCGTTTCAGACGTTACTAAGCTATA
HCoV-229E	151		TATGTGTGT AACAACACTCTGGCTGCAATGTAATGACGTTACAAAAGTGA
HCoV-OC43	151		TATGTGTGT AATGCACCAGGATGTGATGTAATGATGTTACCAAATTGTA
HCoV-NL63	151		TATGTATGT AATGCATCAGGTTGTGGTGTAGTGTGCAAAAAATTGTA
HCoV-HKU1	151		TACGTTTGT AATGCACCTAACTGTGATGTGAGTGTGTCACCAAATTATA
SARS-CoV-2	201		CTTAGGAGGTATGAGCTATTATTGTAATCACATAAACCACCCATTAGTT
SARS-CoV	201		TCTAGGAGGTATGAGCTATTATTGCAAGTCACATAAGCCTCCCATTAGTT
MERS-CoV	201		TTTAGGTGGTATGAGCTACTTTTGTGTAGATCATAGACCTGTGTGAGTT
HCoV-229E	201		TCTTGGAGGTTTGAATTACTGTGTAGACCACAACCACATCTTTCAT
HCoV-OC43	201		TCTAGGTGGTATGTCATATTATTGTGAAGACCATAAGCCACAATATTCAT
HCoV-NL63	201		TCTTGGTGGTTTGAATTACTATTGTACAAATCATAAACCACAGTTGTCTT
HCoV-HKU1	201		TTTGGGCGGTATGCTTACTATTGTGAAAACCATAAACCCATTATTAT
SARS-CoV-2	251		TTCCATTGTGTGCTAATGGACAAGTTTTTGGTTTATATAAAAATACATGT
SARS-CoV	251		TTCCATTATGTGCTAATGGTCAGGTTTTTGGTTTATACAAAACACATGT
MERS-CoV	251		TTCCACTTTGCGCTAATGGTCTTGTATTGCGCTTATACAAGAATATGTGC
HCoV-229E	251		TCCCAGTGTGTTCAAGCTGGTAATGTCTTGGTTTGTACAAAAGTTCTGCT
HCoV-OC43	251		TCAAGTTGTTAATGAATGGTCTGGTTTTTGGTCTATATAACAATCTTGT
HCoV-NL63	251		TTCCATTATGTTCAAGCTGGTAATATATTGGTTTATATAAAAATTAGCA
HCoV-HKU1	251		TTAAGTTAGTTATGAATGGTATGGTCTTGGTTTGTATAACAATCTTGC

**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.